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EVALUATION OF THE NEUROPROTECTIVE ACTIVITY AND COGNITIVE ABILITY OF *AEGLE MARMELOS* ETHANOLIC LEAVES EXTRACT AND ESTROGEN IN ESTROGEN COMPROMISED FEMALE RATS USING SCOPOLAMINE-INDUCED ALZHEIMER'S MODEL

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Keywords:

A. marmelos, Memory impairment, Alzheimer's disease, Oxidative stress, Scopolamine

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ABSTRACT: Alzheimer's disease, the most common form of dementia, is higher in elderly women than men. Escape latency time of Group 3 (Donepezil) and Group 6 (AMEE and Estradiol) is less than group 2 (Scopolamine and letrozole). As shown by the results, the decrease in AChE level in Group 3 (Donepezil) and Group 6 (AMEE and Estradiol) indicates restoration of Ach level in the brain. Increased GSH level was seen in Group 3 (Donepezil) and Group 6 (AMEE and Estradiol16 indicating antioxidant property. The architecture of the neurons in the hippocampus histopathological studies was seen when the combined treatment with estrogen and AMEE was given confirming that our given combination has the potential to improve memory in female rats, particularly those who were estrogen-deficient as in our experimental design. Based on our experiments, we can conclude that Aegle marmelos ethanolic extract, along with estradiol, can improve memory and can be used for further studies in this area.

INTRODUCTION: Alzheimer's disease, the most common form of dementia, is higher in elderly women than men. The biological basis for this heightened risk in women remains to be established; basic and clinical evidence indicate that the rapid decline in estrogen at the onset of menopause has adverse consequences, particularly for the brain [1]. An estimated 0.7% of the global population has dementia, translating to 51.6 million people worldwide. Dementia-related deaths are projected to increase from the current 2.4 million per year to 5.8 million by 2040.

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Pathophysiology of AD is a degeneration of brain neurons due to Aβ overproduction and accumulation of amyloid-β (Aβ), neurofibrillary tau tangles beyond the normal and low concentration of acetylcholine [2, 3]. The deposition of neurofibrillary tangles and Aβ plaques in brain cells raises the generation of reactive oxygen species that causes oxidative stress and cell death [3, 4]. Cognitive function includes memory, attention, creativity, intelligence, and ability to think [5].

The behavioural, cognitive and neuropathological changes observed in AD can be induced by intraperitoneal (i.p) administration of scopolamine in female rats. Current treatment includes aducanumab (Aduhelm; Biogen Inc), the first new drug for the treatment of Alzheimer's disease in 2 decades, approved on June 7, 2021 by the US Food and Drug Administration (FDA) after 20 years [7]. Aducanumab is an amyloid-targeting monoclonal

antibody delivered by monthly intravenous infusions. Aducanumab is titrated to a dose of 10 mg/kg over a 6-month period [8]. Cholinesterase inhibitors and anti- NMDA for symptomatic treatment. FDA currently approves three ChEIs for use in mild-to-moderate AD: donepezil, rivastigmine and galantamine. Memantine is an uncompetitive NMDA receptor modulator that may act to inhibit glutamate-mediated neurotoxicity that develops as neurons die during AD progression [9].

Estrogen is a female sex hormone that regulates many reproductive tissues' growth, differentiation, and function. Young, healthy women tend to have 150 pg/mL estradiol levels, whereas postmenopausal women's estradiol levels average an approximate 10–15 pg/mL. The ovary is the primary source of estrogens in females that are synthesized from acetate and cholesterol in a multi-step pathway utilizing a cytochrome P-450 aromatase as the final step. Estrogen is necessary for memory retention, metabolic balance, and bone health in premenopausal women. The loss of estrogen may accelerate age-related cognitive impairment [10, 11, 12]. Estrogen (17β estradiol) has neuroprotective role, role in neurogenesis, role in neuronal activity, role in synaptic plasticity [13]. Estrogen reduced Aβ levels by reducing the production of Aβ and increasing clearance of Aβ in the presence of AD, causing triggers [14]. Estrogen improves flow of blood in brain regions that are affected by AD thus it is a potent factor that not only prevent blood vessels disease but also improves flow of blood in vessel suffering from disease. Estrogen is important for keeping neurons alive and for repairmen of damaged neurons by AD [15].

