Egypt. J. Chem. **Vol. 68**, No. 1 pp. 511- 526 (2025)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Sesamum Indicum L. Seed Oil Synergistically Enhances The Effectiveness of Acetylcholine Esterase Inhibitor in Managing Scopolamine-Induced Neurological Dysfunction in Rats

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Abstract

This study evaluated the potential synergetic efficacy of sesame oil (SSO) and the acetylcholine esterase inhibitor donepezil in attenuating scopolamine (SCO)-induced neuropathological dysfunction in rats. Thirty-five male Sprague-Dawely rats were assigned into five groups at random. Group 1, the negative control, was given saline. Group 2, the positive control, was given SCO (one mg/kg/day, intraperitoneally). Groups three to five received donepezil, sesame oil, or their combination concomitant with SCO for five weeks. SSO's antiradical and anti-inflammatory activities, levels of tocopherols, phytosterols, lignin, and fatty acid composition were also investigated using DPPH radical, membrane stabilization, and high-performance liquid and gas chromatography methods. Oral administration of donepezil, sesame oil, or their combination to SCO-treated rats decreased acetylcholinesterase activity with a concomitant increase in the acetylcholine content, mitigated the SCO-induced amyloid beta (Aβ) accumulation and restored the reduced brain-derived neurotrophic factor (BDNF) levels in the brains of SCO-treated rats. Furthermore, the tested therapies increased the levels of GSH and Bcl-2 and reduced MDA levels, along with the notable reverting of the elevated pro-inflammatory cytokines, TNF-α, IL-1ß, and IL-6. The group that received combined SSO and donepezil therapy showed more pronounced neuroprotective effects than those treated with SSO or donepezil alone, indicating the prominent synergistic impact of the combination. Sesame oil synergized with donepezil in modulating scopolamine-induced cholinergic dysfunction, amyloidopathy, oxidative stress, and neuroinflammation. It can offer a safe and efficient option for a therapeutic combination that may be very beneficial in clinical practice for the practical management of neurological disorders.

Keywords: *Neurological disorders; Chlonorigenic system; Oxidative stress; Neuroinflammation; Sesame oil; Donepezil*

1. Introduction

Age-related neurological disorders, such as Alzheimer's disease (AD), Parkinson's disease, multiple sclerosis, and stroke, are widespread clinical problems linked to accelerating dysfunction and structural abolishing of functional neurons that cause neuronal cell death, leading to memory impairment and cognitive dysfunction [1]. One of the primary factors contributing to cognitive decline is cholinergic neurotransmission disturbance in the brain [1, 2], such as the increased activity of acetylcholinesterase (AChE) and comparatively reduced acetylcholine (ACh) release in the central nervous system (CNS). Other neurotoxic factors responsible for the deterioration of cognitive impairment are amyloidopathy, inflammatory mediators' expression, mitochondrial dysfunction, and oxidative stress [1-3]. Amyloid-β (Aβ) accumulation induces several lesion events such as tau pathology, synaptic malfunction, inflammation, oxidative stress, and apoptosis, causing neurotoxicity and, eventually, damage to neurons and impairment of cognitive function [4]. Currently, the primary treatment for degenerative brain diseases such as AD is a cholinergic replacement therapy, represented by FDA-approved AChE inhibitors, donepezil, galantamine, and rivastigmine [5]. However, these drugs only provide symptomatic alleviation and have various side effects [6]. Accordingly, research on safer alternative and complementary therapies is ongoing.

Several plant products and their active ingredients have recently attracted considerable interest as possible neuroprotective agents [7]. Sesame (Sesamum indicum L.) is a seasonal oilseed crop that grows in Africa and Asia. Sesame seeds are a valuable source of high-quality oil for both human nutrition and the economy due to the high concentrations of beneficial phytochemicals, like lignans (sesamin, sesamol, sesamolin), tocopherols, phytosterols, and unsaturated fatty acids [8-9]. These bioactive compounds are capable of inhibiting inflammation [10], counteracting oxidative stress [11-12], and exerting neuroprotective effects in various neurotoxic conditions [9-13]. In particular, sesamol, sesamin, and sesamolin are potent antioxidants that protect the rat brain from hypoxia and mitigate ultraviolet- and Fe^{3+}/ascorbate-induced lipid peroxidation [12-13]. Furthermore, long-term sesaminol administration prevented pathogenic Aβ's accretion in the brain of the senescence-

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EJCHEM use only: Received date here; revised date here; accepted date here

DOI: 10.21608/EJCHEM.2019.6778.1566

accelerated mouse-prone 8 [14-15]. Sesaminol also reduces the hyperactivity of the monoamine oxidase enzyme linked tage-related neurological problems, stroke, Alzheimer's, and other neurodegenerative diseases [16].

Both phytochemicals and pharmaceutical agents have unique pharmacological properties. Their combination may enhance each other's effects and maximize the therapeutic benefits while minimizing the adverse effects of pharmaceutical agents [17]. Given that sesame oil and its bioactive constituents can reduce oxidative stress and inflammation and eventually could decrease the accumulation of pathogenic Aβ [14-16] and that acetylcholinesterase inhibitors (donepezil) can inhibit AChE activity [5], the current investigation hypothesis was that the combination of sesame oil and AChE inhibitor drugs might have synergistic effects on the management of neurological ailments. Consequently, this study aimed to investigate the potential synergistic effects of sesame oil and AChE inhibitor drugs on neuroprotection in a scopolamine-treated rat. We also appraised whether this probable neuroprotective effect is mediated through modulation of the cholinergic system and mitigating oxidative stress and inflammation. Sesame oil's fatty acids, tocopherols, phytosterol composition, and antioxidant and anti-inflammatory *in vitro* capacities were evaluated.

2. Materials and Methods

2.1. Cold-Pressing Sesame oil extraction

Sesame seeds were kindly provided from the Field Crops Institute, Agricultural Research Center, Egypt. Sieves were used to separate impurities, including dirt, sand, stones, spoiled seeds, and other surplus materials. Then, an oil presser (Dulong DL ZYJ-06, Jiangxi, China) was used to squeeze sesame seeds (1 kg) at 40 °C. The resultant oil was preserved in brown glass bottles impervious to light and kept cold at 4 °C until needed [18].

2.2. In *vitro* analysis

2.2.1. Fatty acids evaluation using gas chromatography (GC)

The detection and quantification of fatty acids in the sesame seed oil were conducted using the GC flame ionization detection (GC FID) method. Fatty acid methylation was accomplished by reacting fats and methanol with 2M potassium hydroxide [19]. Then, fatty acid methyl esters (FAMEs) were investigated utilizing a gas chromatograph (Agilent Technologies 7890B GC) outfitted with an FID. The Zebron ZB-FAME column (0.25 mm internal diameter x 60 m x 0.25 µm film thickness) was used to separate the FAMEs. Hydrogen gas was used as a carrier at a 1.8 mL/min flow rate at a split-1:50 mode, with an injection volume of one µL. The temperature program was 100 °C for 3 min, rising at 2.5 °C /min to 240 °C and held for 10 min. The injector and detector (FID) were held at 250 °C and 285 °C, respectively. The relative peaks of the fatty acids were identified by comparing the fatty acids' retention times with those of the reference mixture that underwent the same analytical process. The entire area was determined and used to compute the percentage of each area beneath every peak, corresponding to each fatty acid.

2.2.2. Phytochemicals analysis

2.2.2.1. Saponification

Ethanolic potassium hydroxide was used to saponify the lipids in the sesame oil sample. The unsaponifiable fraction was removed using 1:1 diethyl ether to petroleum ether extraction and evaporated at 40°C [20].

2.2.2.2. Sample derivatization

The unsaponifiable fraction was reacted with 50 µL pyridine and 50 µL silylation reagent [bis (trimethylsilyl) trifluoroacetamide (BSTFA) + trimethylchlorosilane (TMCS), 99:1] to derivatize the functional groups in the sample to trimethylsilyl groups (TMS), before GC analysis.

2.2.2.3. GC/MS analysis

The Agilent GC-MS system was fitted with a 7890B gas chromatograph and a 5977A mass spectrometer detector. The column in GC was DB-5MS (film thickness: 0.25 µm and 30 m x 0.25 mm internal diameter). Hydrogen was utilized as the carrier gas with a one mL/min flow rate and one µL volume for spitless injection. The temperature schedule was 60 °C for 1 minute, increasing at 10 °C /min to 320 °C and continued for 10 minutes. The temperature of the injector and detector was maintained between 300 and 320 °C. Mass spectra were produced using electron ionization (EI) at 70 eV, employing an m/z 50-600 spectrum range and a six-minute solvent delay. The mass temperature was 230°C while Quad was 150 °C. Various components were identified by matching the Wiley and NIST Mass Spectral Libraries' data with the spectrum fragmentation pattern [21].

2.2.3. Vitamin E analysis

Vitamin E in sesame oil was measured using an Agilent HPLC system (HP 1200 series, USA) that had an HPLC pump (Maxi-Star K-1000 from Knauer, Berlin, Germany), an auto-injector (Spark, AJ Emmen, The Netherlands), an ultraviolet spectrophotometer detector (Knauer, Berlin, Germany), and Computer programs (Version 1.6 of Euro Chrom 2000, Knauer, Berlin, Germany). Vitamin E was analyzed by infusing 20 µL of diluted oil aliquots into the C18 Agilent Column (4.6 mm x 250 mm internal diameter, 5 µm). The column was eluted isocratically with the mobile phase (35:65 acetonitrile: methanol) at a 1.2 mL per minute flow rate. The diode-array detection (DAD) adjustment was at 325 nm. The Fluorescence detector was set at wavelength 290/330 nm (Excitation/Emission), and the column temperature was constantly kept at 40 °C [22]

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2.2.4. Sesame oil scavenging activity against 1,1-diphenyl-2-picrylhydrazyl radical (DPPH)

The potential of sesame oil to scavenge free radicals was assessed by 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) [23]. Three milliliters of varying concentrations of sesame oil in ethanol (3.9, 7.8, 15.6, 31.2, 62.5, 125, 250, 500, 1000 µL/mL) was mixed with one milliliter of 0.1 mM solution of DPPH in ethanol. After thoroughly shaking, the mixture was set aside for 1/2 an hour at ambient temperature. After that, a UV-VIS Milton Roy spectrophotometer was used to measure absorbance at 517 nm. Ascorbic acid was utilized as the reference standard, and the experiment was performed in triplicate. The sample concentration required to neutralize 50% of the DPPH free radical (The 50% inhibitory concentration, IC50) was computed using the curve of logarithmic dose inhibition. The DPPH scavenging % was estimated using the following equation:

$$\text{Percent inhibition or DPPH scavenging (\%)} = \frac{A0 - A1}{A0} \times 100$$

A0 indicates the absorbance of the control reaction, and A1 denotes the absorbance when the ascorbic acid or sesame oil was present.

