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Ethanolic extracts of *Sterculia guttata*: Exploring the neuroprotective effects on memory and cognitive impairment in scopolamine-induced Alzheimer's disease rats

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Abstract: The progressive form of Alzheimer's disease (AD) is a neurological condition marked by decline in older people's memory and cognition. Scopolamine is a behavioral technique that is frequently used to study cognitive disorders, such as Alzheimer's disease. This investigation aimed to determine the protective effects of ethanolic extracts derived from *Sterculia guttata* (ESG) on neurological & pathological changes induced by Scopolamine in rats with Alzheimer's. The ESG procured through a 48-hour hot maceration, followed by column chromatography, isolation and characterization using techniques such as FTIR, 1HNMR, 13CNMR and mass spectra. A flavonoid called Diosmin was identified in the extract. Rats were segregated into five groups: normal, scopolamine, scopolamine + Donepezil, scopolamine + ESG (200mg per kg orally), & scopolamine + ESG (400mg per kg orally) for a study of 14 day duration. Memory & learning abilities were assessed using the rectangular maze and Cook's pole climbing model. Additionally, biochemical parameters and brain histology were analyzed. ESG treatment mitigated scopolamine-induced changes in acetylcholinesterase, dopamine, serotonin, glutamate, and GABA levels, suggesting neuroprotection. These findings propose that ethanolic extracts of *Sterculia guttata* (ESG) show promise as effective preventive or therapeutic agents due to their potential for neuroprotection & cognitive enhancement in AD.

Keywords: Alzheimer's disease, *Sterculia guttata* extracts, rectangular maze and Cook's pole climbing model.

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INTRODUCTION

AD is the predominant neurodegenerative ailment, marked by a progressive deterioration in cognitive abilities and memory as time passes. By the year 2023, it is anticipated that around 6.7 million individuals in the US who are sixty-five years of age or older would be affected by AD. of this population, 73% will be seventy-five years of age or older. Approximately 10.7 percent of people aged above 65 are affected by the disease. Alzheimer's is clearly noticeable by the presence of hyperphosphorylated tau protein tangles inside neurons, plaques of β-amyloid peptide both inside and outside neurons, and a reduction in acetylcholine (ACh) levels, which contribute to cognitive and memory dysfunction. The pathogenesis of Alzheimer's involves the deposition of amyloid plaques, activation of inflammatory responses, increased oxidative stress and decreased levels of steroid hormones, among other factors. Different signaling pathways, such as phosphatidylinositol-3-kinase (PI3K) and their downstream intermediates, such as protein kinase-B, also referred to as Akt, glycogen-synthase-kinase-3β (GSK-3β), cAMP-response element-binding protein (CREB), brain-derived neurotrophic factor (BDNF) and tropomyosin-related kinase receptor-B (TrKB), are responsible for the cognitive and memory dysfunctions caused by Aβ (Sally & Nevien *et al*., 2023).

Current therapy for Alzheimer's disease only provides symptomatic relief, but there is no cure. To culminate the side effects of current therapy researchers are in search of natural alternatives. Many natural products and plant extracts have been investigated for treating Alzheimer's disease. Plants that possess high levels of antioxidants, anti-inflammatory, anti-amyloidogenic and anti-cholinesterase qualities are effective in treating AD. Examples include *Curcuma longa, Bacopa monnieri, Convolvulus pluricaulis, Centella asiatica, Ginko biloba, Zingiber officinalis, Allium sativum*. Secondary plant metabolites with anti Alzheimeric activity include alkaloids (Caffeine, huperzine A, berberine, crytolepine, betaine, galantamine, sophocarpine), lignans, tannins, steroids (diosgenin), triterpenoids (artemisnin, ginkolide, cinsenoside, thymoquinone), flavonoid (cinnamaldehyde, apigenin, luteolin, curcumin, resveratrol, kaempferol, naringin, catechin, mangiferin and quercetin) and polyphenols. (Bui and Nguyen., 2017, Deng *et al.*, 2023). Research studies on rats with aqueous and methanol extract of *Ashwagandha* (Kuboyama *et al*., 2014), alcoholic extract of *Trifolium resupinatum* (Mardi S et al., 2023), extracts of *Evolvulus alsinoides* (Mettupalayam Kaliyannan Sundaramoorthy and Kilavan Packiam., 2020), alcohol extract of *Bacopa monnieri*, treatment with oil-diluted cannabis extract in patients (Palmieri and Vadala., 2023) with their antioxidant properties, anti-inflammatory properties of *Uncaria tomentosa* shown

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recovery from functional impairment and neurodegenerative diseases (Gregory *et al*., 2023), Lutein , a dietary antioxidant significantly ameliorated the effects of scopolamine on escape spatial learning and memory (Chirag P et al., 2021). Despite numerous studies on Alzheimer's disease treatment, finding a promising intervention for curing the disease remains a formidable challenge.

Sterculia guttata Roxb, commonly known as the bloody drop ordure tree and synonymously referred to as *Astrodendrum malabaricum* Dennst or the 'Hirik' tree, belongs to the Malvaceae family. Various species within the Sterculia genus have exhibited biological functions like antimicrobial, anti-inflammatory, antioxidant, & cytotoxic properties have been reported for various species within the Sterculia genus. The CNS depressant, anti-epileptic and mosquito larvicidal activities of *Sterculia guttata* seeds have been noted. The distribution of *Sterculia guttata* encompasses local regions in Assam, Andaman and Maharashtra in India, as well as Sri Lanka (Srivastava and Mehrotra., 2013, Katade *et al.*, 2009, Katade *et al*., 2004). The primary goal of this research was "to investigate the potential of ethanolic extracts of *Sterculia guttata* (ESG) as a powerful neuroprotective agent against AD.

MATERIALS AND METHODS

Collection of Plant material

The leaves of *Sterculia guttata* specimen was identified and authenticated by Dr. Jose Mathew, Assistant Professor, Department of Botany, Sanatana Dharma College, Alappuzha, Kerala, India. The voucher specimen was identified as *Sterculia guttata*, Family: Malvaceae. Voucher Number: DS-001/2019

Preparation of extract

The leaves of *Sterculia guttata* were gathered from Palode and its environs in Thiruvananthapuram, Kerala, India. Following the gathering process, the leaves underwent a meticulous cleaning procedure involving two rounds of washing with distilled water, followed by drying in a shaded area. After the leaves were completely dried, they were ground into a fine powder and then placed in receptacles that had been sterilized. In the process of ethanol extraction, the powdered leaves were inserted into a thimble made of durable filter paper or cellulose. This thimble was then placed in the designated chamber of a Soxhlet apparatus. This setup allowed for continuous extraction. The extraction process was carried out for approximately 48 hours or until the ethanol passing through the siphoning mechanism became colorless, indicating the extraction's completion. After collection, the solvent was evaporated from the extracts, and the samples were prepared for further analytical studies (Bagavan *et al*., 2008).

