

ARTICLE

Open Access



Omija (*Schisandra chinensis*) extract and soybean mixture to enhance memory function through the gut-brain axis: network pharmacology and in vivo validation of multi-target mechanisms

Yu Yue^{1†}, Hye Jeong Yang^{2†}, Myung-Sunny Kim², Ting Zhang¹, Chen Li¹, Min Jung Kim², Dai-Ja Jang^{2*} and Sunmin Park^{1,3*} 

Abstract

With the growing prevalence of memory impairment and limited treatment options, this study evaluated the cognitive-enhancing potential of an Omija (*Schisandra chinensis* (Turcz.) Baill) 50% ethanol extract and soybean mixture (OSM). By integrating network pharmacology with in vivo validation, we aimed to elucidate the molecular mechanisms and therapeutic efficacy of OSM. Network pharmacology was used to identify core pathways and targets associated with the memory-enhancing effects of Omija (gomisin, schizandrin) and soybean. To validate these findings, scopolamine-induced memory-impaired rats (2 mg/kg/day for 8 weeks) through the parasympathetic nervous system (PNS) suppression were administered OSM (100, 250, or 750 mg/kg/day) or dextrin (positive control) alongside a high-fat diet. Behavioral, metabolic, and molecular evaluations were conducted. Network analysis identified key targets involved in neuroinflammation including interleukin (IL)-17 and nuclear factor Kappa B (NF- κ B) signaling and neurotransmitter regulation (dopamine synapse), including acetylcholinesterase (AChE), prostaglandin-endoperoxide synthase-2 (PTGS2), glycogen-synthase kinase-3 β (GSK-3 β), solute carrier family 6 member 3 (SLC6A3; dopamine transporter), and plasminogen activator (PLAU). In vivo, OSM at 250 and 750 mg/kg significantly improved memory performance across passive avoidance, Y-maze, Morris water maze, and novel object recognition tests. These improvements were associated with suppressed neuroinflammatory genes (NF- κ B, PTGS2, TNF- α , IL-17, IL-1 β), enhanced cholinergic and dopaminergic balance (reduced AChE activity, increased dopamine), improved glucose metabolism, and upregulated neurotrophic factor expression (brain-derived neurotrophic factor, ciliary neurotrophic factor). Furthermore, OSM enhanced hippocampal insulin signaling (increased phosphorylation of Akt and GSK-3 β), improved intestinal morphology, and increased portal vein butyrate levels, suggesting modulation of the gut-brain axis. In conclusion, OSM enhances memory through multiple central (cholinergic, dopaminergic, neurotrophic) and peripheral (anti-inflammatory, metabolic) mechanisms via activating the gut-brain axis. These findings support

[†]Yu Yue and Hye Jeong Yang Equal contribution.

*Correspondence:

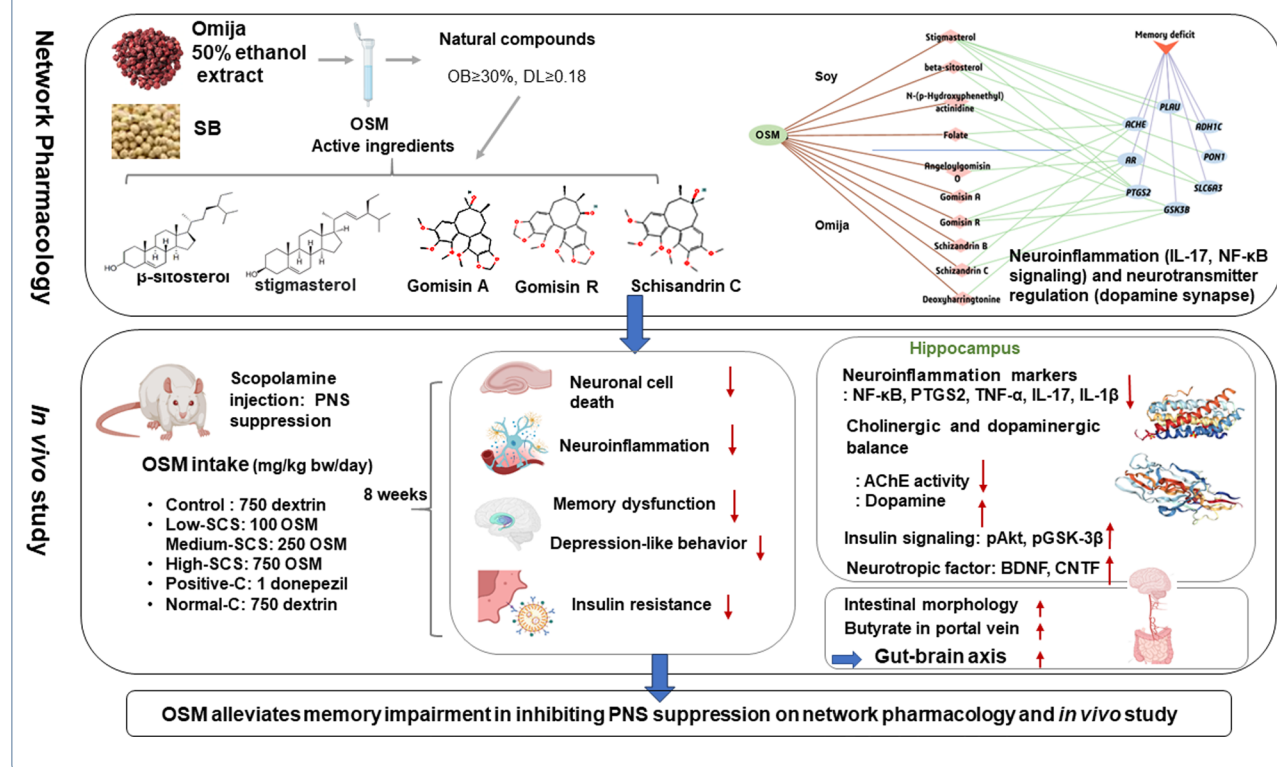
Dai-Ja Jang
djjang@kfri.re.kr
Sunmin Park
smpark@hoseo.edu

Full list of author information is available at the end of the article

OSM (250–750 mg/kg) as a promising candidate for cognitive enhancement potentially by inhibiting PNS suppression, warranting further clinical investigation.

Keywords *Schisandra chinensis* fruit, Soybean, Memory improvement, Network pharmacology, Neuroinflammation, Gut-brain axis

Graphical abstract



Introduction

Memory impairment is a hallmark of neurodegenerative disorders and age-related cognitive decline, posing a significant global health burden with limited therapeutic options [1]. Conventional pharmacological interventions, such as acetylcholinesterase inhibitors (donepezil) and N-methyl-D-aspartate receptor antagonists (memantine), provide transient symptomatic relief and are often associated with adverse effects, including gastrointestinal disturbances, dizziness, and headache [2, 3]. Given the multifactorial pathology of cognitive decline, including cholinergic dysfunction, oxidative stress, and neuroinflammation, there is a need for safer, multi-target therapies derived from natural products [4].

Traditional herbal formulations offer a promising avenue for neuroprotective drug discovery. Among these, Omigalsu is a traditional Korean beverage composed of *Schisandra chinensis* (Turcz.) Baill. (Omija) and soybean (Glycine max). This formulation has been traditionally

used to alleviate diabetic symptoms according to the Donggubogam, a seminal text on traditional Korean medicine. Previous research has demonstrated that both Omija and soybeans possess antioxidant, anti-neuroinflammatory, and adaptogenic properties [5, 6]. Since bioactive compounds from Omija show optimal solubility in 50% ethanol, an Omija 50% ethanol extract and soybean mixture (OSM) was developed to validate its potential for improving memory function. However, the mechanisms underlying the combined effects of these components require further exploration.

Some herbal products exhibit poor bioavailability and may lack direct neuroprotective effects [7]. However, emerging evidence suggests that they can indirectly enhance memory function through modulation of the gut-brain axis (GBA), a bidirectional communication network that plays a critical role in brain health and cognitive performance [8]. Among the key mediators of this axis are short-chain fatty acids (SCFA)—metabolites

produced by gut microbiota—that regulate intestinal metabolism, neurotransmitter synthesis, immune responses, and systemic metabolic functions, all of which are linked to learning and memory processes [9, 10]. Importantly, disruption of intestinal barrier integrity has been associated with cognitive impairment, highlighting the therapeutic relevance of gut-targeted strategies. Despite this, the specific effects of OSM on intestinal morphology, portal vein SCFA concentrations, and goblet cell populations—factors closely tied to memory improvement via the GBA—remain poorly understood.