2.2.5. In *vitro* anti-inflammatory activity of sesame oil

2.2.5.1. Preparation of erythrocyte suspension

Fresh whole blood (3 mL) samples were drawn into heparinized tubes from healthy human participants who hadn't taken Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) for at least three weeks before the collection date. The whole blood was added to the same volume of Alsever solution (0.5 % citric acid, 0.42 % sodium chloride, 2 % dextrose, and 0.8 % sodium citrate) and then kept at 4°C for a day. After that, the mixtures underwent a 3000 ×g centrifugation for ten minutes.The packed cell volume was reconstituted with(10 mM,pH 7.4) phosphate-buffered saline to obtain a 40% (v/v) RBC suspension at 30 °C.

2.2.5.2. Hypotonic solution-induced erythrocyte hemolysis

The sesame oil's membrane stabilization activity was evaluated by inducing erythrocyte hemolysis with a hypotonic solution. The sample was composed of 0.5 mL stock erythrocyte (RBCs) suspension added to five milliliters of hypotonic (50 mM) sodium chloride solution in sodium phosphate-buffered saline (10 mM, pH 7.4) containing the oil in ethanol or indomethacin (as positive control) in different concentrations. The negative control sample was composed only of 0.5 milliliters of RBCs added to the hypotonic-buffered saline solution. After 10 min incubation at ambient temperature, they underwent a 10-minute 3000 × g centrifugation. The supernatant's absorbance has been identified at 540 nm. The percentage of membrane stabilization or hemolysis inhibition was computed using the following equation:

$$\text{Inhibition of hemolysis (membrane stabilization\%)} = OD1 - OD2/OD1 \times 100 \quad [24]$$

OD1 represented the hypotonic-buffered saline solution's optical density alone;OD2 represented the sample's optical density in a hypotonic solution. The IC_{50} value was the sample concentration needed to prevent 50% RBC hemolysis under testconditions.

2.3. In *vivo* study

2.3.1. Animals

A total of 35 male Sprague-Dawley rats (180 g±20) were procured from the Medical Research Centre breeding unit (located at Ain Shams University in Cairo, Egypt). Each animal resided in a metal cage at a temperature of 24±2°C and a 12-h light-dark cycle. All rats had unrestricted access to lab chow and water for seven days as an adaptation period and during the trial phase. The animal experiment had been carried out in compliance with the committee on institutional animal ethics. It was endorsed by the Ethics Committee, Faculty of Women for Arts Science and Education, Ain Shams University (approval no. ASU/W/Sci-1532309002).

2.3.2. Experimental design

Five groups of animals (7 rats/group) were assigned at random as follows:

1. Negative control group: Rats were injected intraperitoneally (*i.p*) with physiological saline (0.9% sodium chloride, 1 mL/ Kg body weight/ day).
2. Positive control group: Rats were injected intraperitoneally with scopolamine (1 mg/kg/ day, *i.p*) disintegrated in saline daily [25].
3. Donepezil group: Rats received donepezil HCl at an oral dose of 3 mg/kg [26], afterward scopolamine (1 mg/kg/ day, *i.p*) after 40 min.
4. Sesame oil group: Rats were given a 5 mL/kg body weight oral dosage of sesame oil [27], followed by scopolamine (1 mg/kg/ day, *i.p*) after 40 min.
5. Donepezil + sesame oil group: Rats received donepezil (3 mg/kg body weight/ day, *p.o*) and sesame oil (5 mL/kg body weight, *p.o*), then scopolamine (1 mg/kg/ day, *i.p*).

   The aforementioned treatments persisted for five weeks; the animals were then decapitated. The brain was separated on ice and kept at -80°C until use.

2.3.3. Biochemical Analysis

2.3.3.1. Estimation of acetylcholine esterase activity, brain acetylcholine, and brain-derived neurotrophic factor (BDNF) levels

The brain sample homogenate (10% W/V) was homogenized with 0.1 M phosphate-buffered saline (PBS) and chilled with ice. Then, the homogenates underwent a 15000 g centrifugation. The supernatants were gathered to estimate AChE activity and ACh and BDNF levels using ELIZA kits (Wkea Med Supplies Corp, China), adopting the manufacturer's instructions.

2.3.3.2. Estimation of brain amyloid-β

Brain tissue was homogenized using five volumes of extraction buffer (1% CHAPS in Tris-buffered saline (TBS), pH 7.6). After homogenization, the mixture was left to stand for at least three hours on ice. Then, it was centrifuged for 20 minutes at

4°C at 70,000 rpm. Subsequently, the supernatant was diluted using "4, EIA buffer" in the kit. After dilution, the supernatant was used to measure amyloid-β using ELISA kits containing monoclonal antibodies specific to rat amyloid-β (IBL America) based on the instructions provided by the manufacturer.

2.3.3.2. Estimation of tumor necrosis factor-α, interleukin-1ß, and interleukin-6

To determine the brain inflammatory cytokines (TNF-α, IL-1ß, and IL-6), Two volumes of 0.01M PBS with 0.05% Tween-20 were used to homogenize 100 mg of brain tissue. After homogenization and 20 minutes of 10,000 g centrifugation at 4°C, the resulting supernatant was collected. Then, TNF-α, IL-1ß, and IL-6 levels were measured utilizing ELISA kits (IBL Co., Ltd Japan) containing TNF-α, IL-1β, and IL-6 rat-specific monoclonal antibodies in compliance with the manufacturer's instructions.

2.3.3.3. Estimation of brain MDA, GSH, and Bcl-2:

In ice-cold tri-hydrochloride buffer (pH 7.2), an additional 100 mg of brain tissue was homogenized. The homogenate underwent a ten-minute, 800 g centrifugation, and the supernatant was then centrifuged at 12,000 g for fifteen minutes. The supernatant was used to determine GSH contents utilizing 5,5′-dithionitrobenzoic acid to produce the yellowish 5-thio-2-nitrobenzoic acid that was colorimetrically measured at 412 nm [28]. The thiobarbituric acid assay was employed to determine the MDA levels [29]. Bcl-2 levels were determined using ELIZA kits (IBL America), adopting the manufacturer's guidelines.

2.3.3.4. Estimation of brain total protein:

Brain total protein was measured using typical spectrophotometric techniques reported by Henry et al. [30].

2.3.4. Statistical Analysis

Statistical analysis was carried out by adopting a one-way analysis of variance (ANOVA) and Tukey's multiple comparisons test. Significantity was defined as $P < 0.05$. GraphPad Prism software, version 9, was used for all statistical tests. The values were presented as means±SEM. Pearson's correlation study employed the same software program where the difference was considered significant at $P < 0.0001$.

3. Results

3.1. Fatty acid composition

The fatty acid (FA) composition of sesame oils is displayed in Table (1). The results revealed that the sesame oil's main fatty acids are linoleic (42.36%), oleic (40.21), palmitic acid (9.58%), and stearic acid (5.99%). Similar levels of FAs content in sesame oils were also documented in previous studies [31-32]; the authors reported that the sesame oil's FAs content included linoleic acid (39–46%), oleic acids (36–43%), palmitic acid (8.2–10.3%), stearic acid (4.63–6.4%). Figure (1) illustrates the GC-FID chromatogram obtained for sesame seed oil fatty acids.

Table 1: Fatty acid composition in sesame oil

Peak	RT*	Name	C	Area	Area Sum %
1	26.886	Palmitic acid	C16:0	6178436.14	9.58
2	28.003	Palmitoleic acid	C16:1	80251.15	0.12
3	29.591	Margaric acid	C17:0	38371.2	0.06
4	32.469	Stearic acid	C18:0	3863865.65	5.99
5	33.318	Oleic acid	C18:1	25922915.2	40.21
6	35.024	Linoleic acid	C18:2	27310910.75	42.36
7	36.957	Linolenic acid	C18:3	229229.05	0.36
8	37.396	Arachidic acid	C20:0	439047.14	0.68
9	38.087	*cis*-11-Eicosenoic acid	C20:1	117061.17	0.18
10	42.186	Behenic acid	C22:0	101637.97	0.16
11	42.78	Erucic acid	C22:1	113406.31	0.18
12	43.846	Eicosapentaenoic acid	C20:5	5329.89	0.01
13	46.723	Lignoceric acid	C24:0	70109.65	0.11

*Retention time

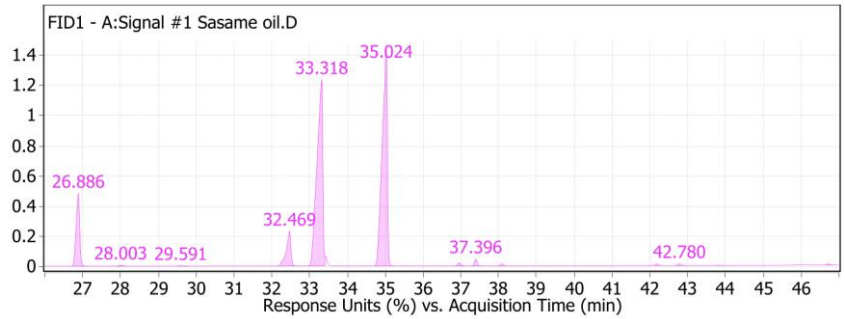

Figure 1. GC-FID chromatogram obtained for sesame seed oil fatty acids

3.2. Lignins and phytosterol composition in sesame oil

Table (2) lists the unsaponifiable matters identified in sesame oil. The major phytosterols identified in sesame oils are beta sitosterol trimethylsilyl ether, campesterol, isofucosterol, phytol, stigmasterol, trimethylsiloxycitrostadienol, and brassicasterol. The two lignins, Sesamol and (+)-sesamin, were also detected in sesame oil. The total ion chromatograms of the unsaponifiable materials in sesame oils are highlighted in Figure (2).

Table 2: The unsaponifiable matter (USM) identified in sesame oil

Peak	RT	Name	Formula	Area	Area Sum %
1	9.271	Sesamol, TMS derivative	C10H14O3Si	942117.14	4
2	17.697	Phytol, TMS derivative	C23H48OSi	1741537.46	7.4
3	23.965	Gamma.-Tocopherol, TMS derivative	C31H56O2Si	137503.41	0.58
4	25.222	(+)-Sesamin	C20H18O6	310449.17	1.32
5	25.541	Brassicasterol, TMS derivative	C31H54OSi	237820.17	1.01
6	25.591	Campesterol, TMS derivative	C31H56OSi	2986003.59	12.69
7	25.735	Stigmasterol, TMS derivative	C32H56OSi	918847.81	3.9
8	26.123	Beta.-Sitosterol trimethylsilyl ether	C32H58OSi	14091188.57	59.88
9	26.204	Isofucosterol, O-TMS	C32H56OSi	1791611.1	7.61
10	26.523	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-TMS derivative	C33H58OSi	64572.55	0.27
11	26.705	Androstane-11,17-dione, 3-[(trimethylsilyl) oxy]-, 17-[O-(phenylmethyl)oxime], (3.alpha.,5.alpha.)-	C29H43NO3Si	47626.92	0.2
12	27.068	Trimethylsiloxycitrostadienol	C33H58OSi	263007.37	1.12

The compounds are displayed according to elution order in the DB-5MS column. The identification process involved comparing the mass spectrum (MS) data and retention indices (RI) with the NIST Mass Spectral Library (2011), Wiley Mass Spectral Data Registry, Eighth Ed.