Animals

Adult "Wistar rats, of any gender, weighing between 150 & 200 grams, were acquired from the University College of Veterinary and Animal Houses in Mannuthy, Kerala. The subjects were accommodated at the Central Animal House of the JKK Munirajah Medical Research Foundation Annai JKK Sampoorani Ammal College of Pharmacy (approval number: 1158/CPCSEA) while adhering to a twelve-hour light-dark cycle. Both the control and experimental groups were provided with unlimited access to food as well as water. The food given to them consisted of pellets obtained from Sri Venkateswara Enterprises in Subramanya Nagar, Bangalore, India. The animal experimentation protocols followed the ethical requirements set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), as outlined by the" Ethical Committee IAEC registration number JKKMMRFCP/IAEC/2021/019.

Experimental groups

The anti-Alzheimer activity of ESG was assessed by categorizing rats into 5 separate groups, with every group including six rats (n=6/group):

Group I: Received CMC (1ml/kg, p.o.)
Group II: Received scopolamine (1mg/kg, i.p.)
Group III: Received donepezil (5mg/kg, p.o.)
Group IV: Received ESG (200mg/kg, p.o.) + Scopolamine (1mg/kg, i.p.)
Group V: Received ESG (400mg/kg, p.o.) + Scopolamine (1 mg/kg, i.p.)

Table 1 details the grouping of rats and the corresponding drug treatments administered to evaluate anti-Alzheimer activity employing the elevated plus maze model and Cook's pole climbing test. The statistical data is displayed "as the average ± SEM for 6 animals in every group. The differences in the means were examined employing one-way ANOVA, followed by Dunnett's post hoc analysis. Group I results were compared to the results of Groups II, III, IV and V. The significance levels are denoted as ns (not significant), *P<0.05 (significant), **P<0.01 (moderately significant), & ***P<0.001 (very significant").

Study of anti-Alzheimer activity of ESG in rats via rectangular maze model (Goverdhan et al., 2012)

The goal of this research was to evaluate the effectiveness of ESG in preventing AD in rats. The study used a rectangular maze model to assess the rats' learning as well as memory abilities in the face of scopolamine-induced dementia. The apparatus utilized in the study consisted of an enclosed rectangular box with two chambers, labeled X (entry chamber) and Y (reward chamber), positioned at opposite ends. Wooden sticks were strategically placed to create a twisting corridor (Z) connecting chambers X and Y.

During the 14-day study, on the initial day, the rats were familiarized with the maze for 10 minutes. Subsequently, the rats underwent four consecutive training trials per day on the 2nd to 14th days in the rectangular maze. Rats were placed in chamber X for each trial and observations were recorded as soon as the rats left the chamber. The learning score (TRC) represents the duration it took for the rats to reach chamber Y, and it was measured for each trial. The daily learning score was calculated by taking the average of the four trials. Lower scores and higher scores suggest effective and defective learning respectively in rats. The time taken to reach chamber Y was noted on the 1st, 7th, and 14th day. A shortened TRC was interpreted as an improvement in memory. The comprehensive findings are displayed in table 2 and depicted in fig. 1.

Study of the anti-Alzheimer activity of ESG in rats by Cook's pole climbing test (Sagar et al., 2021)

The Cook's pole climbing test was" utilized to determine the anti-Alzheimer activity of ESG in the presence of scopolamine-induced cognitive impairment. Rats were trained according to the specified protocol.

During the test, a mild electric foot shock of 1.5 mA applied through the grid floor after a 5-second 2.85 kHz buzzer signal. The voltage magnitude (5 to 10V) was calibrated "to stimulate the rat to quickly move away from the floor after receiving a shock and ascend the pole.Both the buzzer and the foot shock were immediately turned off once the rat successfully climbed the pole. The escape latency, representing the time taken to climb the centered wooden pole by the rat", was recorded in seconds as the endpoint measure. Each rat underwent at least three such trials with a 2-minute interval per day for the entire 14 days of drug treatment. Rats managing to escape foot shocks within an average of 30 seconds were considered to have experienced a conditioned avoidance response. The duration it took for the escape response to occur was measured in seconds on the 1st, 7th, & 14th days following the administration of the medication. The comprehensive findings are available in table 3 and are visually depicted in fig. 2.

Estimation of brain neurotransmitters levels in the ESG in rats

On the 14th day, immediately following the behavioral assessments using the elevated plus maze model "and Cook's pole climbing test, the rats were humanely sacrificed (euthanized) through cervical dislocation under xylazine + ketamine anesthesia (16 mg + 100 mg/kg). The brain was meticulously removed from the skull and then crushed to produce a brain homogenate with a concentration of 10% w/v in ice-cold 0.1 M phosphate buffer (pH 7.4). After being homogenized, the mixture was centrifuged at a speed of 3000 revolutions/minute for a" duration of 10 minutes in a chilled centrifuge kept at a temperature of 4°C. The supernatant obtained was divided and portions were used to measure the levels of several neurotransmitters in the brain, like acetyl cholinesterase (AChE) activity, GABA, Glutamate, dopamine and serotonin. The data collected are displayed in table 4 and visually depicted in fig. 3.

For histopathological studies, the hippocampal region of the brain was extracted from randomly selected rats after anesthesia with xylazine+ ketamine (16+100mg/kg i.m.). The extracted tissue was fixed in a 10% neutralized formalin buffer for 24 hours. Subsequent to washing in tap water, further processing for histo pathological studies was conducted following the specified procedure. The results and images of the histo-pathological studies are displayed in fig. 3.1-3.5

Isolation and identification of active constituents from leaves of sterculia guttata roxb

The plant extract was solubilized in methanol and absorbed onto silica gel with a particle size of 60–120 mesh. After removing the solvent through evaporation, the remaining substance was placed onto a silica gel column (100-200 mesh) that had been pre-treated with hexane. The elution process was carried out in a progressive manner using hexane, gradually increasing the polarity by introducing mixes of hexane and ethyl acetate (varying from 90:10 to 20:80), and eventually using pure ethyl acetate. Further elution was conducted with ethyl acetate: chloroform mixtures (ranging from 95:5 to 70:30), then with chloroform in various proportions (ranging from 100% chloroform to 70:30 chloroform: methanol). A total of 96 fractions were collected. To identify pure compounds, fractions were monitored using TLC, revealing similar fractions in numbers 82 -91 (designated as compound-1). Fractions with comparable retention factor (Rf) values were merged, and the solvent was removed by evaporation under reduced pressure. The resulting crude materials underwent purification with activated charcoal in hot methanol, followed by overnight storage at room temperature. The obtained solids underwentmelting point (m.p), mass spectrometry, FT-IR, 13C-NMR, and 1H-NMR analyses.