Scopolamine, a muscarinic acetylcholine receptor antagonist, impairs memory by increasing acetylcholinesterase (AChE) activity and reducing acetylcholine availability, mimicking key aspects of cognitive deficits [11, 12]. Scopolamine-induced cognitive impairment is also associated with increased gut permeability, making it a suitable model for investigating the GBA in neurodegeneration [13]. High-fat diets (> 40% energy from fat) are increasingly prevalent in modern societies and have been independently associated with cognitive decline and memory impairment through mechanisms including neuroinflammation, oxidative stress, and altered neurotransmitter function in animal, clinical, and epidemiological studies [14–16]. The combination of a high-fat diet and scopolamine creates a dual-challenge model that better reflects the multifactorial nature of cognitive impairment, where dietary factors compound cholinergic dysfunction commonly observed in neurodegenerative conditions.

The present study investigated the therapeutic potential of a novel herbal combination, OSM, for memory improvement using an integrative approach that applied network pharmacology to identify compound-target-pathway interactions and predict the multi-target therapeutic mechanisms of Omija and soybean [17]. The predicted mechanisms were validated through *in vivo* studies in a scopolamine and high-fat diet-induced memory impairment model. The experimental validation focused on three key aspects: (1) memory function and associated molecular mechanisms in the hippocampus as suggested by network pharmacology, (2) neuroinflammatory responses and neurotrophic factor expression, and (3) GBA modulation through intestinal morphology and portal vein SCFA levels. This integrated approach aimed to elucidate the multi-target mechanisms of OSM in memory enhancement and establish its therapeutic potential through both central and peripheral pathways.

Materials and methods

Network pharmacology analysis

Bioactive components of Omija and soybean were identified through comprehensive searches of databases

including the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), PubChem, Chemical Database of Bioactive Molecules and Linked Data (ChEMBL), and FooDB. The search encompassed the terms: “*Schisandra chinensis*,” “Wu Wei Zi,” “Omija,” “*Glycine max*,” “soy isoflavones,” and “soybeans”. Compounds were selected based on oral bioavailability ($\geq 30\%$) and drug-likeness (≥ 0.18) criteria. The target genes for these compounds were retrieved from multiple databases, including TCMSP, GeneCards®, and Online Mendelian Inheritance in Man (OMIM®). Alzheimer’s disease (AD)-related genes were identified from DrugBank, GeneCards®, and DISGENET using a relevance score threshold of ≥ 30 . The intersection between OSM-related genes and AD-associated genes was analyzed and visualized using Venn diagrams.

A comprehensive drug-component-disease-target network was constructed using Cytoscape 3.8.2 to visualize the interactions between bioactive compounds and their molecular targets. Network topology analysis identified key therapeutic targets based on connectivity metrics, with genes showing a higher-than-average degree considered critical for memory dysfunction progressing to AD. Protein–protein interaction analysis was performed using the search tool for neighboring genes (STRING) database with a confidence score threshold of ≥ 0.9 . The networks were analyzed using Cytoscape’s Network Analyzer function to identify the key target proteins influenced by both Omija and soybean.

Pathway and functional enrichment analyses were conducted using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) databases to elucidate the biological significance of the identified targets. These analyses mapped molecular interactions and categorized targets based on biological processes, molecular functions, and cellular components, with significance thresholds of $p < 0.05$ for GO and $q < 0.05$ for KEGG pathways. The analysis particularly focused on identifying overlapping and complementary pathways that might explain potential synergistic mechanisms between Omija and soybean in memory function enhancement.

Molecular docking analysis

Molecular docking studies were performed to evaluate the binding interactions between the identified compounds and their protein targets. Three-dimensional protein structures were obtained from the Research Collaboratory for Structural Bioinformatics (RCSB) protein data bank (PDB), selecting those with the highest resolution and quality scores. Protein structures were preprocessed using the Discovery Studio software to remove water molecules and co-crystallized ligands

[18]. AutoDock Tools v1.5.6 was used to prepare protein receptors and ligand molecules in the Protein Data Bank, Partial Charge, and Atom Type (PDBQT) format, followed by molecular docking analysis using AutoDock Vina v1.2 [19]. Binding interactions and poses were visualized and analyzed using Discovery Studio, providing detailed insights into the molecular mechanisms of action.

OSM mixture production

Dried Omija (*Schisandra chinensis*, #HSS-19) and soybeans were cultivated in Mungyeong-si, Korea and the Mungyeong Agricultural Technology Center obtained them. The dried Omija was subjected to ethanol extraction using 50% (v/v) ethanol at 70 °C for 3 h. The Omija extract was concentrated under reduced pressure and lyophilized to obtain a dry powder. Soybeans were processed through the following sequential steps: hydration with purified water, dehulling, steaming, and lyophilization. The final OSM product (#HSS-11) was prepared by homogenizing the lyophilized Omija extract powder with the processed soybean powder at a weight ratio of 5:1 (Omija 50% ethanol extract: soybean powder, w/w based on solid content). The OSM was dissolved in methanol, and lignans quantified using high-performance liquid chromatography (HPLC, Waters Co., Milford, MA, USA) with a Gemini C18 column (150×2.0 mm; 5 µm; Phenomenex, Torrance, USA), 280 nm detection, 0.8 mL/min flow rate, and a 55-min acetonitrile gradient. Reference compounds (schizandrin, daidzin, and genistin) with >98% purity were sourced from Sigma-Aldrich Co. (St. Louis, MO, USA).

Animals and experimental design

The sample size was calculated using G*Power software (version 3.1.9.7, Universität Düsseldorf, Germany) based on a one-way ANOVA design. Parameters included a medium effect size ($f=0.4$), a significance level (α) of 0.05, and a statistical power ($1-\beta$) of 0.80. The analysis indicated that a minimum of 10 animals per group was sufficient to detect statistically significant differences among groups. No specific inclusion or exclusion criteria for animals or data points were established a priori. All animals initially enrolled in the study completed the experimental procedures without complications. Consequently, no animals or data points were excluded at any stage of the experiment or analysis. Each experimental group consisted of 10 rats ($n=10$), and all animals were included in the final statistical analysis.

All experimental protocols adhered to the National Institutes of Health (NIH) Guidelines for Animal Care (Institute of Laboratory Animal Resources, 1996) and received approval from Hoseo University's Animal Care

and Use Committee (HSUIACUC-24-009). Sixty male Sprague–Dawley rats (8 weeks old, 189 ± 8.4 g) were obtained from Daehan Bio Inc. (Eum-Sung, Korea) and individually housed under controlled environmental conditions (23 °C, 60% humidity, and 12-h light/dark cycle). All animals were maintained on a high-fat diet (43% energy from lard, 37% carbohydrates, 20% proteins) based on a modified, polyphenol-free AIN-93 formula (CJ Co., Seoul, Korea), with added vitamins and minerals. Food and water were provided ad libitum throughout the experimental period.

After a 1-week acclimatization period, the animals were randomly assigned to six experimental groups ($n=10$ per group). Memory impairment was induced by daily intraperitoneal injections of scopolamine hydrobromide (2 mg/kg body weight; Sigma-Aldrich, St. Louis, MO, USA) for 8 weeks in all groups except the Normal-control (Normal-C) group. The combination of a high-fat diet and scopolamine was designed to create a dual-challenge model that better reflects the multifactorial nature of cognitive impairment, where dietary factors compound cholinergic dysfunction commonly observed in neurodegenerative conditions. The Normal-C group received equivalent volumes of saline via intraperitoneal injection and served as the control for the high-fat diet condition without cholinergic dysfunction. The OSM mixture was administered orally at designated doses daily.

The experimental groups were as follows: Normal-C (saline injection+750 mg/kg dextrin), Control (scopolamine+750 mg/kg dextrin), Low-OSM (scopolamine+100 mg/kg OSM), Medium-OSM (scopolamine+250 mg/kg OSM), High-OSM (scopolamine+750 mg/kg OSM), and Positive-control (Positive-C; scopolamine+1 mg/kg donepezil). Randomization was performed using a computer-generated random sequence, ensuring unbiased allocation. To minimize confounding variables, animals were housed individually, with cage positions rotated weekly. Behavioral testing and sample collection were performed in a randomized order. Blinding was applied during outcome assessment and data analysis, with assessors unaware of group assignments. Each diet formulation was labeled with a coded number, and the animal care staff were blinded to the contents and treatment allocation of each diet. Body weight and food intake were measured weekly to calculate food efficiency for weight gain.