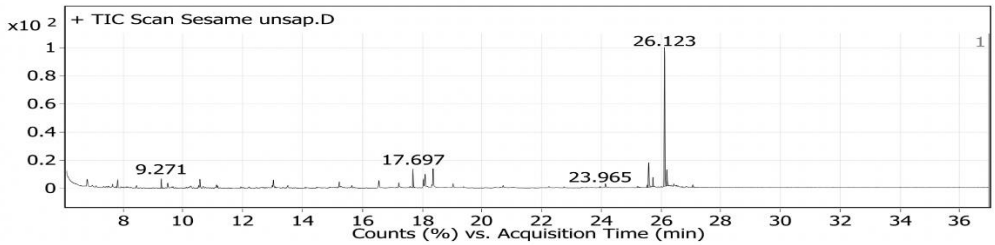

Figure 2. Total ion chromatogram of unsaponifiable matter detected in sesame oil.

3.3. Vitamin E content in sesame oil

Figures (3 A and B) demonstrate the HPLC chromatogram of vitamin E standard and sesame oil. The HPLC analysis revealed that Vitamin E content in sesame oil is 13.84 µg/mL.

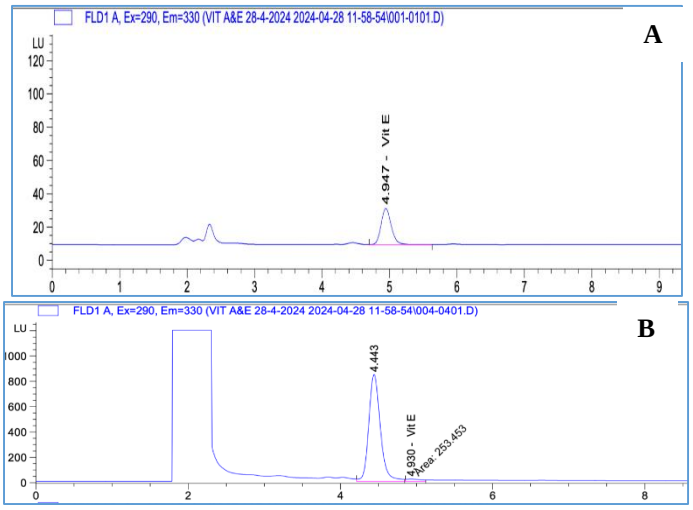

Figure 3. HPLC chromatogram of vitamin E in **A**) calibration standard solution and **B**) sesame oil sample

3.4. DPPH radical scavenging activity of sesame oil

Data illustrated in Figure (4) show that sesame oil exhibited dose-dependent DPPH radical scavenging. The scavenging potency was shown to increase with increasing sesame oil concentration. The greatest scavenging percent for sesame oil (93.0%) was observed at a concentration of 1000 µL/mL, followed by 500 µL/mL (90%) and 250 µL/mL (85%), respectively. On the other hand, the concentration of 1.95 µL/mL gave the lowest scavenging potency, with an average of 39.2%. Meanwhile, ascorbic acid showed a 98.3% inhibition for 1000 µg/mL and 41.9% for 1.95 µg/mL. Sesame oil had an IC50 value of 5.47±0.41. However, ascorbic acid's IC_{50} value was 5±0.47 µL/mL.

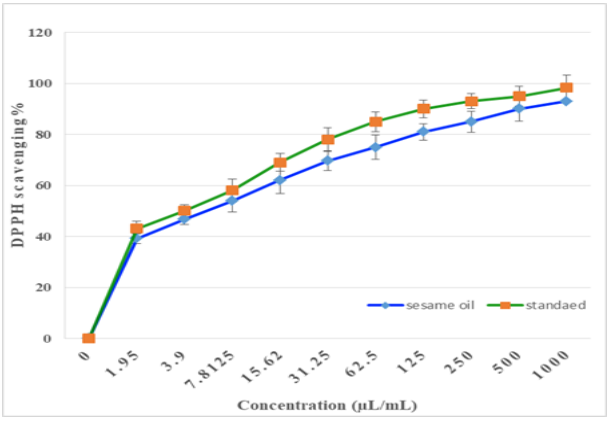

Figure 4. Radical scavenging activity of sesame oil. Data are expressed as mean±SEM

3.5. In vitro anti-inflammatory activity of sesame oil

Figure (5) shows that sesame oil significantly stabilized RBC membranes at different concentrations (1000 to 3.9 µL/mL). The percentage of hemolysis inhibition of sesame oil at a concentration of 1000 µL/mL was 99.8%, which was higher than that of indomethacin (97%) at the same concentration. However, as the sesame oil or indomethacin concentrations decreased, the percentage of inhibition of hemolysis was observed to be reduced. Indomethacin's IC50 value was 6.93±0.91 µL/mL, whereas sesame oil's IC_{50} value was 5.08±0.98 µL/mL.

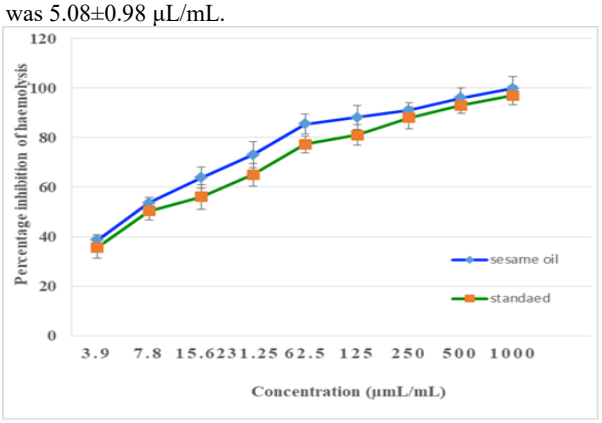

Figure 5. In vitro anti-inflammatory activity of sesame oil. Data are expressed as mean±SEM

3.6. Effect of donepezil and sesame seed oil treatments, alone or in combination, on the cholinergic system in scopolamine-treated rats

Scopolamine administration resulted in a remarkable decline in ACh levels (33.5%, $p<0.001$) and substantial elevation in AChE activity (46%, $p<0.001$) in rat brain tissue compared to that in control. However, such alterations were mitigated by donepezil, sesame oil, and combined donepezil and sesame oil administration, evidenced by the marked increase in ACh levels (14.5%, 12.229% $p<0.05$, and 31%, $p<0.001$, respectively) and the significant suppression in AChE activity (15.6%, 12.7%, $p<0.05$ and 23%, $p<0.001$, respectively) compared to scopolamine-treated rats. Notably, the concomitant administration of donepezil and sesame seed oil was more effective in protecting against scopolamine-induced cholinergic dysfunction than the donepezil or sesame seed oil monotherapy (Figures 6A and B).

3.7. Effect of donepezil and sesame seed oil treatments, alone or in combination, on amyloid-β accumulation in scopolamine-treated rats

Figure (6C) demonstrates that intraperitoneal scopolamine injection instigated a remarkable ($p<0.0001$) Aβ accumulation in brain tissue (1.727-folds) compared to the negative control. Such pathological alteration was attenuated ($p<0.01$) in rats treated with donepezil (34.33%) and sesame seed oil (29.49 %) compared to the positive control. Furthermore, rats who received combined donepezil and sesame seed oil therapy exhibited a more remarkable reduction in Aβ levels (38.6%) than those who received the monotherapy.

3.8. Effect of donepezil and sesame oil treatments, alone or in combination, on brain BDNF levels in scopolamine-treated rats

Figure (6D) displays the alterations in the brain BDNF levels in the different experimental groups. The scopolamine-treated group elicited a significant decline in the BDNF levels (31.27%, $p<0.001$) in the brain tissue compared to the negative control group. Meanwhile, oral administration of donepezil, sesame oil, and their combination markedly alleviated such alterations as demonstrated by the significant ($p<0.01$) increase in brain BDNF levels by 21.22%, 18.84%, and 21.9%, respectively, compared to the positive control.

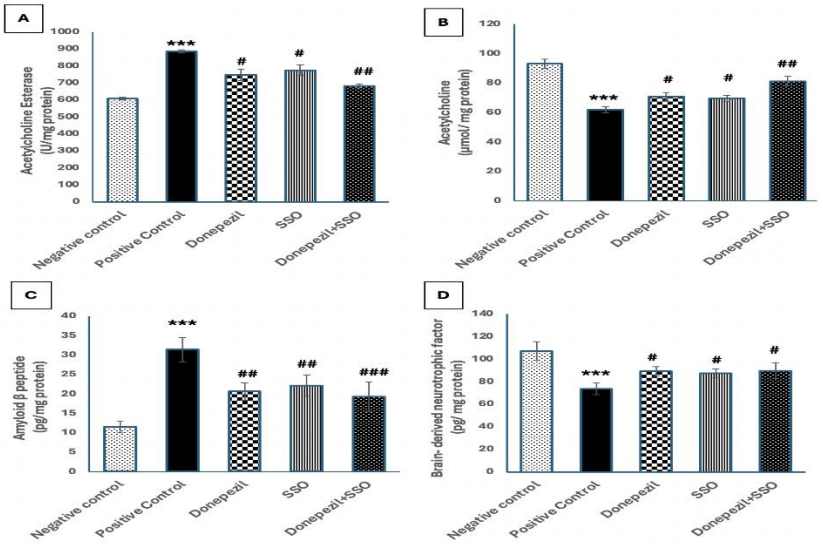

Figure 6. (A) Acetylcholine esterase activity, (**B**) acetylcholine, (**C**) amyloid ß peptide, and (**D**) BDNF levels in brain tissues of negative control, positive control, donepezil, sesame oil (SSO), and donepezil + SSO groups. Data are expressed as mean±SEM for seven rats/group. One-way ANOVA-Tukey's multiple comparison tests were performed. $^{***}p < 0.001$ compared with the control group; $^{\#}p < 0.05$ and $^{\#\#}p<0.01$ other treated groups compared with the positive control group

3.9. Effect of donepezil and sesame oil treatments, alone or in combination, on the oxidative status in scopolamine-treated rats

Data presented in Figures (7A and B) exhibit that scopolamine injection incited oxidative stress manifested by the elevation in the brain MDA (84.3%, $p<0.001$) levels concomitant with a marked reduction (73.9%, $p<0.001$) in brain GSH levels as compared to the negative control group. Oral administration of donepezil, sesame seed oil, and their combination attenuated the scopolamine-induced oxidative stress, evidenced by the marked ($p<0.001$) reduction in brain MDA (21.11%, 19.21%, and 27.5%, respectively) and significant ($p<0.001$) enhancement in GSH levels (31.5%, 29.58% and 51.04%, respectively) as compared to the positive control. It is worth mentioning that the co-administration of donepezil and sesame oil was more efficacious in modulating the scopolamine-induced oxidative stress than the monotherapy.