Estrogen is important for protection against programmed cell death, antioxidant and anti-inflammatory actions in brain [16]. Estrogen exerts biological effects through its receptors. ERα acts as a transcription factor in the reproductive system and during human development and ERβ is widely expressed in the cortex, hippocampus and other brain areas associated with learning and memory [17]. *Aegle marmelos* are used traditionally for many diseases. It has an antioxidant mechanism: *A. marmelos* shows radical scavenging activity due to flavonoids and OH group in phenol. It protects against ROS and free radicals by scavenging them and converting them into less reactive molecule [18]. Acetylcholinesterase mechanism: *A. marmelos* inhibit acetylcholinesterase, an enzyme that degrades acetylcholine into choline and acetate. Loss of acetylcholine results in cholinergic hypofunction, which in turn causes memory impairment in patients of AD. Thus, by suppressing AChE, acetylcholine levels rise, thus improving cognitive functions in AD patients [19].

MATERIALS AND METHODS:

1.1. Chemicals and Drugs: Scopolamine and donepezil were obtained from the School of Pharmaceutical Sciences, Shri Guru Ram Rai University, Dehradun, India. Letrozole and estradiol were purchased from Sigma-Aldrich, India. All the chemicals used were of analytical grade.

1.2. Animals: The protocol was reviewed and approved by the Institutional Animal Ethics Committee, School of Pharmaceutical Sciences, Shri Guru Ram Rai University, Dehradun, India (Approval no. 264/CPCSEA/IAEC/2021/10). Female albino Wistar rats (250-300 g, 5-6 months old) were obtained from the animal house of Shri Guru Ram Rai University, Dehradun. The animals were housed in polypropylene cages with standard housing conditions of 23 ± 2 °C temperature and 60–65% relative humidity in a natural light/dark cycle, provided food and water *ad libitum*. The protocol was reviewed and approved by Institutional Animal Ethics Committee, School of Pharmaceutical Sciences, Shri Guru Ram Rai University, Dehradun, India (Approval no. 264/CPCSEA/IAEC/2021/10) Ethical standards were maintained for carrying out all the experimental procedures on animals.

1.3. Preparation of Ethanolic Extract: Fresh leaves of *A. marmelos* were collected from the herbal garden of, the School of Pharmaceutical Science, Shri Guru Ram Rai University, Dehradun, India in march and were authenticated from the Botanical Survey of India (BSI) Dehradun, India (Ref no. Tech./Herb(Ident.)/2020-2021/612). A Herbarium specimen was submitted at BSI. The leaves were washed and shade dried for one week and then coarsely powdered. Next, the powdered material was soaked in ethanol, and occasional stirring was done for one week later. The extract

was strained and evaporated under reduced pressure and was stored at 2–4 °C protected from sunlight until further studies. The ethanolic extract of *A. marmelos* was dissolved in saline prior to pharmacological studies.

1.4. Experimental Design and Grouping of Animals: Female Albino Wistar rats to be used in each group will be 6 (n=6). Randomly selected female Wistar rat will be 36.

Group I: Normal saline (10 ml/kg body weight) will be administered as a vehicle for 14 days in female Albino Wistar rat.

Group II: Scopolamine 1mg/kg i.p [105] and Letrozole (1mg/kg p.o [20] for 14 days in female Albino Wistar rat .

Group III: Scopolamine and Letrozole followed by Donepezil 5 mg/kg i.p [21] for 14 days in female Albino Wistar rat.

Group IV: Scopolamine and Letrozole followed by Estrogen 2.4 µg/kg i.p [22] for 14 days in female Albino Wistar rat.

Group V: Scopolamine and Letrozole followed by AMEE *Aegle Marmelos* Ethanolic Extract (400mg/kg p.o [21] for 14 days in female Albino Wistar rat.

Group VI: Scopolamine and Letrozole followed by estrogen (2.4 µg/kg i.p) and AMEE (*Aegle Marmelos* Ethanolic Extract*)* (400mg/kg p.o) for 14 days in female Albino Wistar rat. The doses of AME were selected based on a previous study.