Memory assessment

Memory function was assessed using a series of behavioral tests. On testing days, scopolamine was administered 50 min prior to the initiation of each behavioral assessment. The passive avoidance test utilized a light–dark shuttle box where the rats underwent two 8-h acquisition

trials with electric stimulation (75 V, 0.2 mA, 50 Hz). Retention was tested after 16 h by measuring the latency to enter the dark chamber, with a maximum cutoff time of 600 s. Spontaneous alternation behavior was assessed using a Y-maze (50.5×20×20 cm) during 8-min sessions, with performance calculated as the percentage of correct alternations over total arm entries.

The novel object recognition test began with 3 days of habituation, during which the rats explored the testing arena for increasing durations (5–20 min daily). On the fourth day, the rats explored two identical objects for 10 min, followed by a 1-h interval before testing. During the test phase, one object was replaced with a novel item, and exploration was recorded for 5 min. The recognition index was calculated based on the time spent exploring the new object relative to the total exploration time.

Spatial memory was evaluated using the Morris water maze test over 5 days. The rats learned the platform location in Zone 5 during the first 2 days, with the final assessment on day 5 measuring latency, visit frequency, and duration in the target zone, using a maximum cutoff time of 600 s.

Oral glucose tolerance (OGTT) and intraperitoneal insulin tolerance tests (IPITT)

Overnight-fasted rats received an oral dose of glucose (2 g/kg body weight), and blood glucose levels were measured at 10-min intervals up to 90 or 120 min using a commercial glucose assay kit (Asan Pharmaceuticals, Korea). Serum insulin levels were determined at 0, 20, 40, and 90 min post-glucose administration using an ultra-sensitive insulin ELISA kit (Crystal Chem, USA). On the following day, after a 6-h fasting period, rats were intraperitoneally injected with insulin (1 IU/kg body weight). Blood glucose levels were then measured every 15 min for 90 min to assess insulin sensitivity.

Sample collection and biochemical analysis

Following the completion of behavioral tests and a 16-h fast, samples were collected under ketamine/xylazine anesthesia. Blood was drawn from the portal vein and inferior vena cava, while brain tissue was collected from ten rats per group, with six samples frozen at −70 °C and four fixed in 4% paraformaldehyde. Cecal contents were collected and stored at −70 °C, and epididymal and retroperitoneal fat masses were measured. Serum liver damage index including aspartate aminotransferase (AST) and alanine aminotransferase (ALT), were measured in colorimetry kits (Asan Pharmaceuticals, Seoul, Korea).

Serum inflammatory markers (tumor necrosis factor [TNF]- α , interleukin [IL]-1 β) were analyzed using enzyme-linked immunoassay (ELISA), while the hippocampal tissue was lysis with RIPA buffer for

measurements of triglycerides, cholesterol, lipid peroxides, glycogen, AChE activity using corresponding kits (Elabscience, Huston, TX, USA) and phosphorylation of Akt and GSK-3 β using Western blot. The total RNA of the hippocampus was extracted with Trizol and cDNA was made from it. mRNA expression analysis for ciliary neurotrophic factor (*CNTF*), brain-derived neurotrophic factor (*BDNF*), *TNF- α* , and *IL-1 β* was performed using real-time PCR with β -actin as a reference gene.

Equal amounts of protein (20–30 μ g) in the hippocampal lysate were separated by SDS-PAGE and transferred onto PVDF membranes. Membranes were blocked with 5% non-fat milk in TBS-T and incubated overnight at 4 °C with primary antibodies against phosphorylated Akt (pAkt, Ser473) and phosphorylated GSK-3 β (pGSK-3 β , Ser9). After washing, membranes were incubated with HRP-conjugated secondary antibodies and visualized using enhanced chemiluminescence (ECL). Band intensities were quantified using ImageJ and normalized to total Akt, total GSK-3 β , or β -actin as loading controls.

Histological analysis

The brain and intestinal tissues underwent histological examination following standard procedures. The brain Sects. (10 μ m) were stained with cresyl violet to assess damage to the hippocampal CA1 region. Large intestine Sects. (5 μ m) were processed using both hematoxylin and eosin (H-E) staining for structural analysis and Alcian Blue/Periodic Acid-Schiff (AB-PAS) staining for mucin-producing goblet cells. The histological evaluation included measurements of mucosal length, crypt dimensions, cell damage scoring on a 0–5 scale, and quantification of goblet cells. All microscopic analyses were performed using a Zeiss Axiovert microscope with the Dilation X-ray Imager (DIXI) Imaging solution.

Serum SCFA analysis

Serum short-chain fatty acid concentrations were determined using gas chromatography. Samples were processed by adding acid ethanol and the supernatants were collected after centrifugation at 15,000 rpm. The SCFA in the supernatants was measured using a Clarus 680 GAS chromatograph (PerkinElmer) equipped with an Elite-Free Fatty Acid Phase (FFAP) capillary column (30 m×0.25 mm×0.25 μ m). Acetate, propionate, and butyrate were quantified using external standards from Sigma Co.

Statistical analysis

Statistical analyses were performed using SAS version 7 (Cary, NC, USA). Data are presented as means±standard deviations. The normality of data distribution was assessed using univariate analysis, and all datasets met

the assumption of normality. Group comparisons were conducted using one-way ANOVA, followed by Tukey's post hoc test for multiple comparisons when significant main effects were observed. A p -value of <0.05 was considered statistically significant.

Results

Network pharmacology analysis of Omija and soybean

Compound identification and target gene selection.

We identified 130 bioactive compounds in Omija and 116 in soybean through comprehensive database mining. After filtering based on pharmacokinetic parameters (oral bioavailability $\geq 30\%$ and drug-likeness ≥ 0.18), we selected eight compounds from Omija, including deoxy-harringtonine, gomisins A, R, schizandrin B, and C, along with 13 compounds from soybean, such as stigmasterol, beta-sitosterol, and daidzein-4,7-di-glucoside (Supplementary Table S1).

The analysis of the selected compounds using multiple databases identified 48 unique target genes for Omija and soybean combined (Supplementary Table S2). We compared these targets against 358 memory impairment-related genes obtained. This comparison revealed eight overlapping targets (Fig. S1A).

Network analysis and protein–protein interaction (PPI) analysis. The compound–target network analysis (Fig. 1a) revealed that gomisins R from Omija and both stigmasterol and beta-sitosterol from soybean demonstrated the highest connectivity. Each herb showed distinct targeting patterns: Omija and soybean targeted the acetylcholine esterase (*ACHE*) prostaglandin-endoperoxide synthase 2 (*PTGS2*), glycogen synthase kinase-3 β (*GSK-3 β*), and androgen receptor (*AR*) genes, while soybean additionally affected the solute carrier family 6 member 3 (*SLC6A3*), plasminogen activator (*PLAU*, urokinase), alcohol dehydrogenase 1C (class I), gamma polypeptide (*ADH1C*), and paraoxonase 1 (*PON1*) genes.

The PPI network analysis identified *PTGS2* and *ACHE* as central hub genes in the network. *PTGS2* showed significant interactions with *AR*, *GSK3B*, and *PLAU*, while *ACHE* demonstrated strong connections with *PON1* and *SLC6A3*, indicating their crucial roles in the therapeutic effects (Fig. 1b, S1B).

Functional enrichment analysis. GO enrichment analysis highlighted significant enrichment in endopeptidase activity, catecholamine binding, nuclear receptor activity (involving ESR-1), AR, and nuclear receptor subfamily 3 group C member 2 [NR3C2]), G-protein-coupled neurotransmitter receptor activity (including *ACHE*, cholinergic receptor muscarinic 1 and 2 [CHRM1], and CHRM2), and protein kinase A catalytic subunit binding (adjusted $P=1 \times 10^{-4}$)

(Fig. 1c). The KEGG pathway analysis revealed significant involvement in the neurodegeneration pathways, IL-17 signaling, nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) signaling, and dopaminergic synapse modulation (Fig. 1d; $P=0.049$). The signaling pathways of NF- κ B and IL-17 are shown (Fig. S1c, d). Both signaling pathways were involved in releasing inflammatory markers, such as TNF- α and IL-1 β . These findings collectively suggest that Omija and soybean may work synergistically to enhance memory function through multiple mechanisms, including cholinergic neurotransmission regulation, neuroinflammation modulation, and neuroprotection.

Molecular docking. Among the proteins encoded by these genes, *PTGS2*, *ACHE*, and *GSK3B* lowered binding energy (< -9 kcal/mol) with Omija and soybean including schizandrin B, schizandrin A, gomisins C, stigmasterol, beta-sitosterol, folate (Table 1). The binding of schizandrin B and gomisins R with *PTGS2*, schizandrin C and gomisins R with *GSK-3 β* , and gomisins A and schizandrin C with *AChE* are shown in Fig. 1e, f, and S1E, respectively.