3.10. Effect of donepezil and sesame oil treatments, alone or in combination, on the brain B-cell lymphoma (Bcl-2) levels in scopolamine-treated rats

Figure (7C) shows that the levels of the antiapoptotic protein Bcl-2 dramatically declined (30.3%) in the scopolamine-treated group compared with the negative control group. In contrast, the donepezil, sesame seed oil, and donepezil+SSO groups exhibited significant protective effects against a scopolamine-induced decline in Bcl-2, manifested by significant increases in Bcl-2 levels by 24.5%, 14.35%, and 28.15%, respectively, as compared to the positive control. These results indicate that co-administration of donepezil and SSO produced a more prominent attenuation of apoptosis in the brains of scopolamine-treated rats than monotherapy (Figure 7C).

3.11. Effect of donepezil and sesame oil treatments, alone or in combination,on inflammatory cytokines in rat brain tissues

As shown in Figure (8), scopolamine treatment significantly provokes neuroinflammation, evidenced by the marked upsurge in the concentrations of TNF-α (98.5%), IL-1β (10.19-folds), and IL-6 (1.117-folds) in rat brain tissues compared to the positive control ($p < 0.0001$). Such alterations were significantly modulated by the tested therapy indicated by the substantial reduction in TNF- α, IL-1β and IL-6 levels by oral administration of donepezil (27.7%, $p<0.001$, 42.37%, $p<0.001$, and 19.5%, $p<0.01$, respectively), sesame oil (26.4%, 39.03%, and 20.37%, respectively, $p<0.001$) and the combined donepezil and sesame oil (31.27%, 58.7%, and 32.1%, respectively, $p<0.001$). Notably, the co-administration of donepezil and sesame oil produced the most noticeable modulatory effects against scopolamine-induced neuroinflammation compared to the treatment with donepezil or sesame oil alone.

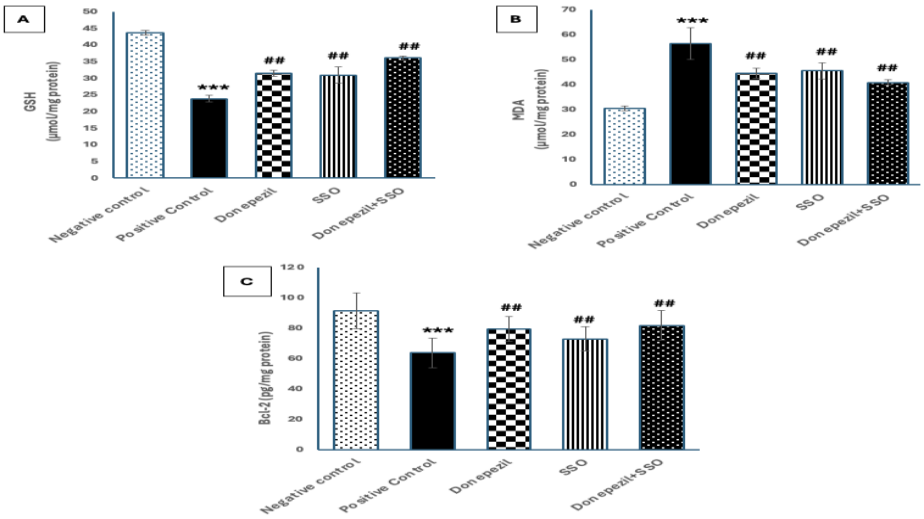

Figure 7. (**A**) Reduced glutathione (GSH), (**B**) malondialdehyde (MDA), and (**C**) B-cell lymphoma (Bcl-2) levels in brain tissues of negative control, positive control, donepezil, sesame oil (SSO), and donepezil + SSO groups. Data are expressed as mean±SEM for seven rats/group. One-way ANOVA-Tukey's multiple comparison test was performed. ***p < 0.001 compared with the control group; ##p<0.01 other treated groups compared with the positive control group.

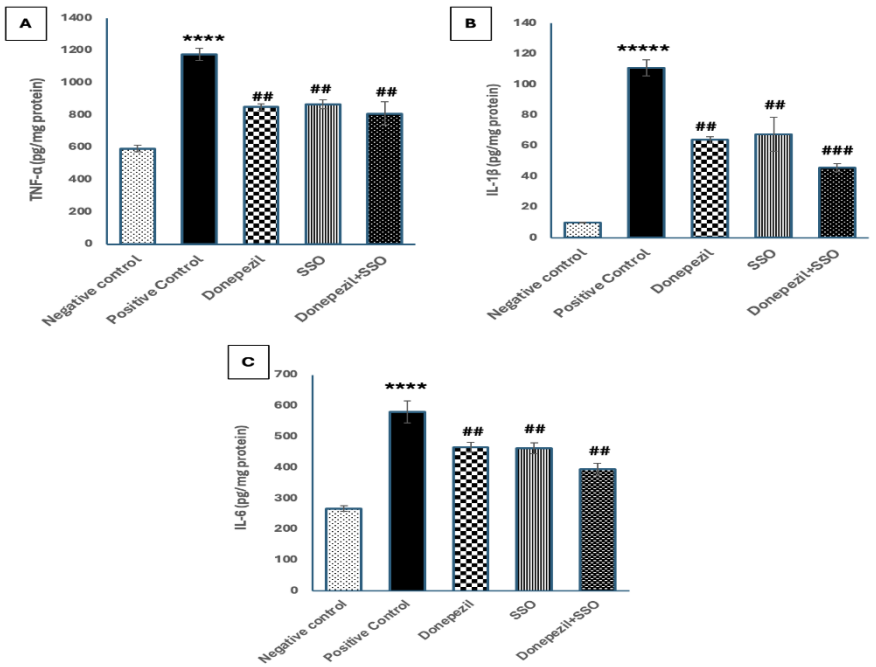

Figure 8. (A) Tumor necrosis factor-alpha (TNF-α), (B) interleukin-1beta (IL-1β), and (C) interleukin-6 (IL-6) levels in brain tissues of negative control, positive control, donepezil, sesame oil (SSO), and donepezil + SSO groups. Data are expressed as mean±SEM for seven rats/group. One-way ANOVA-Tukey's multiple comparison tests were performed. ****p < 0.0001 compared with the control group; ##p<0.01 other treated groups compared with the positive control group

3.12. Correlation studies

The correlation analysis exhibited a positive correlation between AChE activity and amyloid-β accumulation in brain tissues. Furthermore, there was a positive correlation between AChE and the lipid peroxidation marker (MDA). A robust direct correlation was also observed between AChE and the inflammatory cytokines in brain tissue. On the other hand, a negative correlation was found between AChE and the levels of ACh, BDNF, GSH, and Bcl-2, Figure (9).

Figure (10) presents the possible correlations between brain ACh levels and the measured biochemical parameters. A positive correlation was found between ACh and BDNF, GSH, and Bcl-2 levels in brain tissues. Meanwhile, a negative correlation was found between the level of ACh in the brain and the MDA and inflammatory marker levels.

Additionally, the correlation between brain amyloid β accumulation and BDNF levels, oxidative stress markers, apoptosis, and inflammation was analyzed and illustrated in Figure (11). Amyloid β was positively related to the brain MDA and the inflammatory cytokines and negatively related to BDNF, GSH, and Bcl-2 levels. There are positive significant correlations

between BDNF and GSH and Bcl-2 levels. Conversely, negative correlations are found between BDNF levels, lipid peroxidation, and inflammatory indicators (Figure 12).

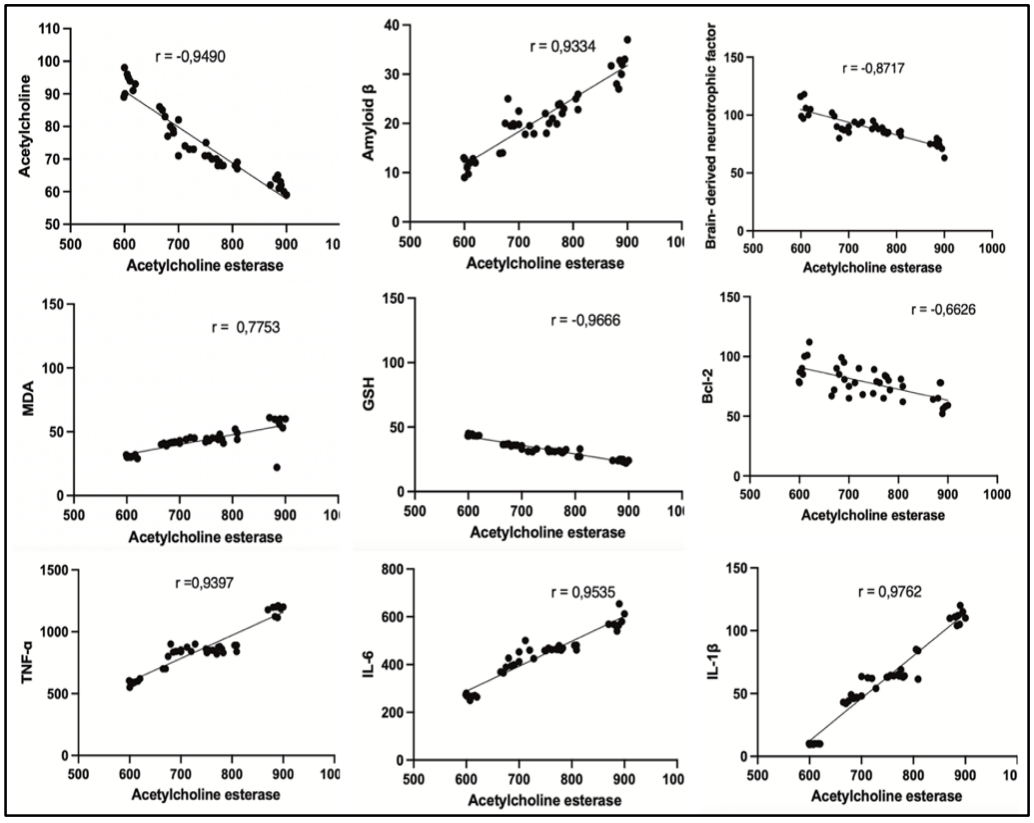

Figure 9: Correlation between brain acetylcholine esterase activity and all parameters at p<0.0001