The melting points were determined using an uncorrected Kohfler melting point instrument. The IR spectra were obtained by utilizing KBr discs on a Perkin Elmer FTIR spectrophotometer 1650. Nuclear magnetic "resonance (NMR) spectra were acquired using a JEOL spectrometer operating at a frequency of 400megahertz. The solvent used for the measurements was deuterated dimethyl sulfoxide (DMSO-d6). The acquisition of mass spectra was performed employing a JEOL-Accu TOF JMS-T 100 LC mass spectrometer. Column chromatography utilized silica gel 100-200 mesh (Merck), while TLC was conducted on silica gel 60PF254 plates.

RESULTS

Table 1: Treatment groups in Rectangular Maze model

S. No.	Treatment groups	Rats treated with
1	Group I(Control)	CMC 0.5%suspension (1ml/kg, p.o.)
2	Group II	Scopolamine (1mg/kg, i.p.)
3	Group III (Standard)	Donepezil (5mg/kg, p.o.) + Scopolamine (1mg/kg,i.p.)
4	Group IV	ESG - 200mg/kg p.o. + Scopolamine (1mg/kg,i.p.)
5	Group V	ESG - 400mg/kg p.o. + Scopolamine (1mg/kg,i.p.)

*** Scopolamine (1mg/kg,i.p.) 1h after" donepezil / ESG administration

Table 2: Results of the anti-Alzheimeric activity of ESG in rats" in the rectangular maze model

Group/Treatment	Transfer Latency (sec)		
	Day 1	Day 7	Day 14
Group – I	46.51±1.02	43.16±2.26	42.66±3.44
Group – II	50.30±4.24*	63.5±5.23***	97.17±6.11***
Group – III	45.52±3.09*	28.11±2.02***	17.11±1.08***
Group IV	46.80±4.42*	32.01±3.12**	22.5±2.91**
Group – V	45.01±1.09*	28.03±1.82***	18.28±1.63***

Group II "was contrasted with Group I and Groups III, IV and V were contrasted with Group II. Data were analyzed statistically by using one-way ANOVA followed by Dunnett's test as the post hoc. *P<0.05, **P<0.01, ***P<0.001 compared with negative control.

Table 3: Results of the anti-Alzheimeric activity of ESG in rats according to Cook's pole climbing test

Group/Treatment	Escape Latency (sec)		
	Day 1	Day 7	Day 14
Group – I ("Control- CMC 0.5% suspension -1ml/kg, p.o.)	22.67±3.45	21.43±4.14	21.56±2.21
Group – II (Negative control- Scopolamine-1 mg/kg, i.p.)	32.52±2.46*	44.83±5.32***	48.77±5.11***
Group – III(Donepezil-5 mg/kg, p.o.+ Scopolamine-1mg/kg, i.p.)	22.42±3.11*	16.16±1.08***	10.33±2.24***
Group– IV(ESG-200 mg/kg p.o.+ Scopolamine (1mg/kg, i.p.)	22.67±4.45*	16.52±4.12**	13.36±3.31**
Group – V (ESG- 400mg/kg p.o.+ Scopolamine (1mg/kg, i.p".)	22.32±3.05*	14.27±1.06**	11.58±2.05***

Group II "was contrasted with Group I and Groups III, IV,and V were contrasted with Group II. Data were analyzed statistically by using one-way ANOVA followed by Dunnett's test as the post hoc. *P<0.05, **P<0.01, ***P<0.001 compared with negative control.

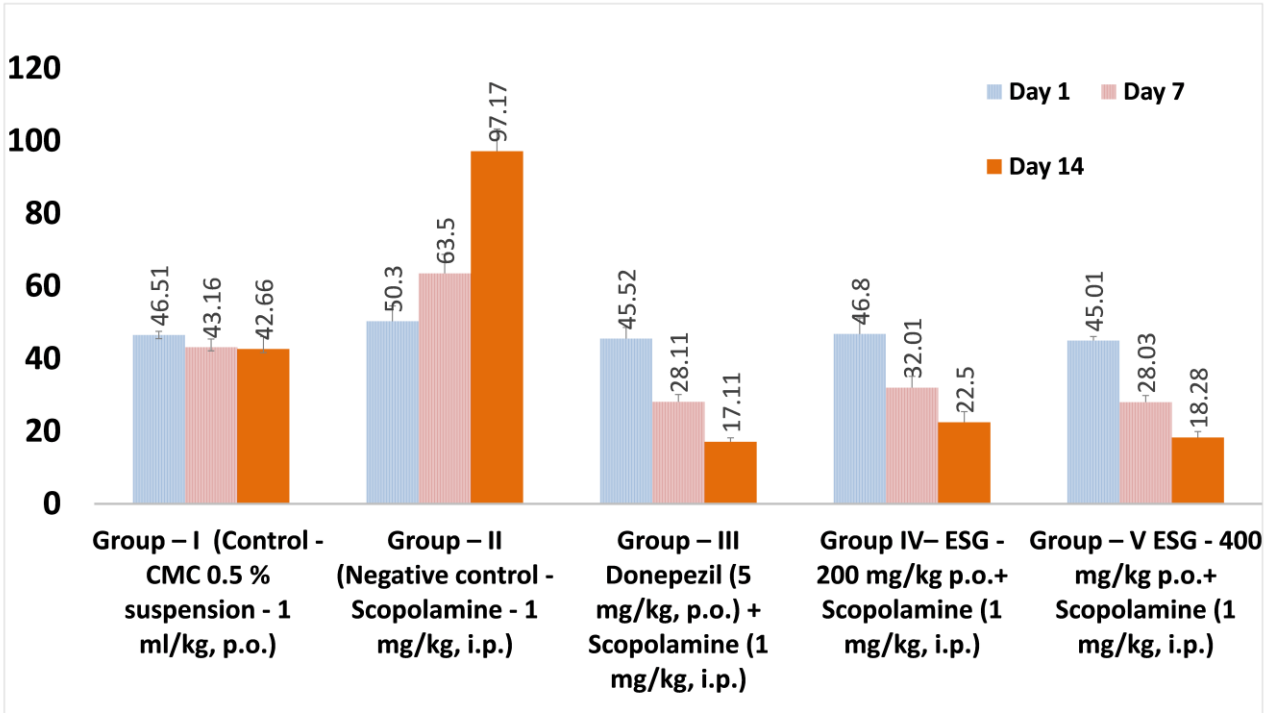

Fig. 1: Demonstrates the results of anti-Alzheimeric activity of ESG in rats" in the rectangular maze model according to the transfer latency (sec).