Bioactive compound content in Omija and soybean

The retention times and peaks of the bioactive compounds from HPLC analysis are shown in Fig. S2. The quantities of the compounds detected per 1 g OSM were: 8.83 ± 0.045 mg schizandrin; 0.15 ± 0.0 mg daidzein; and 0.24 ± 0.006 mg genistin.

OSM effect on energy metabolism and liver function in vivo

Body weights at the end of the interventions and weight gain during the interventions were significantly lower in the Normal-C compared to the Control group. There was a significant reduction in weight in the OSM-treated groups and the Positive-C group ($P < 0.05$; Table 2). Food efficiency showed corresponding patterns, being higher in the Normal-C versus the Control group, and the treatments with OSM prevented this decrease. There was no significant difference in the visceral fat mass between the Control and Normal-C groups. The visceral fat content was significantly reduced after the treatment with Medium- and High-OSM and the Positive-C group ($P < 0.05$; Table 2).

Serum ALT levels were significantly elevated in the Control group versus the Normal-C ($P < 0.05$), while AST levels remained unchanged between the groups. Both liver damage markers decreased dose-dependently with the OSM treatment, and there was no evidence of hepatotoxicity (Table 2).

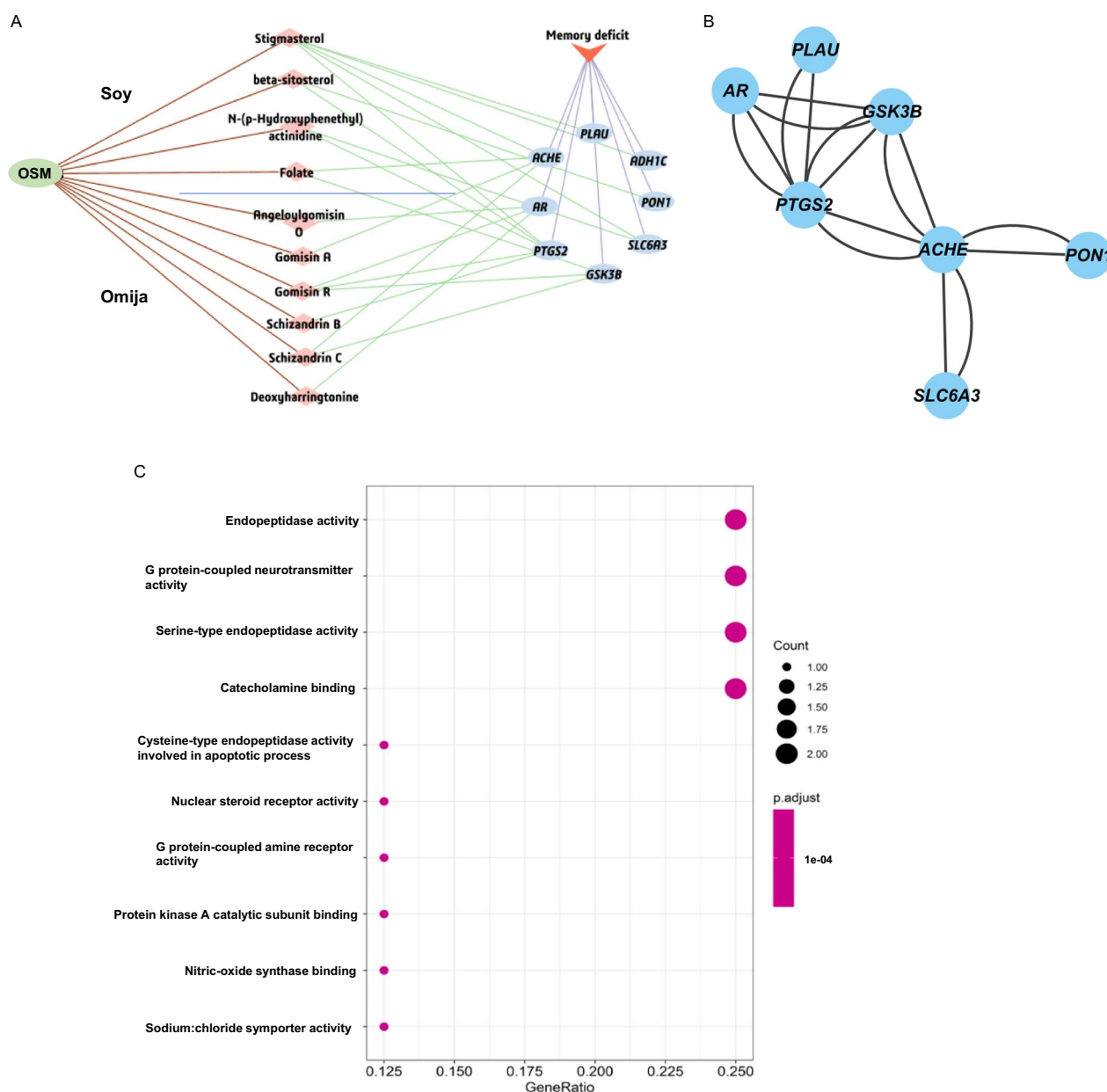


Fig. 1 Network pharmacology between the bioactive compounds of Omija (*Schisandra chinensis* (Turcz.) Baill) and soybeans and the molecular targets of memory impairment. **a** Network between the bioactive compounds of Omija and soybeans (pink diamonds) and the molecular targets (blue eclipses) linked to memory deficit. The lines indicate the interaction between the Omija bioactive compounds and the molecular targets of memory deficit. Network pharmacology analysis was conducted using Cytoscape-3.8.2, National Resource for Network Biology, National Institute of Health, Bethesda, Maryland, USA (<http://www.cytoscape.org>). **b** Interaction of molecular targets linked to memory deficit. **c** The interaction counts of Omija and the soybean ingredients with molecular targets in the Gene ontology (GO) enrichment analysis. **d** The interaction counts of Omija and soybean ingredients with molecular targets in the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis. **e** Molecular docking between the molecular target of acetylcholinesterase (AChE) with schizandrin B. **f** Molecular docking between the molecular target of glycogen synthase kinase-3 β (GSK-3 β) with gomisin R. PTGS2, prostaglandin-endoperoxide synthase 2 (known as Cyclooxygenase-2); GSK3B, glycogen synthase kinase-3-Beta; ACHE, acetylcholinesterase; PON1, paraoxonase 1; SLC6A3, solute carrier family 6 member 3 (known as dopamine transporter); AR, androgen receptor; PLAU, plasminogen activator, urokinase; IL-17, interleukin 17; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells