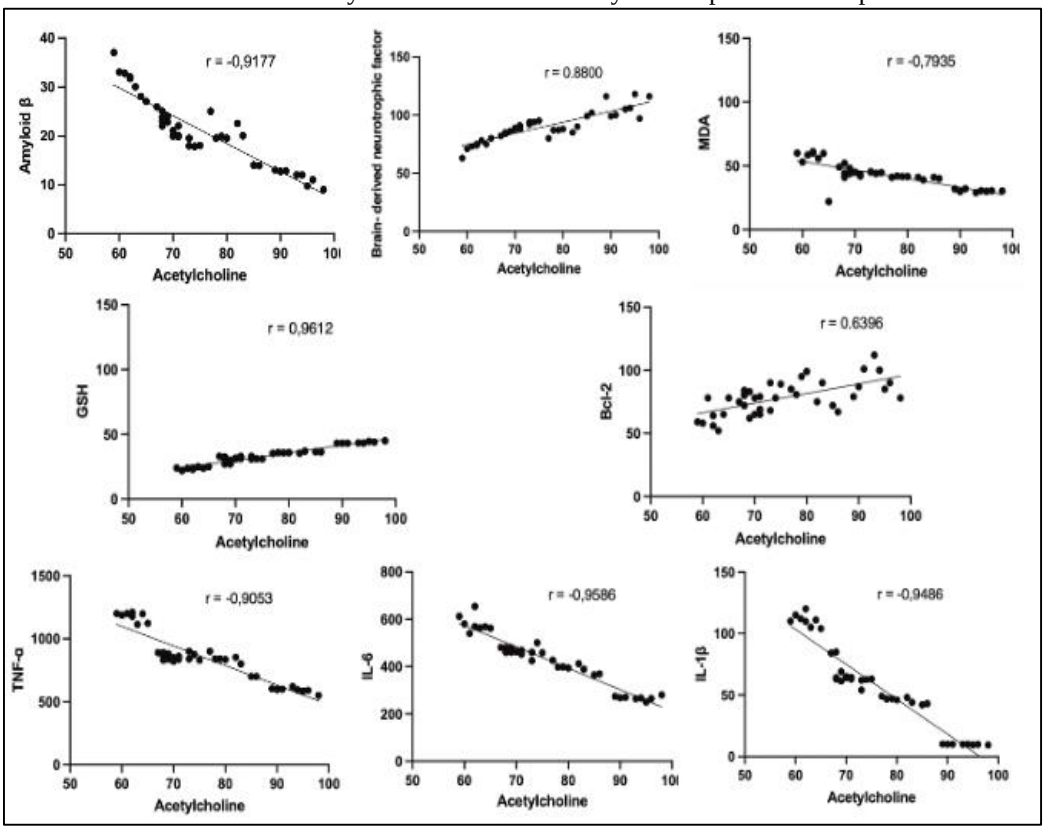

Figure 10: Correlation between brain acetylcholine levels and all parameters at p<0.0001

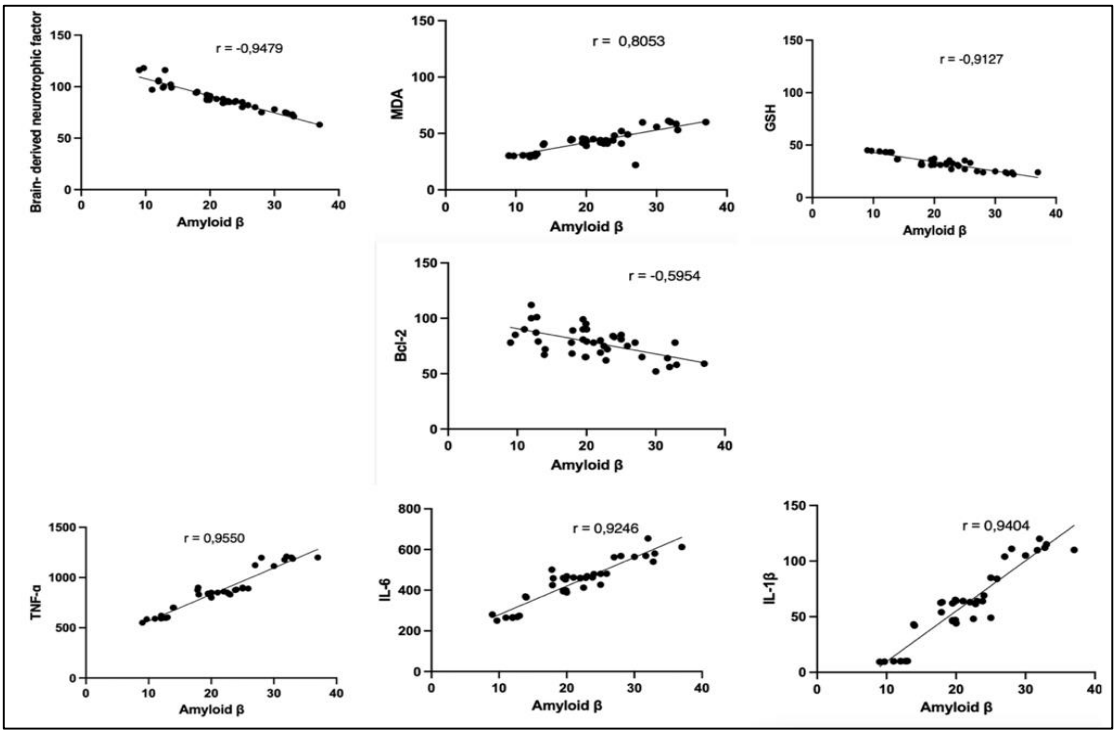

Figure 11: Correlation between brain amyloid β and all parameters at p<0.0001

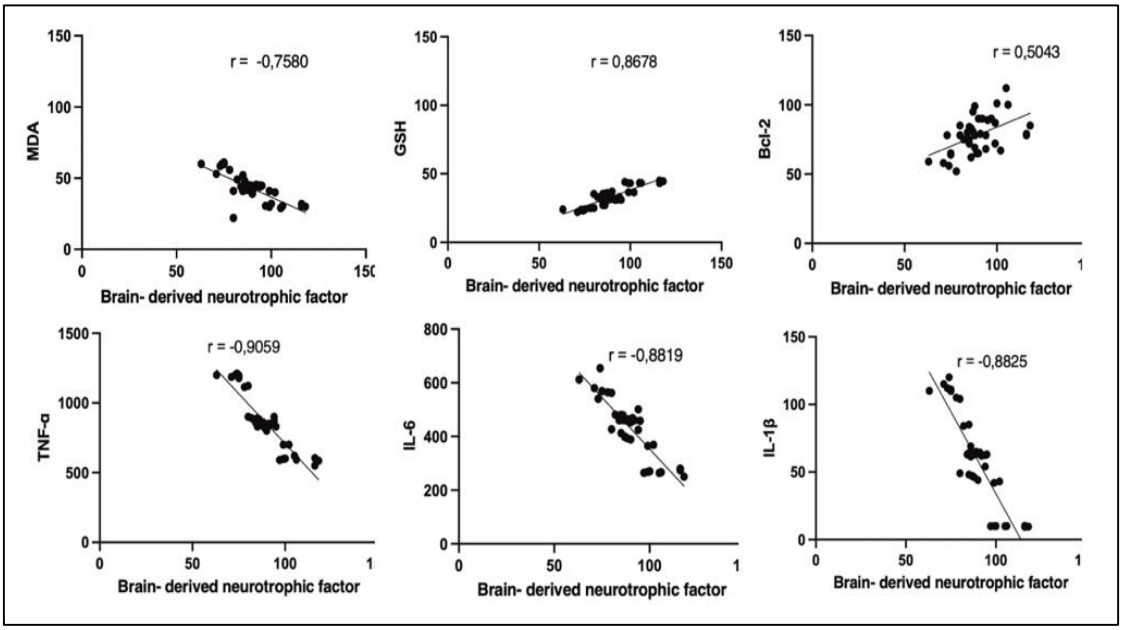

Figure 12: Correlation between brain BDNF and all parameters at p<0.0001

Discussion

This study evaluated the potential synergetic efficacy of sesame oil and the acetylcholine esterase inhibitor donepezil to attenuate scopolamine-induced neuropathological dysfunction in rats. The noteworthy finding of this study was that sesame oil synergistically potentiated the neuroprotective effects of donepezil. This protective effect was mediated by modulation of the cholinergic system, mitigation of amyloidopathy, increasing the neurotrophic factor, BDNF, inhibition of inflammation, suppression of oxidative stress, and augmentation of the anti-apoptotic protein, Bcl-2, in a scopolamine-induced neurological disorder rats model.

Scopolamine, a competitive muscarinic ACh receptor antagonist, affects the expression of several genes involved in muscarinic receptor cascades, cell differentiation, and apoptosis in rodent brains. It, therefore, causes substantial memory decline in animals and humans since deterioration and dysfunction of the cholinergic neurons are intimately linked to cognitive impairments [33]. Accordingly, scopolamine has become widely accepted as an experimental model of neurodegenerative disorders and is used to screen for neuroprotective drugs [34]. Thus, in this study, scopolamine (1 mg/kg

body weight) was used to induce neurological dysfunction in rats. Donepezil (3 mg/kg body weight) was also used as a reference conventional drug [26]. Donepezil is a reversible and specific AChE inhibitor that is currently accepted for the symptomatic relief of AD; it is thought to prevent the neurotransmitter acetylcholine (ACh) from being broken down and reimburses for the deficiency of ACh in the brain [35].

In accordance with earlier research, the results of this investigation verified that daily scopolamine injection increased AChE activity and decreased ACh levels in brain tissue compared with the controls. Treating rats with donepezil and sesame oil attenuated these scopolamine-induced neurochemical alterations. Noteworthy, sesame oil synergistically boosts the effect of donepezil in restoring the balance of the cholinergic system in scopolamine-treated rats. Comparable results were formerly reported concerning the effects of scopolamine [33-34], donepezil [26, 35], and sesame oil [9, 36]. Previous studies highlight the improved impacts of sesame oil and its lignan on cholinergic system regulation. Mohamed et al. [36] noted that administering sesame oil to rats intoxicated with $AlCl_3$ significantly reduced the activity of AChE, followed by improved locomotor activity, cognition, and memory function. Sesamin ameliorates cognitive function in lipopolysaccharide (LPS)-induced neuroinflammation and rat models with diabetes by alleviating AChE levels [37]. Sesamol restrains cholinergic system dysfunction by suppressing AChE activity and boosting ACh and choline acetyltransferase activity [38]. Consistently, the results of this study suggest that sesame oil boosted the effect of donepezil in modulating the cholinergic system reactivity in scopolamine-induced neurological disorders in rats [9, 39].