Table 4: Results of the estimation of the effect of brain neurotransmitters on the anti-Alzheimer activity of ESG in rats

Group/Treatment	AChE (µmol/min/g of tissue)	Dopamine (µmol/min/g of tissue)	Serotonin (ng/g tissue)	Glutamate (µmol/min/g of tissue)	GABA (µmol/min/g of tissue)
Group – I	0.209±0.045	16.93±0.829	0.412±0.022	17.2±1.116	0.312±0.014
Group – II	0.726±0.035***	10.11±0.537***	0.532±0.031**	21.52±2.124**	0.112±0.014***
Group – III	0.447±0.041**	15.21±0.731**	0.314±0.013**	10.03±1.011***	0.312±0.011**
Group – V	0.564±0.037*	14.32±0.225*	0.323±0.034*	13.92±0.312**	0.225±0.232*
Group- V	0.421±0.020**	15.48±0.819**	0.312±0.007**	11.46±0.511***	0.313±0.021**

The values of Group II were contrasted with those of Group I and those of Groups III, IV, and V were compared with those of" Group II. Data were analyzed statistically by using one-way ANOVA followed by Dunnett's test as the post hoc. *P<0.05, **P<0.01, ***P<0.001 compared with negative control.

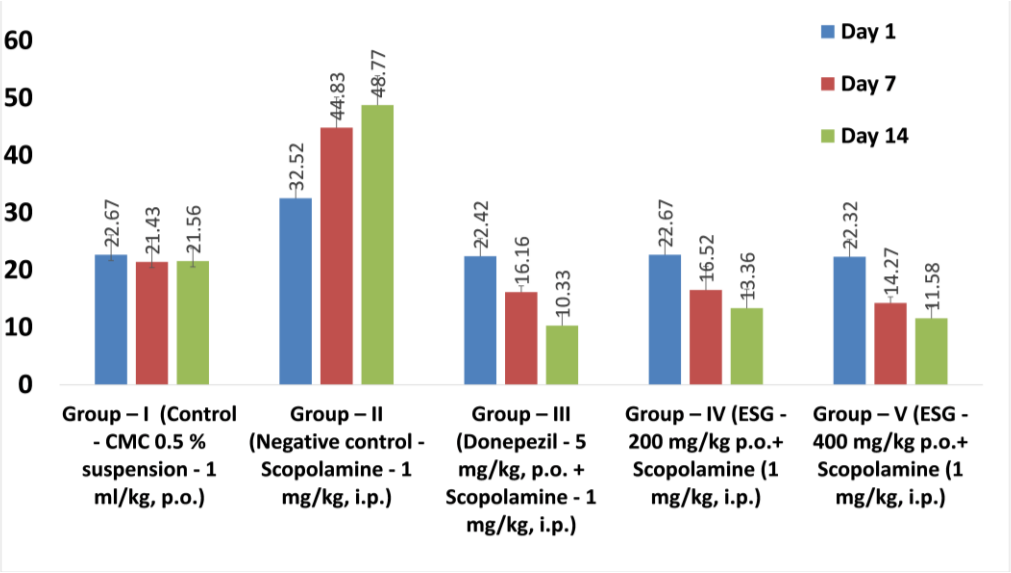

Fig. 2: Shows the results of anti-Alzheimeric activity of ESG in rats as measured by Cook's pole climbing test according to escape latency.

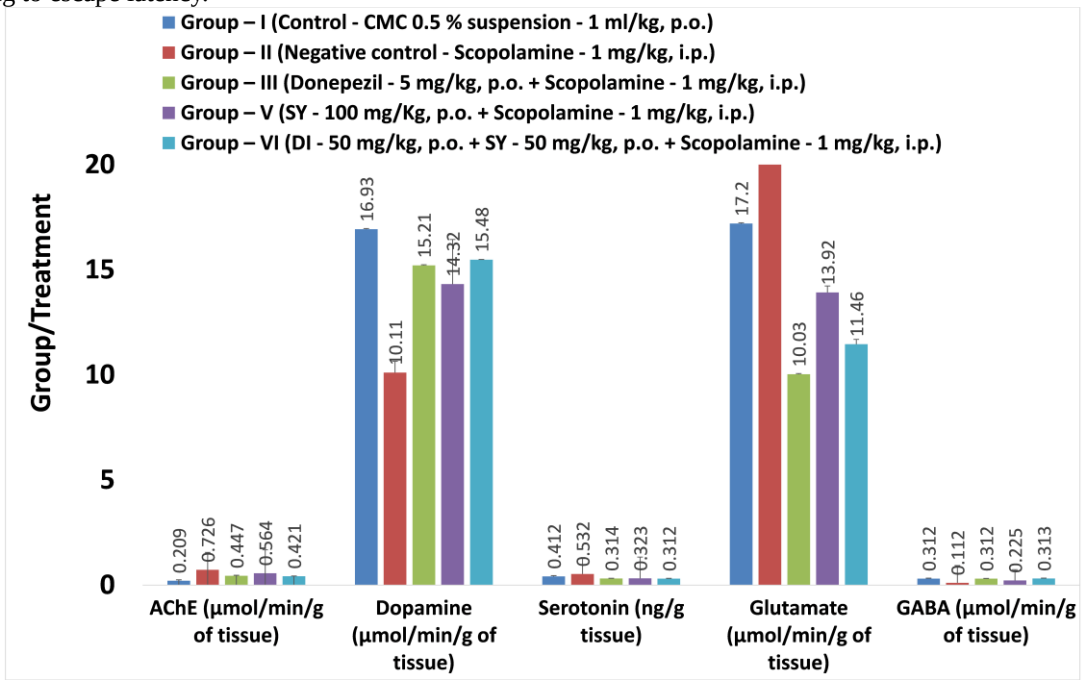

Fig. 3: Results of histopathological examination of brain tissues H&E staining, magnification X 40