1.5. Experimental Induction of AD: To induce amnesia, scopolamine (1 mg/kg i.p.) was injected into female rats 1 h before the start of behavioral tests. Scopolamine is a well-known anticholinergic drug and acts by blocking the muscarinic Ach receptor (mAchR) that can interfere with memory deficits by disrupting cholinergic neurotransmission.

1.6. Behavioural Assessment

1.7. Evaluation of Learning and Spatial Memory: A circular water pool with a diameter of 130 cm and a height of 60 cm, filled to 3/4th level with water (27 1 °C), was split into four equal quadrants. In either of the quadrants, a circular platform (10 cm diameter) was submerged 1 cm beneath the water surface and its location was constant throughout the experiment. The platform was rendered undetectable by making the water in the pool opaque using non-toxic paint. During a four-day training phase, animals were given four attempts each day (with a 30-second pause between them) to discover the submerged platform.

The experiment began with the animal being placed in the pool facing the pool wall. The rats were given a maximum of 60 seconds to discover the platform, after which they were permitted to rest for 30 seconds. The time it took the rats to discover the platform (latency time) was recorded in each trial, and the rats that did not find the platform within 60 seconds were educated to do so by the researcher.

Successful memory retention was defined as a substantial reduction in mean latency throughout the training period. A 60-second probing experiment was done on day 5 of the MWM task to measure memory by removing the platform and allowing the rats to swim freely in the pool.

The amount of time rats spent in the target quadrant (where the submerged platform was placed) was tracked to determine the degree of memory consolidation following learning sessions [23].

1.8. Estimation of Biochemical Parameters: All biochemical parameters were performed in the brain homogenate after MWM evaluation. Animals were sacrificed by euthanizing with lethal dose of anaesthesia (diethyl ether) and cervical dislocation.

1.9. Preparation of Brain Homogenate: The brain was promptly removed and cleaned in ice-cold saline. The hippocampus was extracted, and hippocampal tissue was homogenized in a tissue homogenizer with 0.1 M phosphate buffer (pH 7.4). The tissue homogenate (10% w/v) will be centrifuged for 15 min at 10,000g. The supernatant was collected for further analysis of the biochemical parameters listed below.

1.10. Estimation of Brain Cholinesterase Activity: Anti choline esterase enzyme assay-AChE assay was performed by the previously reported method of Ellman *et al*. To 0.05 ml of supernatant, 3 ml of 0.01 M sodium phosphate

buffer (pH 8), 0.1 ml of AChE iodide and 0.1 ml of Ellman reagent will be added.

The absorbance was measured immediately at 412 nm using spectrophotometer. The AChE activity in the samples will be expressed as nmol/mg protein.

1.11. Estimation of GSH: Ellman's technique, published in 1959, was used to calculate GSH in the produced homogenate.

In a nutshell, 1 mL of 4 percent sulfosalicylic acid was added to 1 mL supernatant and digested at 4°C for 1 h. For 15 minutes, the mixture was centrifuged at 1200 g for 15 minutes. One milliliter of supernatant was combined with 2.7 mL of phosphate buffer (0.1 M, pH 7.4) and 0.2 mL of DTNB.

Using a spectrophotometer, the yellow colour generated was measured at 412 nm right away. The standard curve was used to calculate GSH concentration, which was expressed as mol/mg protein.

1.12. Histopathological Studies: Isolated rat brain samples was stored in formalin consisting of rat specimen measures 2.7 X 1.4 X o.5cms. Cut surface unremarkable grossly. Processed in 1 block plus tissue.

1.13. Statistical Analysis- All data are expressed as the mean ± S.E.M. The significant difference was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test. A p-value of <0.05 was calculated as statistically significant.

RESULTS:

2. Effect of AMEE and Estradiol on Scopolamine Induced Memory Impairment in MWM Task: The memory-enhancing effect of AMEE and estradiol are present in **Table 1;** there is an increase in escape latency in the inducer group (Scopolamine and Letrozole) when compare with the control group (p<0.001) of the scopolamine and letrozole group of Alzheimer induce in rats, both showed decreased time to escape on the escape platform.