Accretion of amyloid-β *(*Aβ) results in oxidative stress and apoptosis and consequently harms neurons and deteriorates cognition; it is one of the main pathogenic factors that cause AD [40]. Aβ is produced by the degradation of amyloid-β precursor protein (AβPP) by γ-secretase and β-site amyloid-β-protein precursor cleaving enzyme 1 (BACE-1) [41]. Extensive insoluble amyloid fibril accretion results in amyloid plaque formation and propagation through the brain. Interestingly, it has been observed that administering scopolamine to Wistar rats upregulates AβPP expression and increases Aβ release [42]. Results obtained in the present study confirmed that scopolamine administration (1 mg/kg/day, *ip* for four weeks) increased amyloid deposition in brain tissue. Hernández-Rodríguez et al. [43] reported that the increased hippocampal Aβ deposition in rats could be due to the increased BACE1 expression and activity. It was suggested that the increased BACE1 activity by scopolamine might be associated with the phosphorylation of glycogen synthase kinase 3 (GSK3βP9) [44-45]. Additionally, Koh et al. suggested that elevated Aβ production subsequently phosphorylates GSK-3β, which in turn raises Aβ production. However, sesame oil and donepezil treatments substantially mitigated the scopolamine-induced Aβ accumulation. Furthermore, sesame oil enhanced the effects of donepezil on reducing Aβ accumulation, as demonstrated in the group that received the co-therapy (donepezil and sesame oil). The results of former studies corroborated this finding [14, 36]. Similar results showed that sesame oil attenuated amyloid-β accretion in a rat model of AD [36]. Sesamol and sesaminol improved learning and memory deficits caused by Aβ in the Morris water maze test [14]. Thus, sesame oil in this study may have exerted a part of the neuroprotective effect through lowering Aβ in brain tissue.

Additionally, scopolamine administration demonstrably declined BDNF levels in rat brain tissue compared to the negative control. Meanwhile, these alterations were attenuated by donepezil and sesame oil treatments. Trophic or growth factors, including BDNF, are vital for neurogenesis, differentiation and survival of neurons, and synaptic plasticity [44]. It has been reported that depletion of the cholinergic system leads to a reduced BDNF signaling and BDNF protein level [45]. Thus, the impaired cholinergic system reactivity observed in this study could be related to the decline in BDNF levels in the brain tissue of the scopolamine-treated rat. Furthermore, Oxidative stress, sparked by ROS generation, is assumedly implicated in BDNF decline. Thus, the glutathione and lipid peroxidation alterations observed in this study could account, at least partly, for the corresponding changes in brain BDNF levels. The correlation analysis in this study confirmed the perspective mentioned above; the BDNF levels in rat brains were positively related to ACh levels and negatively related to the AChE and oxidative stress parameters. On the other hand, the current study's results revealed that donepezil and sesame oil administration postponed the decline in BDNF. This beneficial effect could be attributed to restoring cholinergic function [44-46]. A preceding study has revealed that multifarious lignans (including sesamin) upregulate factors involved in neural regeneration. Sesamin enhances BDNF expression in the hippocampal regions of mice exhibiting stress-induced depressive-like behaviors [46]. These results suggest that sesame oil may promote neuroprotection by activating neurogenic factors such as BDNF in scopolamine-treated rats.

Additionally, oxidative stress has been postulated as a potential cause of neurological disorders. The brain requires high oxygen levels to perform its extensive synaptic functions and is highly vulnerable to oxidative stress [47]. Surplus production of reactive oxygen species (ROS) contributes to the pathophysiology of neurodegenerative diseases by inducing neurotoxic processes through lipid- and thiol-dependent peroxidation of cell membranes and suppression of hippocampus plasticity [47]. Furthermore, Aβ1-42 production can amplify the state of oxidative stress. It has been extensively shown that elevated Aβ levels can cause oxidative stress by generating ROS. The generation of ROS by amyloid β results in lipid peroxidation, which compromises membrane permeability and triggers excitotoxicity pathways due to elevated calcium (Ca^{2+}) influx, leading to neurotoxic consequences. This is thought to impact neurotransmission and cognitive processes profoundly [48]. Additionally, it has been proposed that an elevated amount of free radicals and ROS in cells produces more Aβ peptides, which further oxidatively stress and toxically insult neurons. [49]. In line with previous research, the current investigation revealed that scopolamine considerably exacerbated oxidative stress in the rats, as evidenced by an upsurge in MDA levels and a drop in GSH levels compared to the control group [50-51]. Conversely, donepezil and sesame oil reverted the scopolamine-induced oxidative stress, as seen by increased GSH reserves and decreased brain lipid peroxidation. The antioxidative role of sesame oil was established in our *in vitro* study, as shown by its capacity to scavenge the DPPH radical in a dose-dependent manner. These antioxidant properties of sesame oil could be ascribed to the existence of considerable amounts of potent antioxidants such as phytosterols, lignans, and tocopherols in the sesame oil, as evidenced by the chromatographic analysis in this study [52-54]. Several lines of evidence have also indicated the antioxidative role of sesame oil, where sesame oil decreased lipid peroxidation and augmented the antioxidant defense system [52-53].

Inflammation is also a significant contributing factor in the pathogenesis of neurologic ailments. In agreement with many previous studies, this investigation revealed that scopolamine-treated rats provoked intense neuroinflammation, demonstrated by the dramatic upsurge in brain tissue's TNF-α, IL-1β, and IL-6 levels [55]. Robust evidence from human and animal models indicates a close connection between oxidative stress, Aβ accumulation, and inflammation. Indeed, misfolded β-amyloid directly disrupts the outer mitochondrial membrane stability, eliciting a surge in ROS generation [56]. Further, Aβ stimulates the production of pro-inflammatory cytokines, which is essential for the chain of events resulting in cell death [55]. Simultaneously, excessive levels of inflammatory cytokines and ROS have been shown to promote the formation and accretion of Aβ fibrils and the death of neuronal cells. Collectively, these data point to a "vicious cycle" that may gradually worsen the course of the disease and ultimately cause neuronal death. This cycle involves elevated oxidative stress, a surge in inflammatory cytokines, and overproduction of Aβ, further perturbing ROS generation [56]. In light of the above justifications, the results of the current investigation revealed a strong positive correlation between Aβ levels and oxidative stress and inflammatory cytokines markers. On the contrary, sesame oil curtails the upsurge of inflammatory cytokines. Thus, part of sesame oil's beneficial effect could be ascribed to its anti-inflammatory potential [57-58]. The results of the *in vitro* experiment corroborated the sesame oil's *in vivo* anti-inflammatory properties, as sesame oil showed considerable stabilization of RBC membranes at various concentrations. These anti-inflammatory properties could be attributed to a wealth of bioactive phytoconstituents in sesame oil, as proved by GC–MS and HPLC oil analysis.

Apoptosis is one of the primary causes of neurodegeneration. ROS can directly lead to apoptosis responses. Inhibition of apoptosis promotes cell survival, particularly in the aftermath of toxic insult, growth factor deficits, or hypoxia [59]; thus, protective effects against apoptosis and oxidative damage in neurodegenerative diseases are essential in discovering new therapeutic drugs. In this study, scopolamine administration declined the antiapoptotic protein Bcl-2; such alteration was restored nearly to normal levels by treatment with the combined therapy of donepezil and sesame oil. These antiapoptotic effects could be ascribed to sesame oil's marked antioxidant and anti-inflammatory activities [60].

There are several ways to extract oils from seeds, including pressing, solvent extraction, and supercritical fluid extraction. However, these extraction processes could strongly influence oils' nutritional composition and bioactive compound content. In this study, sesame oil was extracted using a cold-pressing method. This method is considered a green technology for extracting edible oils, whereby the bioactive components are maintained because neither thermal nor chemical procedures are used. Owing to possible variations in the composition, properties, and bioactivities of sesame oils extracted by varying procedures, the sesame oil obtained by cold press was subjected to chemical analysis by GC–MS and HPLC to characterize its bioactive constituents. Consistent with earlier research findings, the chromatographic analysis in the current study verified the existence of tocopherols and phytosterols such as beta-sitosterol trimethylsilyl ether, campesterol, isofucosterol, phytol, stigmasterol, trimethylsiloxycitrostadienol, and brassicasterol in sesame oil. The primary unsaturated fatty acids identified in the oil were linoleic and oleic. Hence, we can assume that sesame oil's protective potential for treating neurodegenerative disorders could be ascribed to these bioactive components [8-15]. Numerous *in vitro* and *in vivo* investigations have clearly demonstrated the neuroprotective properties of vitamin E [61-63]. Tocopherols and tocotrienols shield tissue lipids from free radicals by lowering chemical species, including singlet oxygen, peroxyl, hydroxyl, and superoxide radicals. A marked reduction in ROS generation by α-tocopherol has already been shown in macrophages and is related to the suppression of protein kinase C (PKC) [64]. The inhibition of PKC leads to the inhibition of NADPH-oxidase assembly [65] and, thus, reduces the production of superoxide. Besides, numerous studies demonstrate that vitamin E markedly inhibited LPS-induced microglia activation by decreasing nitric oxide production and inducing the expression of IL-1α, TNF-α, and inducible nitric oxide synthase (iNOS) [63]. Furthermore, vitamin E has also been documented to interact with the pro-inflammatory signals in BV2 cells through the cyclooxygenase-2 (COX-2) signaling pathway [66–67]. Furthermore, several reports have indicated that phytosterols have intriguing anti-inflammatory and antioxidant properties. In vitro studies revealed that phytosterols lowered iron-induced lipid peroxidation in platelet membranes [67]. Sesame lignan (including sesamin and sesamol) possesses anti-inflammatory properties in several neuropathological conditions, including brain ischemia [69], seizures [70], diabetic retinopathy [71], and scopolamine induction [72].

Conclusion

The current study shows that sesame oil synergistically boosted the effect of donepezil in restoring the cholinergic system, preventing amyloid-beta accumulation, increasing the levels of neurotrophins (BDNF), the antioxidant (GSH), and the antiapoptotic marker (Bcl-2) and prevented scopolamine-induced lipid peroxidation and inflammation in scopolamine-induced neurological dysfunction rat model. This improvement may be attributed to the enhancements in the pharma-dynamics or pharma-kinetics properties of donepezil in the presence of sesame oil. Furthermore, sesame oil's bioactive phytochemicals, such as lignins, phytosterols, and tocopherols, which were proven in our *in vitro* study, may act in parallel with donepezil as coadjuvant agents for preventing and treating neurological disorders. The present study's findings may justify the synergistic intervention between food therapy and pharmacotherapy to alleviate the increasing incidence of neurodegenerative diseases.

Declarations

Competing interests

The authors declare that they have no competing interests.

Funding

This research received no specific grant from any funding agencies in the public, commercial, or not-for-profit sectors.

Availability of data

All data generated or analyzed during this study are included in this published article.

Ethics approval

All methods in the current research were approved by the Ethics Committee, Faculty of Women for Arts Science and Education, Ain Shams University (approval no. ASU/W/Sci-1532309002).

Consent for publication

Not applicable.

References

[1] Kesidou E, Theotokis P, Damianidou O, Boziki M, Konstantinidou N, Taloumtzis C, Sintila SA, Grigoriadis P, Evangelopoulos ME, Bakirtzis C, Simeonidou C. (2023). CNS ageing in health and neurodegenerative disorders. *J Clin Med.* 14;12(6):2255. doi: 10.3390/jcm12062255.

[2] Zhang, L., Seo, J. H., Li, H., Nam, G., & Yang, H. O. (2018). The phosphodiesterase 5 inhibitor, KJH-1002, reverses a mouse model of amnesia by activating a cGMP/cAMP response element binding protein pathway and decreasing oxidative damage. *British journal of pharmacology*, *175*(16), 3347–3360. https://doi.org/10.1111/bph.14377

[3] Selkoe, D.J. (2008). Biochemistry and molecular biology of amyloid beta-protein and the mechanism of Alzheimer's disease. *Handb Clin Neurol* 89, 245–260.