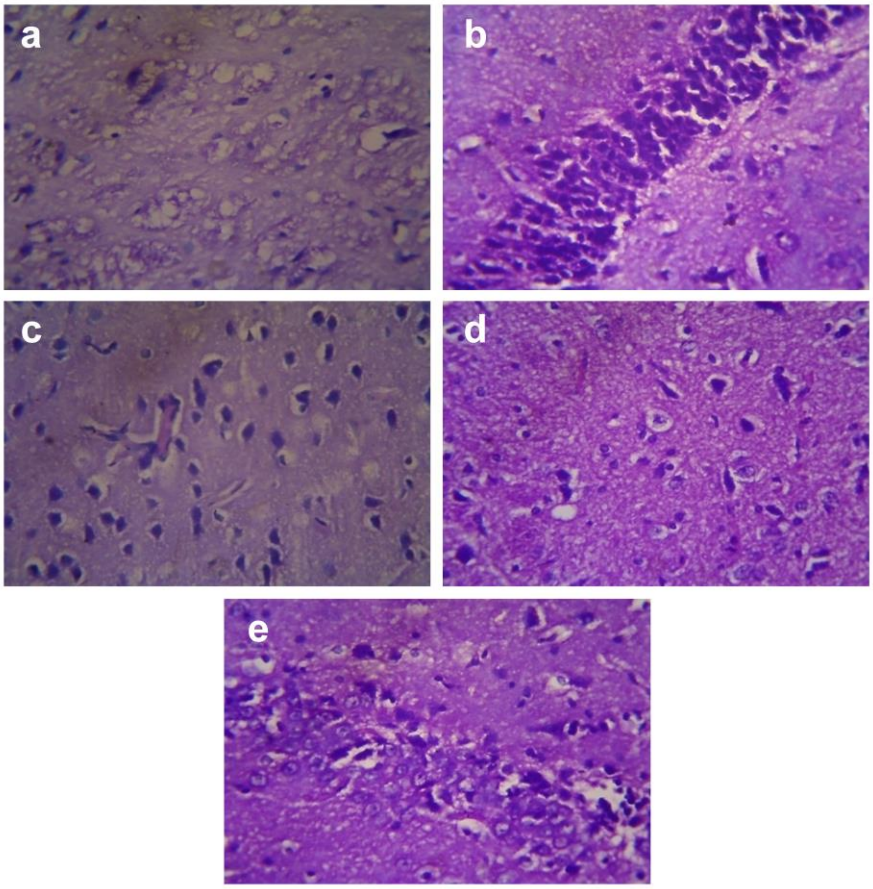

Fig. 4: A: CMC-treated brain cells of rat. B: Scopolamine-treated brain cells of rat. C: Donepezil + Scopolamine treated brain cells of rat. D: ESG -200 mg/kg p.o + Scopolamine treated brain cells of rat. E: ESG -400 mg/kg p.o + Scopolamine treated brain cells of rat.