The group treated with AMEE and estradiol shows a decreased escape latency and significant value (P<0.001), respectively, shown in **Table 1.**

TABLE 1: TREATMENT EFFECT ON ESCAPE LATENCY IN 14TH DAYS (BY MORRIS WATER MAZE)

S. no.	Groups	Mean ± S.E.M
1	Normal Control	23.60± 0.6302
2	Scopolamine+ Letrozole	56.00± 1.133
3	Donepezil	28.30± 0.5600
4	Estradiol	39.42± 0.9175
5	AMEE	35.28± 0.5062
6	AMEE+Estradiol	31.83± 0.8508

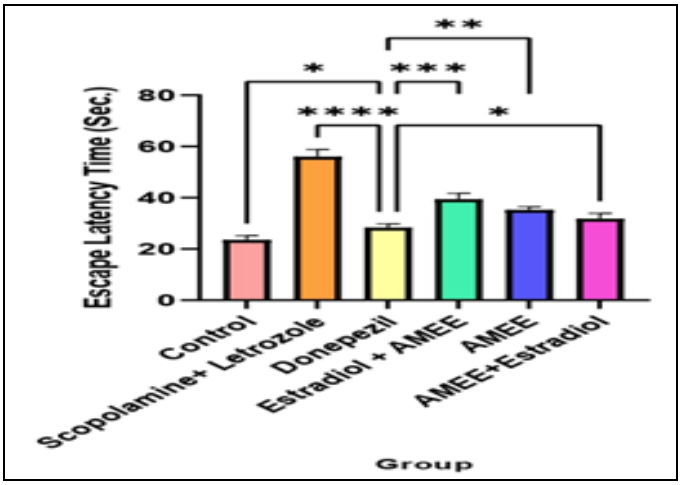

FIG. 1: DATA ARE EXPRESSED AS MEAN ± SEM = STANDARD ERROR OF MEAN **P<0.01, ***P<0.001, ****P<0.0001 WHEN COMPARED WITH THE DONEPEZIL (STANDARD) GROUP.

Control indicate administration of normal saline (10mg/kg) for 14 days, Scopolamine and Letrozole indicates Letrozole(1mg/kg p.o) and Scopolamine (1mg/kg i.p) 30 min after Letrozole administration for 14 days, Donepezil indicate administration of Donepezil (5 mg/kg i.p) for 14 days + Scopolamine 30min after Donepezil administration for 14 days, Estradiol indicate administration of Estradiol (2.4 µg/kg) for 14 days + Scopolamine 30 min after Estradiol administration for 14 days, AMEE indicate administration of AMEE (400mg/kg p.o) for 14 days + Scopolamine 30 min after AMEE administration for 14 days, Estradiol + AMEE indicates Estradiol (2.4 µg/kg) for 14 days and AMEE (400mg/kg p.o) 30 min after Estradiol administration and + Scopolamine 30 min after AMEE administration for 14 days.

2.1. Effect of AME on AChE Activity: There is an increased in AChE activity in disease control group when compared with the control group of the scopolamine and letrozole groups of Alzheimer induced rats, both shows increased in AChE activity.

The group treated with AMEE and estradiol showed decreased activity compared with disease control group. The significant showed in **Table 2.**

TABLE 2: EFFECT OF AMEE AND ESTRADIOL ON SCOPOLAMINE AND LETROZOLE INDUCED ALZHEIMER IN FEMALE RATS USING THE ESTIMATION OF ACHE LEVEL IN µMol

S. no.	Groups	Mean ± S.E.M
1	Normal Control	125.1±0.8794
2	Scopolamine+ Letrozole	195.5±0.7638
3	Donepezil	84.73±0.5018
4	Estradiol	142.9±0.7937
5	AMEE	136.5±0.7638
6	AMEE+Estradiol	92.83±0.9458