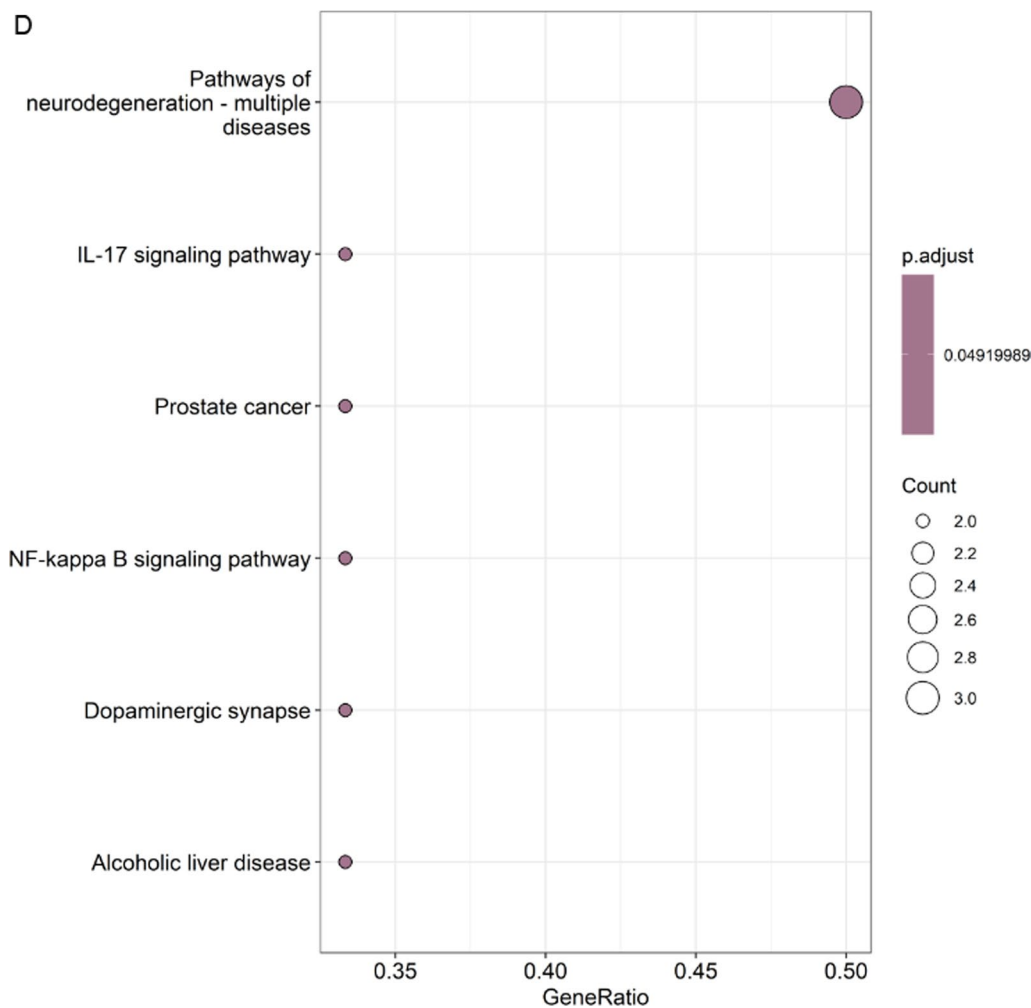


Fig. 1 continued

OSM effect on glucose metabolism

Fasting serum glucose and insulin levels were significantly elevated in the Control group compared to the Normal-C ($P < 0.05$; Table 2). The OSM treatments reduced glucose levels dose-dependently, while only high-dose OSM significantly affected insulin levels. Insulin resistance measured by the Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) was significantly higher in the Control and Positive-C groups, with OSM at medium and high doses reducing it to levels comparable to levels seen in the Normal-C group (Table 2).

In the OGTT, serum glucose levels were significantly higher in the Control and the Positive-C groups compared to the Normal-C group ($P < 0.05$; Fig. 2a). The High-OSM group showed significantly lower glucose levels compared to the Control group. Treatments with medium and high OSM doses significantly reduced glucose AUC compared to the Control group in both the

early phase (0–40 min) and the late phase (40–120 min) (Fig. S3a).

Serum insulin levels during the OGTT were consistently higher in the Control group compared to the Normal-C group. Treatments with medium and high doses of OSM normalized insulin levels at 40 and 90 min, while no significant differences were observed at 20 min (Fig. 2b). Insulin levels in both the early-phase (0–20 min) and late-phase (20–90 min) showed that treatment with high-dose OSM achieved levels equivalent to the Normal-C group (Fig. S3b).

In the IPITT of the rats with 6-h fasting, serum glucose levels were significantly elevated in the Control and Positive-C groups compared to the Normal-C group ($P < 0.05$; Fig. 2c). The Medium- and High-OSM groups showed significantly improved insulin sensitivity, with high-dose treatment achieving glucose levels similar to the Normal-C group. The High-OSM group maintained significantly lower glucose levels throughout the test period. Both the

E

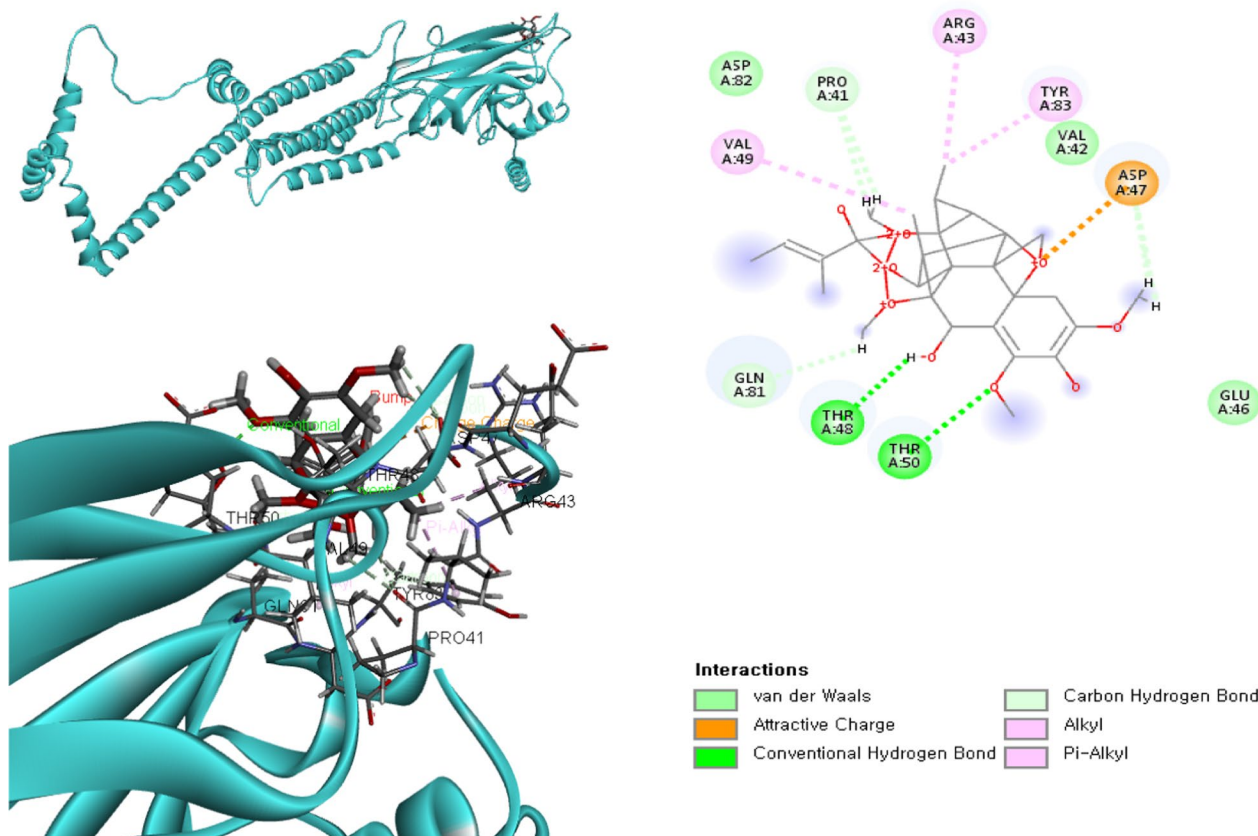


Fig. 1 continued

early-phase (0–30 min) and late-phase (30–90 min) glucose levels confirmed that the treatment with high-dose OSM demonstrated the most pronounced improvement in insulin sensitivity (Fig. S3c).

OSM effect on hippocampal neuronal cell death and memory function

Cresyl violet staining revealed significantly increased neuronal cell death in the hippocampal CA1 region of the Control group compared to the Normal-C ($P < 0.05$). The OSM treatment dose-dependently reduced neuronal cell death, and the High-OSM and Positive-C groups achieved levels comparable to the Normal-C (Fig. 3a).

In the hippocampus, the phosphorylation of Akt and GSK-3 β linked to insulin signaling was attenuated, and it was potentiated in the High-OSM group, better than in the Normal-C group (Fig. 3b). However, their phosphorylation did not differ between the Control and Positive-C groups. By contrast, the phosphorylation of NF- κ B was much higher in the Control group than in the Normal-C group and decreased in the Positive-C and High-OSM groups. The phosphorylation observed in the

High-OSM group was similar to that of the Normal-C group (Fig. 3b).

Memory function was assessed using multiple behavioral tests. In the passive avoidance test, the initial latency to enter the dark chamber was similar across all groups in the first trial (Fig. 4a). At 8- and 24-h post-training (second and third trials), all groups showed increased latency compared to the first trial. Notably, the Positive-C, Medium-, and High-OSM groups demonstrated 1.5–1.7-fold higher latencies than the Control group in the third trial, reaching levels similar to the Normal-C (Fig. 4a).