[4] Bhatia, V., & Sharma, S. (2021). Role of mitochondrial dysfunction, oxidative stress and autophagy in progression of Alzheimer's disease. *Journal of the Neurological Sciences*, 421, 117253. https://doi.org/10.1016/j.jns.2020.117253

[5] Marucci, G., Buccioni, M., Ben, D. D., Lambertucci, C., Volpini, R., & Amenta, F. (2021). Efficacy of acetylcholinesterase inhibitors in Alzheimer's disease. *Neuropharmacology*, *190*, 108352. https://doi.org/10.1016/j.neuropharm.2020.108352

[6] Young, S., Chung, E., & Chen, M. A. (2021). Cardiovascular complications of acetylcholinesterase inhibitors in patients with alzheimer's disease: A Narrative Review. *Annals of Geriatric Medicine and Research*, *25*(3), 170-177. https://doi.org/10.4235/agmr.21.0079

[7] Rehman, M. U., Wali, A. F., Ahmad, A., Shakeel, S., Rasool, S., Ali, R., Rashid, S. M., Madkhali, H., Ganaie, M. A., & Khan, R. (2019). Neuroprotective strategies for neurological disorders by natural products: An update. *Current neuropharmacology*, *17*(3), 247–267. https://doi.org/10.2174/1570159X16666180911124605

[8] Wei, P., Zhao, F., Wang, Z., Wang, Q., Chai, X., Hou, G., & Meng, Q. (2022). Sesame (Sesamum indicum L.): a comprehensive review of nutritional value, phytochemical composition, health benefits, development of food, and industrial applications. *Nutrients*, *14*(19). https://doi.org/10.3390/nu14194079

[9] Kim, Y., Kim, S., Lee, J., Kim, I., Oh, E., Kim, W., Lee, E., Cho, S., Kim, S., & Lee, H. (2023). Lignan-rich sesame (sesamum indicum l.) cultivar exhibits in vitro anti-cholinesterase activity, anti-neurotoxicity in amyloid-β induced sh-sy5y cells, and produces an in vivo nootropic effect in scopolamine-induced memory-impaired mice. *Antioxidants*, *12*(5). https://doi.org/10.3390/antiox12051110

[10] Deme P., Narasimhulu C.A., Parthasarathy S. (2018). Identification and evaluation of anti-inflammatory properties of aqueous components extracted from sesame (*Sesamum indicum*) oil. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.*1087:61–69.

[11] Ruankham W., Suwanjang W., Wongchitrat P., Prachayasittikul V., Prachayasittikul S., Phopin K. (2019). Sesamin and sesamol attenuate H2O2-induced oxidative stress on human neuronal cells via the SIRT1-SIRT3-Foxo3a signaling pathway. *Nutr. Neurosci.* 24:90–101. doi: 10.1080/1028415X.2019.1596613.

[12] Prasad N.R., Mahesh T., Menon V.P., Jeevanram R., Pugalendi K.V. (2005). Photoprotective effect of sesamol on UVB-radiation induced oxidative stress in human blood lymphocytes in vitro. *Environ. Toxicol. Pharmacol.* 20:1–5. doi: 10.1016/j.etap.2004.09.009

[13] Hassanzadeh, P.; Arbabi, E.; Rostami, F. (2014**).** The ameliorative effects of sesamol against seizures, cognitive impairment and oxidative stress in the experimental model of epilepsy. *Iran. J. Basic Med. Sci, 17,* 100.

[14] Katayama S., Sugiyama H., Kushimoto S., Uchiyama Y., Hirano M., Nakamura S. (2016). Effects of sesaminol feeding on brain aβ accumulation in a senescence-accelerated mouse-prone 8. *J. Agric. Food Chem.* 64:4908–4913. doi: 10.1021/acs.jafc.6b01237

[15] Keowkase R., Shoomarom N., Bunargin W., Sitthithaworn W., Weerapreeyakul N. (2018). Sesamin and sesamolin reduce amyloid-β toxicity in a transgenic caenorhabditis elegans. *Biomed. Pharmacother.* 107:656–664. doi: 10.1016/j.biopha.2018.08.037.

[16] Chen, X., Drew, J., Berney, W., & Lei, W. (2021). Neuroprotective natural products for alzheimer's disease. *Cells*, *10*(6). https://doi.org/10.3390/cells10061309

[17] Siu, W. S., Ko, C. H., Shiu, H. T., Li, K. K., Shum, W. T., Leung, P. C., & Zhang, J. F. (2021). Green tea extract synergistically enhances the effectiveness of an antiresorptive drug on management of osteoporosis induced by ovariectomy in a rat model. *Experimental and Therapeutic Medicine*, *21*(4). https://doi.org/10.3892/etm.2021.9759

[18] Rajagukguk, Y. V., Utcu, M. A., Islam, M., Muzolf-Panek, M., & Tomaszewska-Gras, J. (2022). Authenticity assessment from sesame seeds to oil and sesame products of various origin by differential scanning calorimetry. *Molecules*, *27*(21). https://doi.org/10.3390/molecules27217496

[19] Radwan, O.K. & Ahmed, R.F. (2016). Amendment effect of resveratrol on diclofenac idiosyncratic toxicity: Augmentation of the antiinflammatory effect by assessment of Arachidonic acid and IL-1β levels. J App Pharm Sci, 6 (12): 170-177.

[20] Tsuda K., Sakai K., Tanabe K., Kishida Y. Isolation of 22-dehydrocholesterol from Hypnea japonica. Chem Pharm Bull. 7(6),747 (1959). doi: 10.1248/cpb.7.747.

[21] Lee Y.C., Oh S.W., Chang J., Kum I.H. (. 2004). Chemical composition and oxidative stability of safflower oil prepared from safflower seed roasted with different temperatures. *Food Chem.* 84:1–6. doi: 10.1016/S0308-8146(03)00158-4.

[22] Gimeno, E., Castellote, A., Lamuela-Raventós, R., De la Torre, M., & López-Sabater, M. (2000). Rapid determination of vitamin E in vegetable oils by reversed-phase high-performance liquid chromatography. Journal of Chromatography A, 881(1-2), 251-254. https://doi.org/10.1016/S0021-9673(00)00219-3
[23] Mohamed, M., & Farid, O. (2024). Gastro-Protective, antioxidant, and anti-inflammatory potential of three vegetable oils against indomethacin-induced gastric ulceration in rats: in vivo and in vitro study. Egyptian Journal of Chemistry, 67(7), 341-359. doi: 10.21608/ejchem.2024.262460.9181
[24] Yesmin, S., Paul, A., Naz, T., Rahman, A. B., Akhter, S. F., Wahed, M. I., Emran, T. B., & Siddiqui, S. A. (2020). Membrane stabilization as a mechanism of the anti-inflammatory activity of ethanolic root extract of Choi (Piper chaba). Clinical Phytoscience, 6(1), 1-10. https://doi.org/10.1186/s40816-020-00207-7
[25] Adeline Yadang, F. S., Nguezeye, Y., Kom, C. W., Diboue Betote, P. H., Mamat, A., Yamthe Tchokouaha, L. R., Taiwé, G. S., Agbor, G. A., & Bum, E. N. (2020). Scopolamine-induced memory impairment in mice: neuroprotective effects of Carissa Edulis (Forssk.) Valh (apocynaceae) aqueous extract. *International Journal of Alzheimer's Disease*, *2020*. https://doi.org/10.1155/2020/6372059
[26] Shin, C. Y., Kim, S., Cha, H., Won, D. H., Lee, Y.,Jang,S. W.,&Sohn, U.D.(2018).The Effects of donepezil, an acetylcholine sterase inhibitor, on impaired learning and memory in rodents. *Biomolecules & Therapeutics*, *26*(3), 274-281. https://doi.org/10.4062/biomolther.2017.189
[27] Saleem, M. T., Chetty, M. C., & Kavimani, S. (2014). Antioxidants and tumor necrosis factor alpha-inhibiting activity of sesame oil against doxorubicin-induced cardiotoxicity. Therapeutic advances in cardiovascular disease, 8(1), 4–11. https://doi.org/10.1177/1753944713516532
[28] Beutler, E., Duron, O. ,Kelly, B. M. (1963): Improved method for the determination of blood glutathione. Lab. Clin. Med. 61: 882-888.
[29] Uchiyama, M. and Mihara, M. (1978): Determination of malondialdehyde precursor in tissues by thiobarbituric acid test. Anal. biochem. 86: 271-278.
[30] Henry, R.J.; Cannon, D.C.,Winkelman, J.W. (1974): Clinical chemistry principles and techniques (Harper and Row) 16-40 |
[31] Kurt C. (2018). Variation in oil content and fatty acid composition of sesame accessions from different origins. *Grasas y Aceites.* 69:e241. doi: 10.3989/gya.0997171.
[32] Rajagukguk, Y. V., Utcu, M. A., Islam, M., Muzolf-Panek, M., & Tomaszewska-Gras, J. (2022). Authenticity assessment from sesame seeds to oil and sesame products of various origin by differential scanning calorimetry. Molecules, 27(21). https://doi.org/10.3390/molecules27217496
[33] Subedi, L., Cho, K., Park, Y. U., Choi, H. J., & Kim, S. Y. (2019). Sulforaphane-enriched broccoli sprouts pretreated by pulsed electric fields reduces neuroinflammation and ameliorates scopolamine-induced amnesia in mouse brain through its antioxidant ability via nrf2-ho-1 activation. *Oxidative medicine and cellular longevity*, *2019*, 3549274. https://doi.org/10.1155/2019/3549274
[34] Afzal, M., Alzarea, S. I., Alharbi, K. S., Alzarea, A. I., Alenezi, S. K., Alshammari, M. S., Alquraini, A. H., & Kazmi, I. (2022). Rosiridin attenuates scopolamine-induced cognitive impairments in rats via inhibition of oxidative and nitrative stress leaded caspase-3/9 and tnf-α signaling Pathways. *Molecules (Basel, Switzerland)*, *27*(18), 5888. https://doi.org/10.3390/molecules27185888
[35] Colovic MB, Krstic DZ, Lazarevic-Pasti TD, Bondzic AM, Vasic VM. (2013). Acetylcholinesterase inhibitors: pharmacology and toxicology. *Curr Neuropharmacol.* 11:315–335. doi: 10.2174/1570159X11311030006.
[36] Mohamed E.A., Ahmed H.I., Zaky H.S., Badr A.M. (2021). Sesame oil mitigates memory impairment, oxidative stress, and neurodegeneration in a rat model of alzheimer's disease. A pivotal role of NF-KB/p38mapk/BDNF/PPAR-γ pathways. *J. Ethnopharmacol.* 267:113468. doi: 10.1016/j.jep.2020.113468.
[37] Kuhad A., Chopra K. (2007). Effect of sesamol on diabetes-associated cognitive decline in rats. *Exp. Brain Res.* 185:411–420. doi: 10.1007/s00221-007-1166-y.
[38] Liu Z., Chen Y., Qiao Q., Sun Y., Liu Q., Ren B., Liu X. (2017). Sesamol supplementation prevents systemic inflammation-induced memory impairment and amyloidogenesis via inhibition of nuclear factor kappa. *Mol. Nutr. Food Res.* 2016;61:1600734. doi: 10.1002/mnfr.201600734.
[39] Tung, B. T., Hai, N. T. & Thu, D. K. (2017). Antioxidant and acetylcholinesterase inhibitory activities *in vitro* of different fraction of Huperzia squarrosa (Forst.) Trevis extract and attenuation of scopolamine-induced cognitive impairment in mice. *Journal of Ethnopharmacology* 198, 24–32, https://doi.org/10.1016/j.jep.2016.12.037
[40] Hardy J., Selkoe D.J. (2002). The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science.* 297:353–356. doi: 10.1126/science.1072994.
[41] Scholz D., Chernyshova Y., Ückert A.-K., Leist M. (2018). Reduced AB secretion by human neurons under conditions of strongly increased BACE activity. *J. Neurochem.* 147:256–274. doi: 10.1111/jnc.14467.
[42] Bihaqi SW, Singh AP, Tiwari M (2012). Supplementation of Convolvulus pluricaulis attenuates scopolamine-induced increased tau and amyloid precursor protein (AβPP) expression in rat brain. Indian J Pharmacol 44:593–598. https://doi.org/10.4103/0253- 7613.100383
[43] Hernández-Rodríguez, M., Arciniega-Martínez, I. M., García-Marín, I. D., Correa-Basurto, J., & Rosales-Hernández, M. C. (2020). Chronic administration of scopolamine increased gsk3βp9, beta secretase, amyloid beta, and oxidative stress in the hippocampus of wistar rats. *Molecular neurobiology*, *57*(9), 3979–3988. https://doi.org/10.1007/s12035-020-02009-x
[44] Miranda, M., Morici, J. F., Zanoni, M. B., & Bekinschtein, P. (2019). Brain-derived neurotrophic factor: a key molecule for memory in the healthy and the pathological brain. *Frontiers in Cellular Neuroscience*, *13*, 472800. https://doi.org/10.3389/fncel.2019.00363