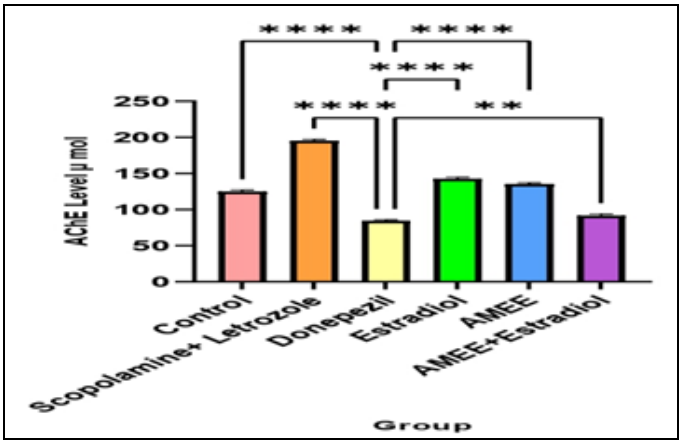

FIG. 2: DATA ARE EXPRESSED AS MEAN ± SEM= STANDARD ERROR OF MEAN **P<0.01, ***P<0.001, ****P<0.0001, WHEN COMPARED WITH THE DONEPEZIL (STANDARD) GROUP.

Control indicate administration of normal saline (10mg/kg) for 14 days, Scopolamine and Letrozole indicates Letrozole (1mg/kg p.o) and Scopolamine (1mg/kg i.p) 30 min after Letrozole administration for 14 days, Donepezil indicate administration of Donepezil (5 mg/kg i.p) for 14 days + Scopolamine 30 min after Donepezil administration for 14 days, Estradiol indicate administration of Estradiol (2.4 µg/kg) for 14 days + Scopolamine 30 min after Estradiol administration for 14 days, AMEE indicate administration of AMEE (400mg/kg p.o) for 14 days + Scopolamine 30 min after AMEE administration for 14 days, Estradiol + AMEE indicates Estradiol (2.4 µg/kg) for 14 days and AMEE (400mg/kg p.o) 30 min after Estradiol administration and + Scopolamine 30 min after AMEE administration for 14 days.

2.2. Effect of AME on GSH: There is a decrease in Glutathione activity in the disease control group when compared with the control group of the scopolamine and letrozole groups of Alzheimer induced rats; both show decrease in Glutathione activity. The group treated with AMEE and estradiol showed increased activity compared with the disease control group. The significance showed in **Table 3.**

TABLE 3: EFFECT OF MEE AND ESTRADIOL ON SCOPOLAMINE AND LETROZOLE INDUCED ALZHEIMER IN FEMALE RATS USING THE ESTIMATION OF GSH LEVEL IN µMol

S. no.	Groups	Mean ± S.E.M
1	Normal Control	18.67±0.8819
2	Scopolamine+ Letrozole	8.167±0.9458
3	Donepezil	21.92±0.7912
4	Estradiol	12.50±0.7638
5	AMEE	14.00±0.9661
6	Estradiol+ AMEE	17.00±0.9661

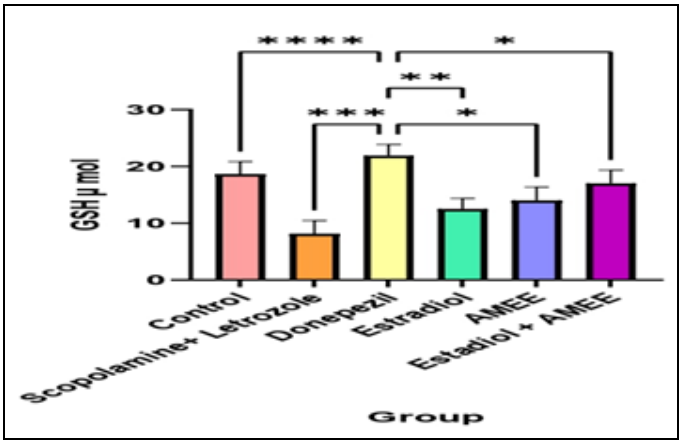

FIG. 3: DATA ARE EXPRESSED AS MEAN ± SEM= STANDARD ERROR OF MEAN NS P>0.05, **P<0.01, ***P<0.001, ****P<0.0001, WHEN COMPARED WITH THE DONEPEZIL (STANDARD) GROUP.