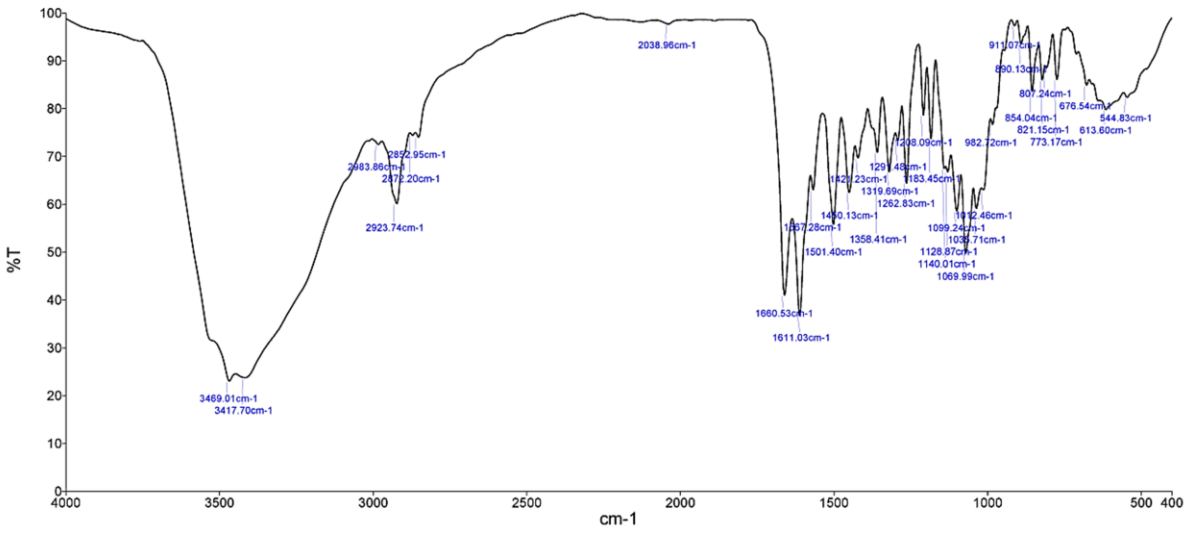

Fig. 5: Results of IR Spectroscopy

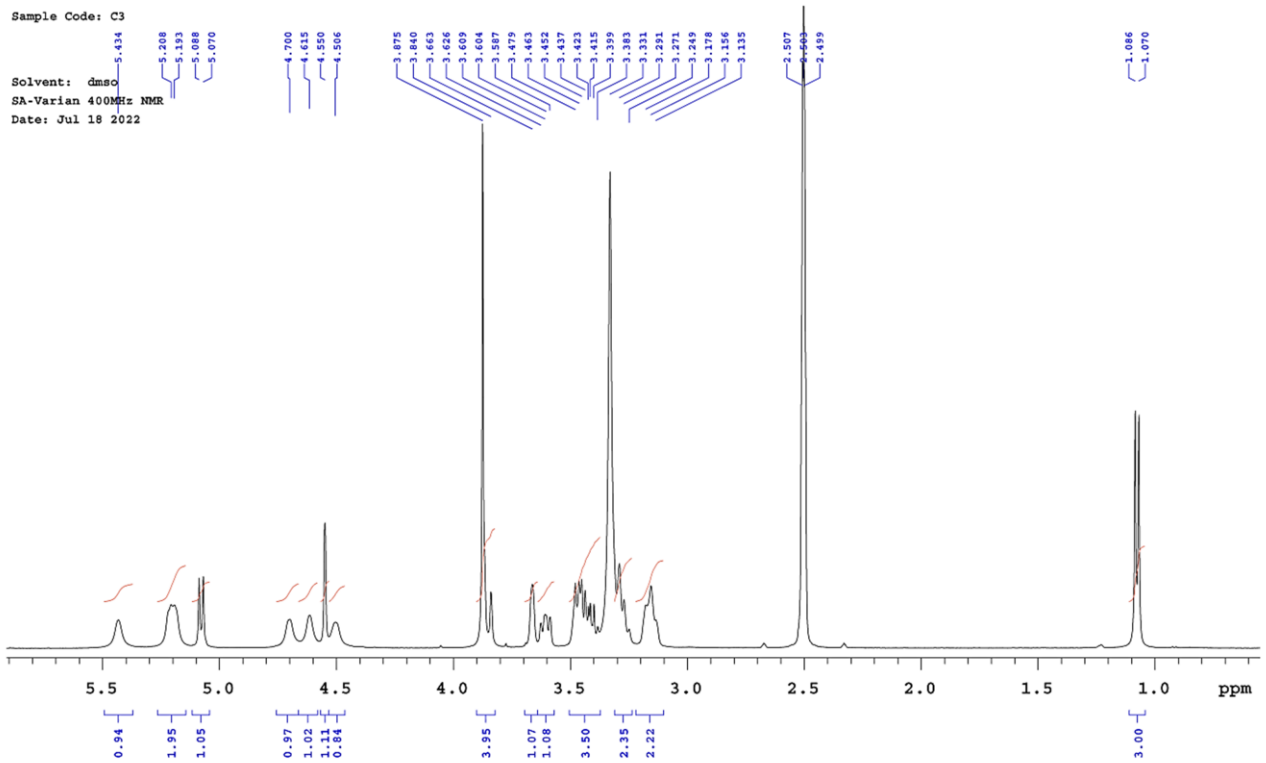

Fig. 6: Results of ^{1}H-NMR

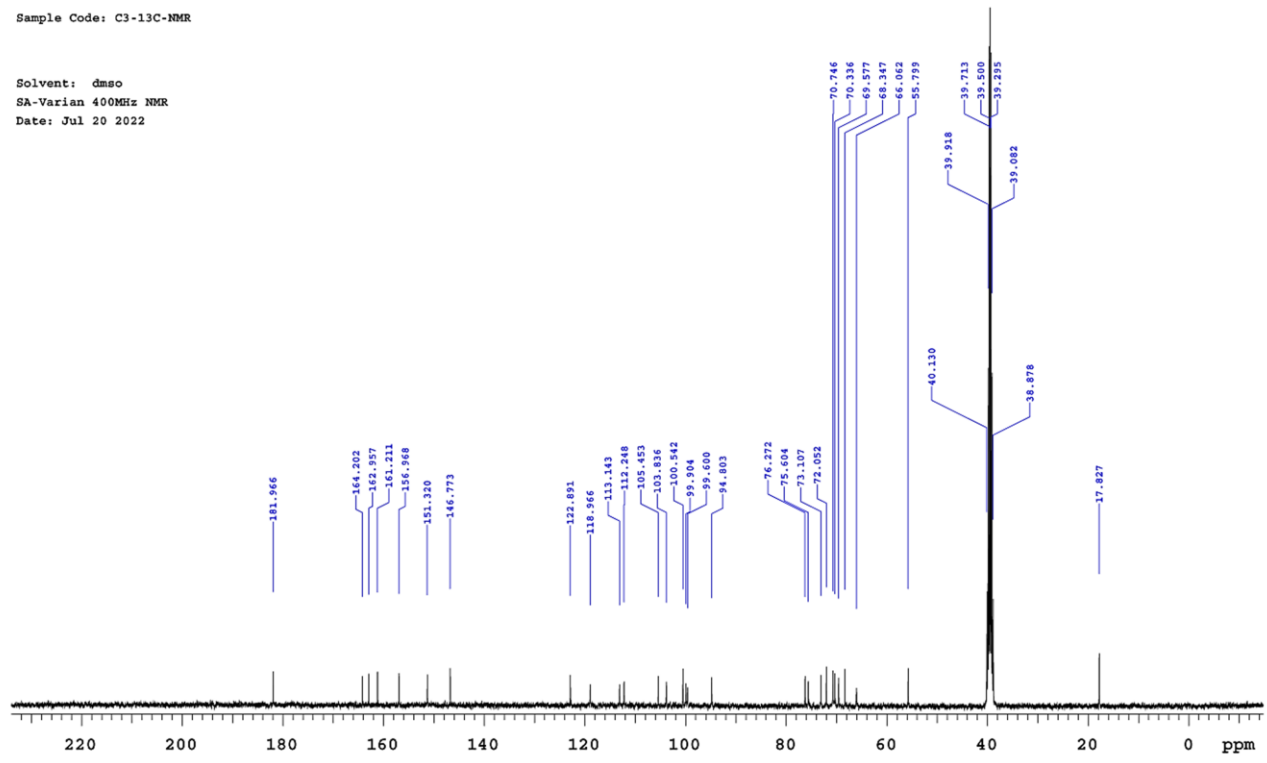

Fig. 7: Resultsof ^{13}C-NMR

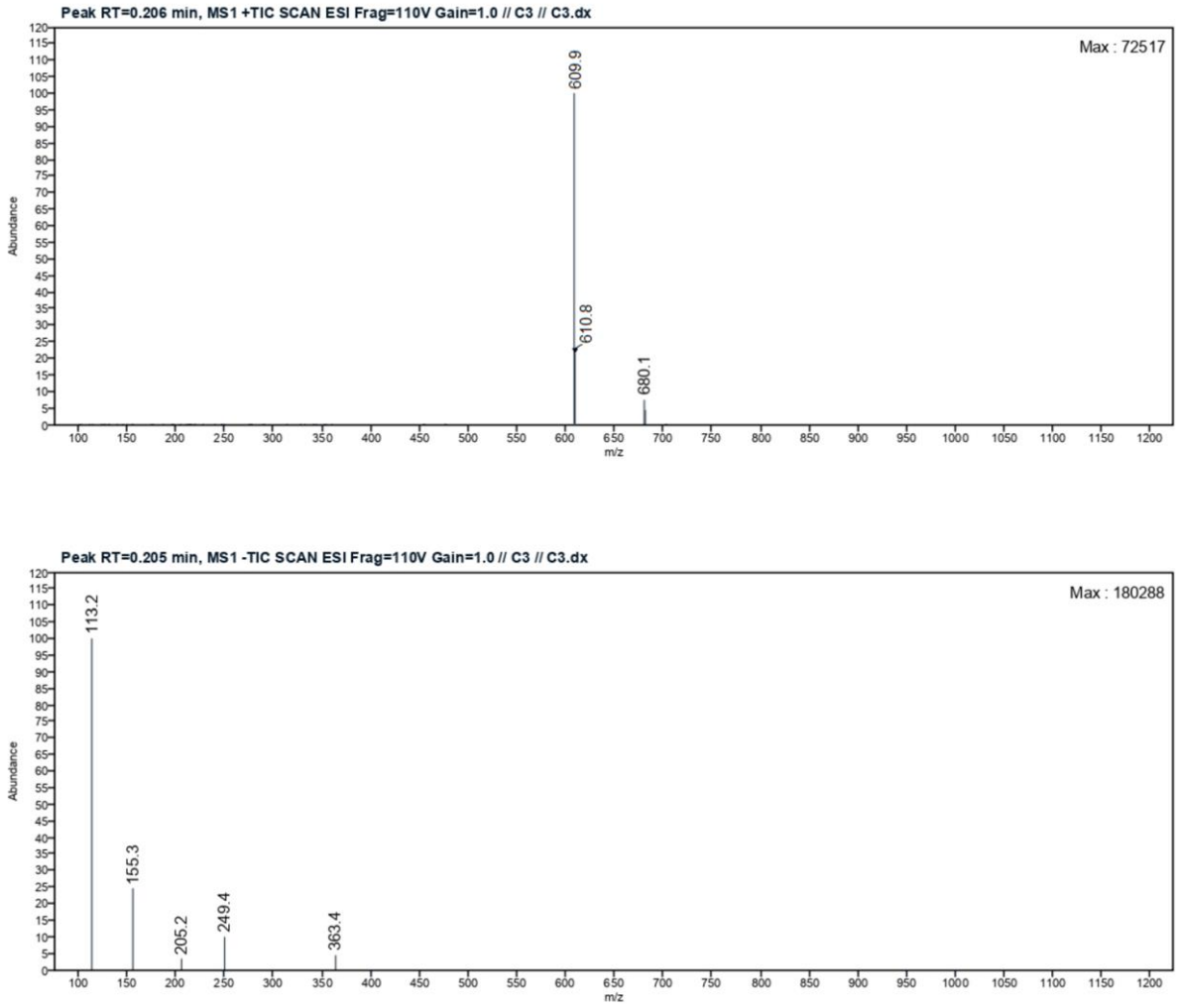

Fig. 8: Results of MASS spectra

Structure of the compound

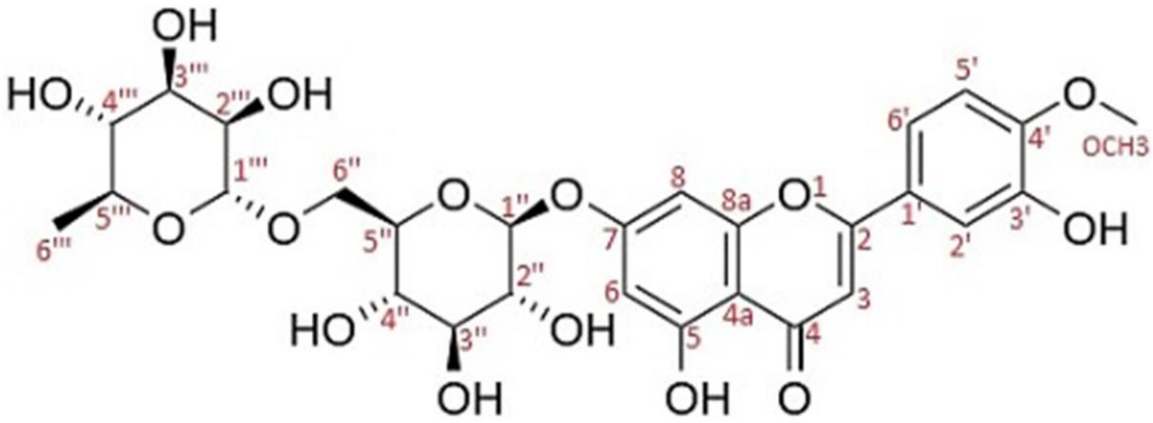

Fig. 9: The structure of compound-1

Results of isolation, identification, characterization of esg

Results of physical constant and characterization

Colorless solid, m.p.: 276 - 278°C.(20)., The FT-IR (KBr cm-1) revealed functional "groups at 3469, 3417cm^{-1}(-OH stretching); 2923cm^{-1}(-CH stretching); 2852cm^{-1} (-CH Stretching); 1660cm^{-1} (-C=O stretching); 1611cm^{-1} (-C=C stretching); 1501cm^{-1} (-CH bending) ; 1069 cm^{-1} (-C-O bending). ^{1}H-NMR:(DMSO-*d6*): δ ppm": 12.92 (1H, s, OH-5), 9.44 (1H, s, OH-3'), 7.56-7.58 (1H, dd, H-6'), 7.44 (1H, d, H-2'), 7.12-7.14 (1H, d, H-5'), 6.82 (1H, s, H-3), 6.76 (1H, d, H-8), 6.46 (1H, d, H-6), 5.43 (1H, s, H-1''), 5.19 -5.20 (1H, d, H-2''), 5.07- 5.08 (1H, d, H-1''), 4.50-4.55 (2H, d, H-1'''), 4.61 - 4.70 (2H, d, H-5''), 3.87 (3H, s, -OCH_3), 3.84 (1H, s, 6"a -H), 3.58-3.66 (2H, m,2'"-H), 3.41-3.47 (4H, m,5'"-H), 3.24-3.29 (2H, m,2"-H), 3.13-3.17 (2H, m, 3"'-,4"-, 4"'-H), 1.07 - 1.08 (1H,d, 6"'-H). ^{13}C-NMR (DMSO-*d6*): δppm 181.96 (C-4), 164.20(C-2), 162.95 (C-7), 