In the Morris water maze test, while platform zone (zone 5) duration showed no significant difference between the Control and Normal-C groups, the Medium- and High-dose OSM treatments significantly increased this duration ($P < 0.05$; Fig. 4b). The platform zone visit frequency was significantly higher in the Positive-C and Normal-C groups (1.8 and 2.1-fold, respectively) compared to the Control ($P < 0.05$; Fig. 4b). All the OSM treatments increased visit frequency above the levels seen in the Control groups. Latency to first platform zone entry was significantly shorter in the Normal-C

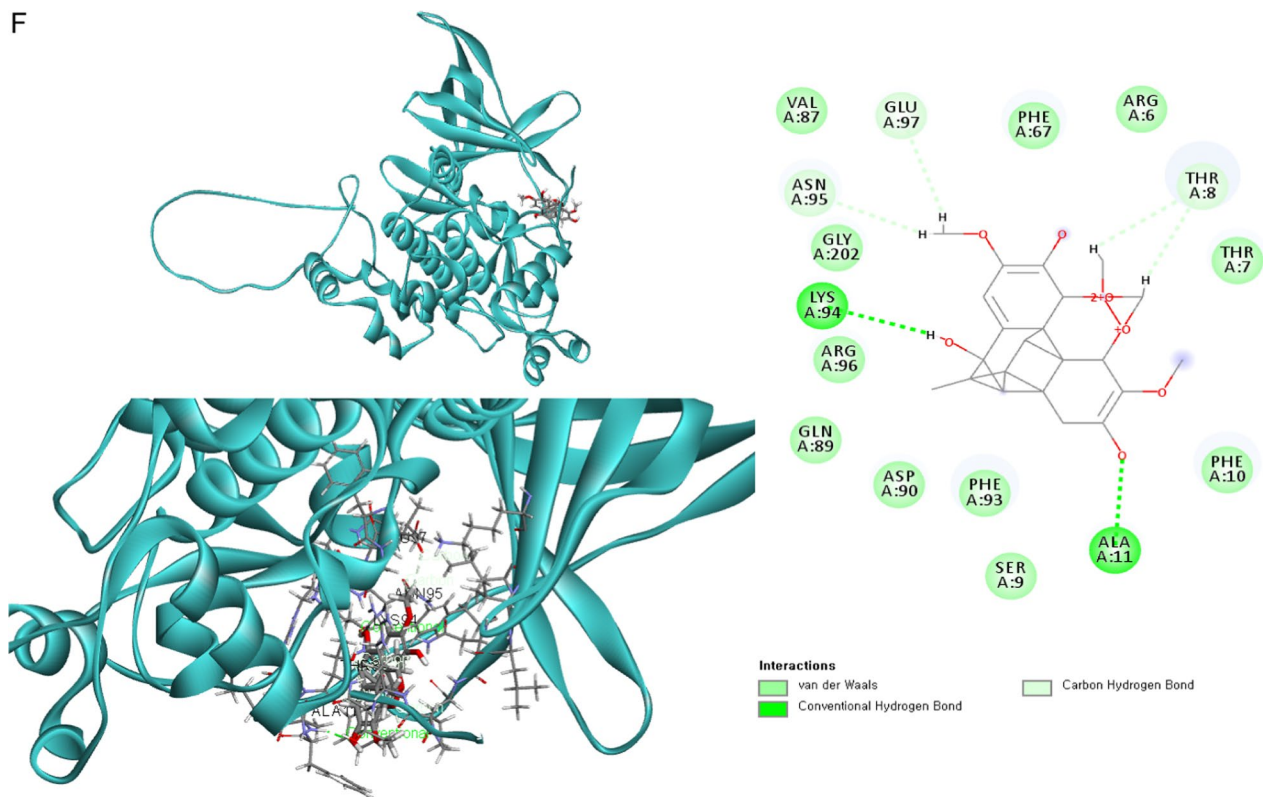


Fig. 1 continued

group versus the Control and Positive-C groups, with the High-OSM group showing the shortest latency (Fig. 4b).

Y-maze performance showed reduced spontaneous alternation in the Control group compared to the Normal-C group ($P < 0.05$; Fig. 4c), and treatment with high dose OSM restored performance to Normal-C levels. Novel object recognition testing revealed a 1.5-fold reduction in the recognition index in the Control group

versus the Normal-C group ($P < 0.05$), while the Positive-C and High-OSM groups maintained indices similar to the Normal-C (Fig. 4c).

OSM effect on hippocampal oxidative stress and neuroinflammation

Associated with Memory Deficits. Markers of systemic inflammation, serum $\text{TNF-}\alpha$, and $\text{IL-1}\beta$, were significantly elevated in the Control group compared to the Normal-C ($P < 0.05$; Table 3). Treatment with Medium- and High-dose OSM significantly reduced these inflammatory markers. While hippocampal triglyceride content showed no significant differences among groups, hippocampal cholesterol levels were significantly higher in the Control group compared to the Normal-C group ($P < 0.05$; Table 3). The levels were further elevated in the Positive-C group. Treatment with OSM reduced them below the Control group levels. Hippocampal glycogen content was lower in the control than in the Normal-C and Medium- and High-OSM increased it ($P < 0.05$; Table 3).

AChE activity was markedly elevated (5.2-fold) in the Control group versus the Normal-C group ($P < 0.05$; Table 3). The activity was significantly lower in the Positive-C and Medium- and High-OSM groups. However,

Table 1 The binding energy between the target protein and primary components in 50% ethanol extract of Omija and soybean mixture (OSM)

	PTGS2	PON1	GSK3B	ADHG1	AChE
Angeloylgomisin-O	−9	−6.6	−8.8	−7.1	−6.5
Gomisin-C	−9.3	−7.9	−9.4	−8.8	−7.8
Schizandrin-B	−9.2	−8.2	−8.5	−6.7	−9
Schizandrin-A	−9.2	−8.3	−9.8	−9.1	−9.1
beta-Sitosterol	−9.5	−7.6	−8.1	−6.8	−6.2
Stigmasterol	−9.3	−7.4	−8.5	−7.7	−9.3
Folate	−8.8	−6.9	−8	−8.5	−10.1

PTGS2, Prostaglandin-Endoperoxide Synthase 2; PON1, Paraoxonase 1; GSK3B, Glycogen Synthase Kinase 3-Beta; ADHG1, Alcohol Dehydrogenase 1G (Class I), Gamma Polypeptide; AChE, Acetylcholinesterase

Table 2 Body weight and glucose metabolism at the end of experimental periods

	Control	Positive-C	Low-OSM	Medium-OSM	High-OSM	Normal-C
Final body weight (g)	340.4 ± 13.5 ^a	299.2 ± 10.5 ^b	329.2 ± 8.62 ^a	322.6 ± 7.96 ^b	299.4 ± 12.9 ^b	325.6 ± 10.1 ^b
Body weight gain (g)	112.4 ± 7.62 ^a	84 ± 9.91 ^c	94.6 ± 9.40 ^{bc}	92.3 ± 10.6 ^b	85.8 ± 11.9 ^{bc}	100.6 ± 5.87 ^b
Food efficiency	6.8 ± 0.73 ^a	5.3 ± 0.53 ^b	5.8 ± 0.85 ^b	5.1 ± 0.57 ^{bc}	4.9 ± 0.86 ^c	5.9 ± 0.56 ^b
Visceral fat (%)	2.82 ± 0.19 ^a	1.97 ± 0.1 ^c	3.04 ± 0.25 ^a	2.48 ± 0.16 ^b	2.4 ± 0.22 ^b	2.98 ± 0.21 ^a
Serum ALT (U/L)	49.2 ± 2.6 ^a	48.3 ± 3.2 ^a	44.3 ± 3.34 ^b	43.7 ± 2.89 ^b	39 ± 2.08 ^c	39 ± 2.09 ^c
Serum AST (U/L)	74.9 ± 4.43 ^a	74.1 ± 1.04 ^a	73.8 ± 3.22 ^{ab}	71.8 ± 1.8 ^b	63.2 ± 3.54 ^c	75.8 ± 4.17 ^a
Fasting serum glucose (mg/dL)	134 ± 9.3 ^a	128 ± 7.1 ^{ab}	121 ± 10.1 ^b	102 ± 8.3 ^c	108 ± 5.3 ^c	117 ± 10.4 ^b
Fasting serum insulin (ng/ml)	0.62 ± 0.04 ^a	0.59 ± 0.04 ^a	0.6 ± 0.08 ^a	0.51 ± 0.06 ^b	0.36 ± 0.04 ^c	0.4 ± 0.05 ^c
HOMA-IR	7.59 ± 0.97 ^a	6.78 ± 0.83 ^a	6.59 ± 1.18 ^a	4.76 ± 0.89 ^b	3.52 ± 0.57 ^c	4.24 ± 0.91 ^{bc}

Food efficiency gain/food intake; Visceral fat = (epididymal fat + retroperitoneal fat)/body weight; HOMA-IR, Homeostasis model assessment for insulin resistance.

Male rats had intraperitoneal injections of scopolamine (2 mg/kg body weight/day) at 30 min after daily feeding of *Omija* (50% ethanol extract; *Schizandra chinensis* BAALL.) plus soybean mixture (OSM) or 1.2 mg donepezil (Positive-control) per kg body weight per day through feeding needle for 6 weeks. The normal-control rats had saline injections without scopolamine

Values represent means ± standard deviations (n = 10)

^{a,b,c} Means on the same row with different superscript letters indicated a significant difference among the groups by Tukey test at P < 0.05

(See figure on next page.)