Control indicate administration of normal saline (10mg/kg) for 14 days, Scopolamine and Letrozole indicates Letrozole (1mg/kg p.o) and Scopolamine (1mg/kg i.p) 30 min after Letrozole administration for 14 days, Donepezil indicate administration of Donepezil (5 mg/kg i.p) for 14 days + Scopolamine 30 min after Donepezil administration for 14 days, Estradiol indicate administration of Estradiol (2.4 µg/kg) for 14 days + Scopolamine 30 min after Estradiol administration for 14 days, AMEE indicate administration of AMEE (400mg/kg p.o) for 14 days + Scopolamine 30 min after Eugenol administration for 14 days, Estradiol + AMEE indicates Estradiol (2.4 µg/kg) for 14 days and AMEE (400mg/kg p.o) 30 min after Estradiol administration and + Scopolamine 30 min after AMEE administration for 14 days.

2.3 Histopathology of Rat Brain (Hippocampus): Histopathological changes in hippocampus region of brain of all the 6 groups studied are shown in **Fig. 4 (A-F).**

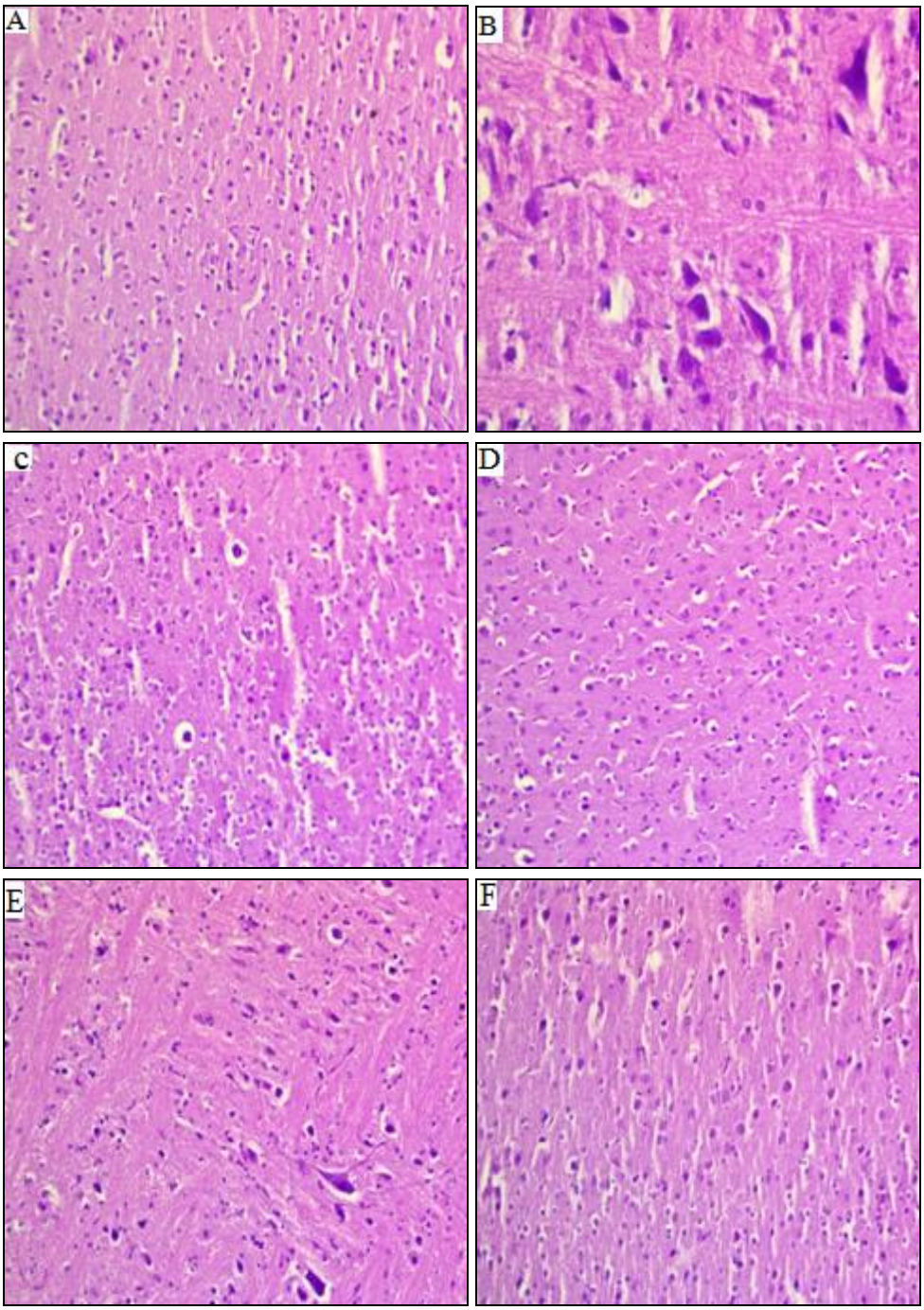

FIG 4: HISTOPATHOLOGY OF RAT BRAIN (HIPPOCAMPUS) AT 200X- A. Normal group: showing normochromatic nuclei. B. Scopolamine and letrozole group: shrinkage of neuronal cell bodies and hyperchromatic nuclei with irregular shape C. Donepezil group: Occasional shrunken irregular hyperchromatic nuclei. D. Estradiol group: a few shrunken hyperchromatic nuclei E. AMEE a few shrunken hyperchromatic nuclei. F. Estradiol and AMEE occasional hyperchromatic nuclei, almost normochromatic nuclei.

In the Normal group, normal-appearing neurons are visible with normochromic nuclei. The Scopolamine and letrozole group shows neurons of variable appearance showing degenerative changes, shrunken irregular hyperchromatic nuclei, showing anisokaryosis and reactive changes. Along with this vascular congestion, moderate to marked lymphoplasmacytic filtration, and edema were also noted. Neuronal damage was seen. Donepezil group has shown neurons of variable appearance

showing occasional degenerative changes, Occasional shrunken irregular hyperchromatic nuclei, and occasional anisokaryosis. Along with these, minimal vascular congestion ad occasional lymphoplasmactic filtration was also noted. Almost normal neuronal damage was seen. Estradiol group has shown neurons of variable appearance showing receding degenerative changes, a few shrunken hyperchromatic nuclei, ocassioal anisokaryosis, and reactive changes. Along with these, moderate vascular congestion and mild lymphoplasmactic filtration were also noted.