161.21 (C-5), 156.96 (C-8a), 151.32 (C-4'), 146.77 (C-3'), 122.89 (C-1'), 118.96 (C-6'), 113.14 (C-2'), 112.24 (C-5'), 105.45(C-4a), 103.83 (C-3), 100.54 (C-1"), 99.90 (C-6), 99.60 (C-1'''), 94.80 (C-8), 76.27 (C-3"), 75.60 (C-5"), 73.10 (C-2"), 72.05 (C-4"), 70.74 (C-4'''),70.33 (C-2'''), 69.57 (C-3'''), 68.34 (C-5'''), 66.06 (C-6"), 17.82 (C-6'''). Calculated for 608.54, $C_{28}H_{32}O_{15}$; EI-MS m/z: 609.12 (M+1). The isolated pattern of the spectrum was identical to that of Diosmin.

DISCUSSION

The primary cause of dementia in late adulthood is AD, a progressive as well as complex neurological disorder.On a pathological level, it is distinguished by the presence of tangled intracellular neurofibrillary structures containing hyperphosphorylated τ-protein within specific regions of the human brain cortex and limbic system. Senile plaques are caused by the aberrant breakdown of amyloid precursor protein (APP) by β- and γ-secretases, which also produces Aβ40 and Aβ42 monomers. These monomers oligomerize and accumulate extra cellular amyloid protein. This pathological landscape is accompanied by weakened transmission by cholinergic neurons, mitochondrial defects, elevated inflammatory mediators, synapse loss and neuronal degeneration (Pohanka *et al.,* 2011). Its clinical manifestations include progressive memory loss and neurocognitive dysfunction. (Nair- Roberts *et al*., 2008).