Fig. 2 Serum glucose and insulin concentrations after administration of *Omija* (*Schizandra chinensis* (Turcz.) Baill.) and soybean mixture (OSM) during oral glucose tolerance tests (OGTT) and intraperitoneal insulin tolerance test (IPITT). **a** Changes in serum glucose concentrations 120 min after the oral administration of 2 g glucose/kg body weight (n = 10). **b** Changes in serum insulin concentrations during the OGTT (n = 10). **c** Changes in serum glucose concentrations 90 min after the intraperitoneal injection of 1 IU insulin/kg body weight during IPITT (n = 10). Each bar represents Means ± standard deviation (n = 10). The scopolamine-induced amnesic rats were fed high-fat diets with Control (750 mg dextrin/kg bw), Low-OSM (100 mg OSM/kg bw), Medium-OSM (250 mg OSM/kg bw), High-OSM (750 mg OSM/kg bw), and positive-control (Positive-C; 1 mg/kg donepezil). Saline-injected (no scopolamine) rats fed high-fat diets with 750 mg dextrin/kg bw were Normal-C. * Significantly different among the groups at each time point at P < 0.05

these levels remained higher than that of the Normal-C group. Lipid peroxidation and superoxide dismutase (SOD) activity were significantly increased in the Control group compared to the Normal-C (P < 0.05; Table 3). These were lower in the Positive-C and the OSM groups. Treatment with high-dose OSM lowered the activity to below the levels seen in the Normal-C group. Since network pharmacology indicated that the dopamine transporter, SLC6A3, was linked to OSM, the dopamine contents in the hippocampus were measured. They were lower in the Control than the Normal-C group and increased in the Positive-C and the Medium-OSM and High-OSM groups (Table 3).

The quantity of hippocampal neuroinflammatory modulators, including *PTGS-2*, *TNF-α*, *IL-17*, and *IL-1β* is suggestive of the impact of OSM on memory impairment. Hippocampal mRNA expression of *PTGS-2* involved in prostaglandin synthesis to modulate neuroinflammation and synaptic plasticity (Table 3) in the hippocampus was higher in the Control than in the Normal-C group. The expression was lower in the Positive-C, Medium-OSM, and High-OSM groups (Table 3). Hippocampal *TNF-α* mRNA expression was significantly elevated (1.3-fold) in the Control group compared to the Normal-C (P < 0.01).

Medium- and High-OSM groups reduced *TNF-α* to levels comparable to the Normal-C (P < 0.05; Table 3). Hippocampal *IL-1β* mRNA in the Control group was also higher than in the Normal-C, with Medium- and High-OSM treatments significantly reducing their expression. Conversely, neurotrophic factors *BDNF* and *CNTF* showed significantly reduced mRNA expression in the Control group compared to the Normal-C (P < 0.05; Table 3). The expression of these neurotrophic factors significantly increased in the Positive-C and OSM groups relative to the Control group (Table 3).

OSM effect on large intestinal morphology and portal vein SCFA

Histological analysis of large intestine morphology revealed significant structural alterations in the Control group compared to the Normal-C group. Treatment with medium- and high-doses of OSM prevented these morphological changes (Fig. 5a, b). Specifically, the length of the colonic epithelium was significantly reduced in the Control group versus the Normal-C group (P < 0.05; Fig. S4B), while in the Positive-C group and after treatment with medium and high doses of OSM, the lengths were maintained comparable to that of the Normal-C group

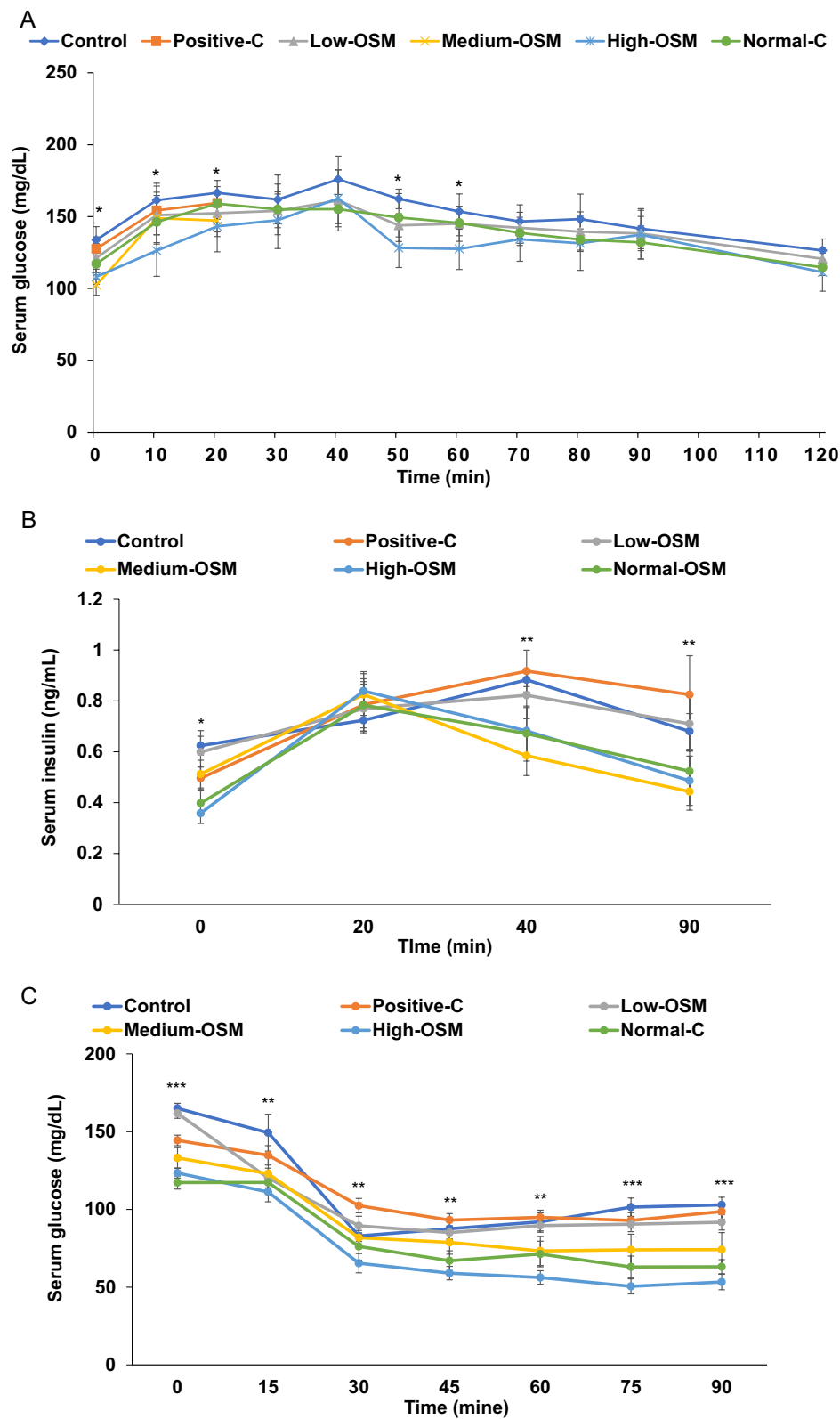


Fig. 2 (See legend on previous page.)

(Fig. S4B). The colonic epithelium width showed a significant increase in the Control group compared to the Normal-C ($P < 0.05$), while treatment with all doses of OSM significantly reduced the width. Crypt height was significantly diminished in the Control group versus the Normal-C group ($P < 0.05$), but this reduction was prevented with high OSM treatment (Fig. S4B).

Goblet cell counts, indicative of mucin secretion capacity, were significantly decreased in the Control group compared to the Normal-C group ($P < 0.05$). All OSM treatments restored counts to levels comparable to the Normal-C (Fig. 5a, b). The number of damaged cells in the large intestine was significantly elevated in the Control group versus the Normal-C ($P < 0.05$), with the OSM treatment significantly inhibiting this increase (Fig. 5a, b).

Analysis of the SCFA in the portal vein revealed that the serum acetate concentrations were similar between the Control and the Normal-C groups. The acetate levels were higher in the Positive-C group, while treatment with medium- and high-dose OSM significantly reduced them compared to the Control ($P < 0.05$; Fig. 5c). Serum propionate concentrations showed no significant differences among groups. Serum butyrate levels were significantly lower in the Control group compared to the Normal-C group ($P < 0.05$; Fig. 5c), with the OSM treatments increasing levels dose-dependently, reaching the maximum concentration in the medium-dose OSM group.