Mild to neuronal damage was seen. AMEE has shown neurons of variable appearance showing minimal degenerative changes, a few shrunken hyperchromatic nuclei, occasional anisokaryosis, and reactive changes. Along with these, moderate vascular congestion and mild lymphoplasmactic filtration also noted. Mild to moderated neuroal damage was seen Estradiol and AMEE have shown neurons of variable appearance showing occasional degenerative changes ad occasional hyperchromatic nuclei. Along with these minimals vascular congestion and mild lymphoplasmactic filtration also noted. Near normal neurons was seen.

DISCUSSION: Scopolamine is an anticholinergic medication that causes dementia by lowering acetylcholine levels, which is a key neurotransmitter for learning and memory. From the behavioral tests, Morris water maze test, it is clearly seen that there was a significant decrease in the transfer latency (The time it taken by rats to discover the platform) in treated groups compared to the scopolamine-treated group. The memory loss effect of scopolamine is more prominent compared to the control group. As compared to normal group scopolamine+ letrozole group shows increased escape latency time. Compared with Donepezil, the higher dose (400 mg/kg p.o.) of AMEE treated groups has excellent performance, indicating neuroprotective effect against scopolamine as seen in **Fig. 1**. Estradiol and AMEE groups have shown a decrease in escape latency compared to scopolamine and estradiol. Administration of a combination of AMEE and estradiol at a dose of 400 mg/kg/p.o. and 2.4 µg/kg i.p for 14 days to animals successfully reversed the learning impairment produced by the scopolamine when compared to the other groups.

ACh is a neurotransmitter important for function in learning and memory, according to research. AChE, an enzyme that degrades acetylcholine into choline and acetate. Loss of acetylcholine results in cholinergic hypofunction, which in turn cause memory impairement in patients of AD. Thus, by suppressing AChE, the level of acetylcholine rises, thus improving cognitive functions in AD patients Scopolamine+ Letrozole group has shown an increased level of AChE level compared to the control group.