Scopolamine, an anticholinergic compound, disrupts cognitive functions by inhibiting acetylcholine activity in the central nervous system through its antagonistic action on muscarinic receptors. This pharmacological agent has been extensively used in both animal models and human studies to induce cognitive impairments, particularly affecting memory performance, thereby serving as a reliable experimental model for studying cognitive deficits. (Gritty *et al*., 2006). Neurotransmitters, which are endogenous biochemical molecules synthesized and released in brain neurons, play a crucial role in transmitting signals within the nervous system. Alterations in neurotransmitter levels have been associated with a number of mental as well as neurological illnesses, including AD, anxiety, depression, and motor dysfunction. (Kotake *et al*., 1985, Mathiyazahan *et al*., 2015, Kumar & Singh., 2015)

ACh, a cholinergic neurotransmitter generated in cholinergic nerve terminals, is essential for memory, learning and mood control, among other cognitive functions. ACh in cholinergic nerve terminals is rapidly hydrolyzed by the enzyme AChE, which prevents ACh-mediated neuronal impulse transmission. Decreased levels of ACh in the brain cause cholinergic activities to be compromised, which exacerbates the clinical signs and symptoms of CNS illnesses like dementia and Alzheimer's disease (Westfall TC & Westfall D., 2011, Benhamu *et al*., 2014).

Gamma-aminobutyric acid (GABA) and dopamine are the neurotransmitters significantly influencing memory retention and cognition. Deficiencies in the GABAergic and dopaminergic systems lead to memory dysfunction in patients with AD. Serotonin (5-HT), a monoamine neurotransmitter, plays a role in various behaviors, including appetite, rhythmic activities, emotional states, mood, muscle contraction, and depression. The serotonergic system is also involved in learning and memory, interacting with cholinergic, dopaminergic, GABAergic, or glutamatergic systems. The excitatory amino acid neurotransmitter glutamate is widely distributed throughout the CNS and has afunction in manylearning, memory and cognitive processes. Nevertheless, excitotoxicity a condition that damages and degenerates neurons can result from elevated glutamate activity (Benhamu *et al*., 2014).

The current investigation aimed to assess the impact of ethanolic extract of *Sterculia guttata* (ESG) on scopolamine-induced AD in rats through behavioral studies aimed at evaluating learning and memory abilities. This was accomplished using the rectangular maze test and Cook's pole climbing test. Rats were intra peritoneally injected with scopolamine (1mg/kg, i.p.) for 14 days to induce memory deficits. Donepezil, a potent, selective and noncompetitive reversible inhibitor of acetylcholinesterase, was used as a reference to increase acetylcholine availability, aiming to improve cognition and memory, particularly in the clinical treatment of severe AD (Choi *et al*., 2021, Lisman *et al*., 2008).

In the rectangular learning and memory maze test, rats administered ESG at 200 mg/kg p.o (Group IV) exhibited major improvements in learning as well as memory properties compared to amnesic control rats. This improvement was demonstrated by a reduction in "the time taken (TRC) for the rats to reach the reward chamber, from 46.08 seconds on Day 1 to 22.50 seconds

on Day 14. Conversely, rats treated with scopolamine (Group II) exhibited a significant increase in" TRC, indicating exacerbated memory impairment (50.30 s on Day 1 and 97.17 s on Day 14) compared to control-treated rats.

Rats in Group III treated with donepezil, also displayed a reduction in TRC (17.11 s on Day 14) compared to Group II (scopolamine alone). Notably, compared to individual therapy, the TRC of "rats treated with ESG - 400mg/kg p.o significantly" improved cognitive function (TRC = 18.28s on Day14).

In the pole climbing conditioned avoidance test, ESG treatment mitigated scopolamine-induced impairment of learning as well as memory, as indicated by elevated time spent in the shock zone. The ESG - 200mg/kg p.o (Group IV) treatment group showed a notable reduction in escape latency (13.36s) in comparison to the diseased control group. Moreover, compared with scopolamine treatment, the 400mg/kg p.o. ESG strongly decreased escape latency (11.58s), similar to the impact of standard donepezil at a dose of 5mg/kg (10.33s), demonstrating efficacy against scopolamine-induced amnesia. The combined dose had a greater effect than the individual doses on memory improvement.

The results from the above two anti-Alzheimer models indicated that treatment with 400mg/kg p.o ESG significantly decreased the transfer latency (TRC) and escape latency as measured in seconds, in response to the memory loss induced by scopolamine. ESG may efficiently improve cognition and memory by acting on cholinergic receptors (AChRs), as evidenced by their nootropic activity in the existence of scopolamine, a muscarinic receptor antagonist. The results suggest that ESG may have an inhibitory effect on the enzyme acetyl cholinesterase, as it has demonstrated anti-amnestic effects similar to those of donepezil, a potent AChE inhibitor.

Histopathological brain tissue sections were examined under 40X magnification using hematoxylin and eosin (H & E) staining. Microscopic examination of the group treated with scopolamine alone revealed severe neuronal damage (neuronal degeneration), along with hippocampal edema, pyknotic cells, and loss of normal nuclei. Compared with the diseased control group, the groups treated with DI (donepezil) and SY (scopolamine) individually showed only mild hippocampal edema. The combination group, treated with both DI and SY, was closer to the control group, protecting neurons from edema and necrosis and preserving normal neuronal architecture and glial cells. Brain sections from rats treated with donepezil showed no histopathological alterations in the hippocampus.

In the current research, scopolamine increased the hydrolytic enzymatic activity of acetyl cholinesterase, thereby decreasing the acetylcholine concentration in the brain. Scopolamine-induced memory impairment results in cholinergic neuronal dysfunction, as confirmed by elevated AChE activity, an enzyme that hydrolyzes ACh, an essential neurotransmitter involved in learning and memory. These findings supported the current study, as scopolamine treatment decreased brain dopamine content after 14 days and its neurophysiological changes in the brain dopamine content were associated with impairments in GABAergic and dopaminergic levels in the cortex and hippocampus.

The extract was isolated and identified by MASS, NMR & IR. The isolated compound obtained was Diosmin, a flavonoid and colorless solid, m.p.276 -278°C; calculated for 608.54, $C_{28}H_{32}O_{15}$; EI-MS m/z: 609.12 (M+1) (Prachi *et al*., 2018).

CONCLUSION

The memory-enhancing effect of ESG in scopolamine-induced Alzheimeric rats appears to be mediated by various mechanisms, including the attenuation of cholinergic deficiency, the amelioration of dopaminergic (DAergic), serotoninergic (serotoninergic), gamma-aminobutyric acid (GABAergic) and glutamatergic neurotransmission and improvements in histopathological changes. Compared with individual doses, the combined dose is notably effective at preventing memory loss and refining cognition in rats with Alzheimer's disease (AD). Additionally, it enhances cholinergic transmission by reducing acetylcholinesterase (AChE) activity, as compared to that in the ESG-treated groups.

While these findings are promising, further pre-clinical studies are essential to delve into the molecular mechanisms that may underpin the anti-amnestic effect of ESG. Deeper insightsinto these mechanisms could lead to possible clinical applications as well as improved understanding of ESG's potential for therapy in the context of AD.

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