[45] Orciani, C. *et al.* (2022). Long-term nucleus basalis cholinergic depletion induces attentional deficits and impacts cortical neurons and BDNF levels without affecting the NGF synthesis. *J. Neurochem.* **163**, 149–167. https://doi.org/10.1111/jnc.15683.
[46] Zhao Y., Wang Q., Jia M., Fu S., Pan J., Chu C., Liu X., Liu X., Liu Z. (2018). (+)-SESAMIN attenuates chronic unpredictable mild stress-induced depressive-like behaviors and memory deficits via suppression of neuroinflammation. *J. Nutr. Biochem.* 64:61–71. doi: 10.1016/j.jnutbio.2018.10.006.
[47] Salim, S. (2016). Oxidative stress and the central nervous system. *The Journal of Pharmacology and Experimental Therapeutics*, *360*(1), 201-205. https://doi.org/10.1124/jpet.116.237503
[48] Butterfield DA, Boyd-Kimball D (2018) Oxidative stress, amyloid β peptide, and altered key molecular pathways in the pathogenesis and progression of Alzheimer's disease. J Alzheimers Dis 62:1345– 1367. https://doi.org/10.3233/JAD-170543
[49] Cheignon, C., Tomas, M., Bonnefont-Rousselot, D., Faller, P., Hureau, C., & Collin, F. (2018). Oxidative stress and the amyloid beta peptide in Alzheimer's disease. *Redox Biology*, *14*, 450-464. https://doi.org/10.1016/j.redox.2017.10.014
[50] Simeonova R, Atanasova M, Stavrakov G, Philipova I, Doytchinova I. (2022). Ex vivo antioxidant and cholinesterase inhibiting effects of a novel galantamine-curcumin hybrid on scopolamine-induced neurotoxicity in mice. *Int. J. Mol. Sci.* **23**, 14843. doi:10.3390/ijms232314843
[51] Hupparage VB, Rasal VP, Patil VS, Patil PP, Mulange SG, Malgi AP, Patil SA, Karade AR. (2020). Ameliorative effect of *Amaranthus tricolor L*. leaves on scopolamine-induced cognitive dysfunction and oxidative stress in rats. *J. Appl. Pharmaceut. Sci.* **10**, 111-120.
[52] Hsu, E., & Parthasarathy, S. (2017). Anti-inflammatory and antioxidant effects of sesame oil on atherosclerosis: a descriptive literature review. *Cureus*, *9*(7). https://doi.org/10.7759/cureus.1438
[53] Imran, M, Khan, MK, Ali, M, Nadeem, M, Mushtaq, Z, Ahmad, MH, et al. (2020). Cold pressed sesame (*Sesamum indicum*) oil. *Cold Press Oils*. 2020:105–11. doi: 10.1016/b978-0-12-818188-100010-4
[54] Oboulbiga, E. B., Douamba, Z., Semporé, J. N., Dabo, R., Semde, Z., Tapsoba, F. W., T., L., Parkouda, C., & Dicko, M. H. (2023). Physicochemical, potential nutritional, antioxidant and health properties of sesame seed oil: A review. *Frontiers in Nutrition*, *10*, 1127926. https://doi.org/10.3389/fnut.2023.1127926
[55] Chong, Y. (1997). Effect of a carboxy-terminal fragment of the alzheimer's amyloid precursor protein on expression of proinflammatory cytokines in rat glial cells. *Life Sciences*, *61*(23), 2323-2333. https://doi.org/10.1016/S0024-3205(97)00936-3
[56] Mondragón-Rodríguez, S., Perry, G., Zhu, X., Moreira, P. I., Acevedo-Aquino, M. C., & Williams, S. (2013). Phosphorylation of tau protein as the link between oxidative stress, mitochondrial dysfunction, and connectivity failure: implications for Alzheimer's disease. *Oxidative medicine and cellular longevity*, *2013*, 940603. https://doi.org/ 10.1155/2013/940603
[57] Cheon, S. Y., Koo, N., Kim, S. Y., Kam, E. H., Nam, J., & Kim, E. J. (2021). Scopolamine promotes neuroinflammation and delirium-like neuropsychiatric disorder in mice. *Scientific Reports*, *11*. https://doi.org/10.1038/s41598-021-87790-y
[58] Choi, J., Lee, E., Jang, H., Cha, Y., Park, Y., & Lee, S. (2021). Allium hookeri extracts improve scopolamine-induced cognitive impairment via activation of the cholinergic system and anti-neuroinflammation in mice. *Nutrients*, *13*(8), 2890. https://doi.org/10.3390/nu13082890
[59] Zeisel, S. H. (2004). Antioxidants suppress apoptosis. The Journal of Nutrition, 134(11), 3179S-3180S. https://doi.org/10.1093/jn/134.11.3179S
[60] Akinrinde, A.S., Oyewole, S.O. & Ola-Davies, O.E. (2023). Supplementation with sesame oil suppresses genotoxicity, hepatotoxicity and enterotoxicity induced by sodium arsenite in rats. Lipids Health Dis 22, 14 https://doi.org/ 10.1186/s12944-022-01760-5
[61] Grundman M. (2000). Vitamin E and Alzheimer disease: The basis for additional clinical trials. Am. J. Clin. Nutr. 71:630–636. doi: 10.1093/ajcn/71.2.630s.
[62] Takahashi T., Nakaso K., Horikoshi Y., Hanaki T., Yamakawa M., Nakasone M., Kitagawa Y., Koike T., Matsura T. (2016). Rice bran dietary supplementation improves neurological symptoms and loss of purkinje cells in vitamin e-deficient mice. Yonago Acta Med. 59:188–195.
[63] Li Y., Liu L., Barger S.W. (2001). Vitamin E suppression of microglial activation is neuroprotective. J. Neurosci. Res. 66:163–170. doi: 10.1002/jnr.1208.
[64] Kamat J.P., Devasagayam T.P. (1995). Tocotrienols from palm oil as potent inhibitors of lipid peroxidation and protein oxidation in rat brain mitochondria. Neurosci. Lett. 11:179–182. doi: 10.1016/0304-3940(95)11812-B.
[65] Lively S., Schlichter L.C. (2018). Microglia Responses to pro-inflammatory Stimuli (LPS, IFNγ+TNFα) and reprogramming by resolving cytokines (IL-4, IL-10) Front. *Cell Neurosci*. 12:215. doi: 10.3389/fncel.2018.00215.
[66] De Rijk M.C., Breteler M.M., den Breeijen J.H., Launer L.J., Grobbee D.E., van der Meche F.G. Dietary antioxidants and Parkinson disease. The Rotterdam study. Arch. Neurol. 1997;54:762–765. doi: 10.1001/archneur.1997.00550180070015.
[67] Van Rensburg S. J., Daniels W. M., Van Zyl J. M., Taljaard J. J. (2000). A comparative study of the effects of cholesterol, beta-sitosterol, beta-sitosterol glucoside, dehydroepiandrosterone sulphate and melatonin on in vitro lipid peroxidation. Metab. Brain Dis. 15, 257–265. doi:10.1023/a:1011167023695
[68] Bakrim, S., Benkhaira, N., Bourais, I., Benali, T., Lee, H., Omari, N. E., Sheikh, R. A., Goh, K. W., Ming, L. C., & Bouyahya, A. (2022). Health benefits and pharmacological properties of stigmasterol. Antioxidants, 11(10). https://doi.org/10.3390/antiox11101912
[69] Dong Y., Benveniste E.N. (2001). Immune function of astrocytes. Glia. 36:180–190. doi: 10.1002/glia.1107
[70] Testa G., Gamba P., Badilli U., Gargiulo S., Maina M., Guina T., Calfapietra S., Biasi F., Cavalli R., Poli G., et al. (2014). Loading into nanoparticles improves quercetin's efficacy in preventing neuroinflammation induced by oxysterols. PLoS ONE. 9:e96795. doi: 10.1371/journal.pone.0096795

[71] Lamar M., Boots E.A., Arfanakis K., Barnes L.L., Schneider J.A. (2020). Vascular disease, alzheimer's disease, and mild cognitive impairment. Oxford Academic; Oxford, UK: 2020. Brain structural alterations common to cardiovascular disease risk factors and alzheimer's dementia; pp. 241–258.

[72] Yun D., Wang Y., Zhang Y., Jia M., Xie T., Zhao Y., Yang C., Chen W., Guo R., Liu X., et al. (2022). Sesamol attenuates scopolamine-induced cholinergic disorders, neuroinflammation, and cognitive deficits in mice. J. Agric. Food Chem. 70:13602–13614. doi: 10.1021/acs.jafc.2c04292.