The donepezil group has shown a decreased level of AChE compared to all groups. Estradiol and AMEE groups have shown a decreased level of AChE compared to Scopolamine+ Letrozole groups. AMEE+ Estradiol group has shown a decreased level of AChE as compared to donepezil and scopolamine+letrozole group, as seen in **Fig. 2**. It was reported that a decrease in AChE activity can improve learning and memory. A combination of AMEE and estradiol improved antioxidant levels when compared to the scopolamine treated group. Glutathione is a major non-protein thiol in living organisms and protects the cellular defense system against the toxic effects of lipid peroxidation.

Due to oxidative stress, the brain is unable to maintain the GSH redox state and oxidized glutathione (GSSG) is concentrated in some regions of the brain. A decrease in GSH level leads to a decrease in cellular levels of α-tocopherol. In the present study, a significant decrease in the levels of non-enzymatic antioxidants like GSH was observed in AMEE and estradiol-treated rats. This could be due to increased utilization of GSH for scavenging the toxic products. Group treated with scopolamine, and letrozole showed a decrease in GSH level as compared to the control group. The donepezil group has shown increased GSH level compared to all other groups. Estradiol and AMEE groups have shown an increased level of GSH as compare to the Scopolamine+Letrozole group but decreased when compared to all other groups. Group treated with estradiol and AMEE has shown an increased level of GSH compared to Scopolamine+ Letrozole, Estradiol group, and AMEE group but decreased level of GSH compared to Donepezil and control group as seen in **Fig. 3**. Rat treated with AMEE and estradiol significantly restored the levels of GSH in the

brain, suggesting the involvement of GSH-dependent detoxification of free radical. These observations reveal that combination of AMEE and estradiol treated groups has enhanced the antioxidant potential.

Histopathological studies of hippocampal region of rats brains of all treated groups were compared with normal group, which showed normal neurons with normochromic nuclei in fibrillary background. There was degeneration of neurons, shrunken hyperchromatic nuclei, showing anisokaryosis and reactive changes, lymphoplasmactic infilteration and edema in scopolamine treated groups. Although no significant reactive changes were seen in the donepezil treated group, almost all neurons were normal. In the estradiol treated group, mild neuronal damage was seen with receding degenerative changes and a few shrunken hyperchromatic nuclei. In the AMEE treated group, mild to moderate neuronal damage was seen with receding degenerative changes and a few shrunken hyperchromatic nuclei. A combination of AMEE and estradiol showed minimal neuronal damage with minimal degenerative changes, occasional shrunken hyperchromatic nuclei and occasional anisokaryosis with minimal vascular congestion and minimal lymphoplasmactic infiltration.

CONCLUSION: In the current work, the effect of AMEE and estradiol on oxidative damage and cognitive deficit induced by i.p scopolamine was investigated in female rats. It was found that, i.p., scopolamine produced neurotoxic effects through the generation of free radicals and decreased acetylcholine levels in the brain by blocking muscarinic cholinergic receptors. Scopolamine is widely used in experimental animals to explore cognitive impairments. The deteriorative effect of scopolamine was shown to be reduced significantly in test groups. Pre-treatment with *A. marmelos* ethannolic leaf extract and estradiol ameliorated oxidative stress, neurodegeneration, cholinergic hypofunction, and memory impairment induced by scopolamine, dose-dependently. Overall, *A.marmelos* and estradiol have the potential to ameliorate the cognitive impairment caused by scopolamine. The escape latency time of Group 3 (Donepezil) and Group 6 (AMEE and Estradiol) is less than group 2 (Scopolamine and letrozole).

As shown by the results, the decrease in AChE level in Group 3 (Donepezil) and Group 6 (AMEE and Estradiol) indicates restoration of Ach level in the brain. Increased GSH level was seen in Group 3 (Donepezil) and Group 6 (AMEE and Estradiol) indicating antioxidant property.

The architecture of the neurons in the hippocampus histopathological studies was seen when the combined treatment with estrogen and AMEE was given confirming that our given combination has the potential to improve memory in female rats, particularly those who were estrogen-deficient as in our experimental design. On the basis of our experiments, we can conclude that *Aegle marmelos* ethanolic extract, along with estradiol has the potential to improve memory and can be used for further studies in this area. Since Alzheimer's is a common old age problem, particularly in postmenopausal women, these studies can be explored in clinical research also for the future benefit of mankind.

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CONFLICTS OF INTEREST: The author has no conflicts of interest.

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