Discussion

This study uniquely integrated network pharmacology analysis with experimental validation to elucidate the mechanisms of OSM in treating memory deficits. Network analysis revealed distinct targeting patterns: Omija and soybean targeted the *ACHE*, *PTGS2*, *GSK-3 β* , and *AR* genes, while soybean additionally affected the *SLC6A3*, *PLAU*, *ADH1C*, and *PON1* genes. In the molecular docking analysis, *PTGS2*, *GSK3B*, and *AChE* demonstrated lower binding energy with the gomisins C, schizandrin-B, and schizandrin-A compounds of Omija while *PTGS2* and *AChE* showed lower binding energy with β -sitosterol, stigmasterol, and folate of soybean. These computational

predictions were experimentally validated in scopolamine-treated rats, where we observed significant alterations in three key pathways: elevated hippocampal *AChE* activity (5.19-fold), increased *PTGS2* mRNA expression (1.61-fold), and decreased dopamine content linked to *SLC6A3* (1.53-fold). The OSM treatment effectively normalized these parameters in a dose-dependent manner. Pathway enrichment analysis identified neuroinflammation, mainly through IL-17 and NF- κ B signaling, as a central mechanism. This was confirmed by elevated hippocampal NF- κ B signaling and inflammatory markers (TNF- α and IL-1 β) in the scopolamine-treated rats, which were significantly attenuated by OSM treatment. This is the first study to demonstrate that the synergistic effects of the two components of OSM operate through distinct. However, complementary molecular targets provide a novel therapeutic approach to improve memory through the simultaneous modulation of the cholinergic, dopaminergic, and inflammatory pathways.

Network pharmacology has emerged as a valuable tool for predicting the therapeutic targets of traditional herbal medicines, with recent studies demonstrating its utility in identifying the mechanism of action of single herbs in memory improvement [20]. Our study advances this approach by successfully predicting and validating the complementary mechanisms of a herbal combination, OSM. The molecular docking results provided mechanistic support for our network pharmacology predictions, with the Omija compounds (gomisin C, schizandrin B, and schizandrin A) showing strong binding affinity to *PTGS2*, *GSK3 β* , and *AChE*, while the soybean compounds (β -sitosterol, stigmasterol, and folate) demonstrated significant interactions with *PTGS2* and *AChE*. These findings suggest that the bioactive compounds in both herbs can effectively modulate the identified target proteins.

While previous network analyses of Omija have identified its effects on cholinergic transmission [21, 22] and studies of soybean have highlighted its anti-inflammatory properties [23], our integrated analysis uniquely revealed their synergistic potential through distinct molecular targets. This synergistic effect was validated in

(See figure on next page.)

Fig. 3 Neuronal cell survival and insulin signaling in the hippocampus post-OSM intervention. **a** Neuronal cells stained by cresyl violet in the hippocampus ($n = 5$). The red scale bar indicated 50 μ m in the upper panels and 10 μ m in the lower panel. The red square box in the upper image panel indicates the part of the hippocampus enlarged to show in the lower panel. **b** Insulin signaling (phosphorylation of Akt and GSK-3 β) and phosphorylation of NF- κ B ($n = 4$). Each bar represents Means $\pm$ standard deviation. a, b Different letters on the bars indicate significant differences at $P < 0.05$. Omija (50% ethanol extract; Schizandra chinensis BAALL.) plus soybean mixture (OSM). The scopolamine-induced amnesic rats were fed high-fat diets with Control (750 mg dextrin/kg bw), Low-OSM (100 mg OSM/kg bw), Medium-OSM (250 mg OSM/kg bw), High-OSM (750 mg OSM/kg bw), and positive-control (Positive-C; 1 mg/kg donepezil). Saline-injected (no scopolamine) rats fed high-fat diets with 750 mg dextrin/kg bw were Normal-C. Akt, protein kinase B; GSK-3 β glycogen synthase kinase 3 β ; NF- κ B, nuclear factor kappa B-light-chain-enhancer of activated B cells

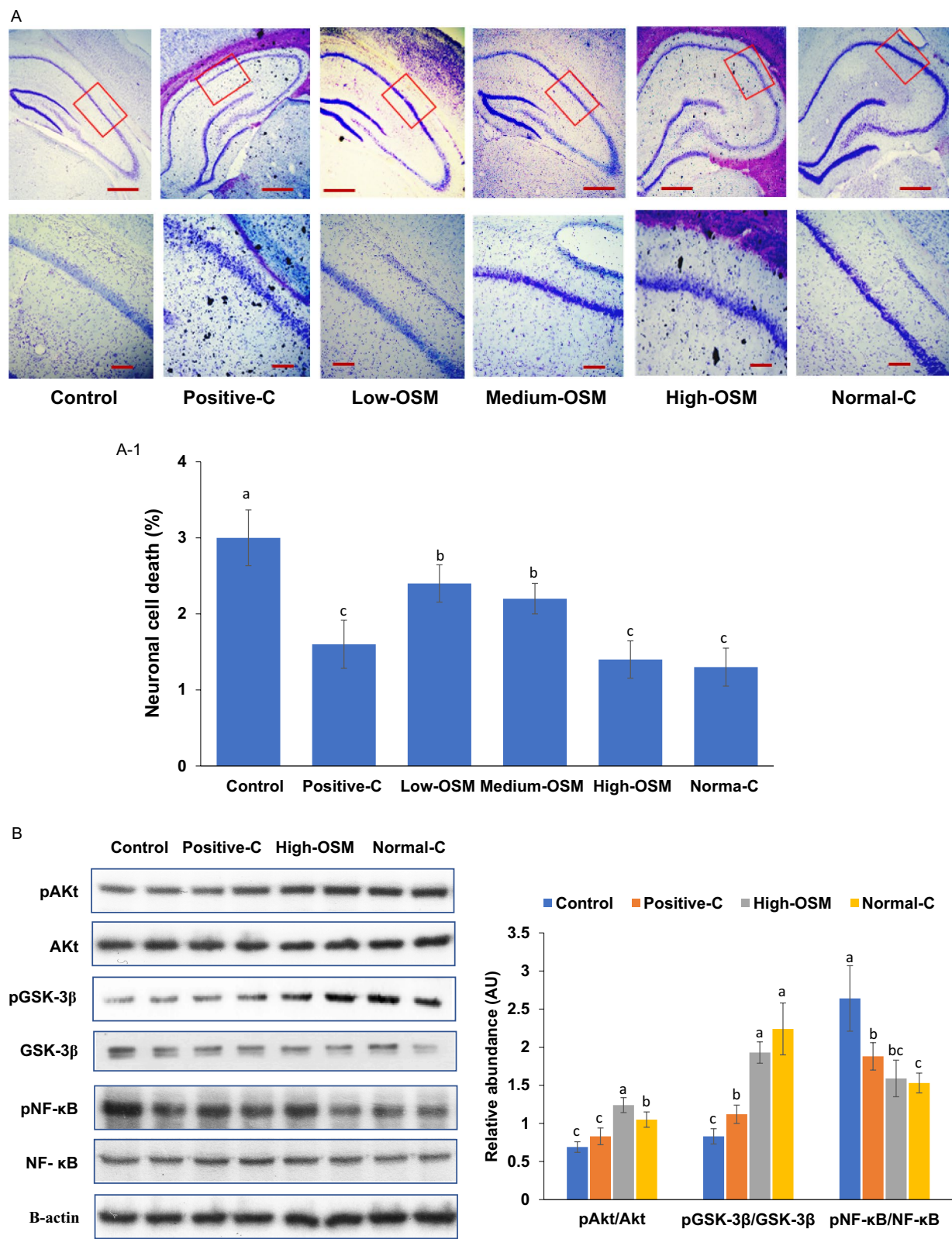


Fig. 3 (See legend on previous page.)

our scopolamine-induced memory impairment model, wherein OSM effectively normalized multiple predicted targets. The strong binding affinity of the Omija compounds to AChE and PTGS2 explains their potent effects on cholinergic function and neuroinflammation, while the soybean compounds complemented these actions through additional PTGS2 inhibition and modulation of the dopaminergic pathways.

Previous studies have demonstrated that scopolamine-induced memory impairment primarily involves cholinergic dysfunction and increased oxidative stress in the hippocampus [13, 24]. Our findings extend this understanding by showing that *PTGS2* expression upregulation and NF- κ B activation creates a pro-inflammatory environment in the hippocampus, leading to increased *TNF- α* (1.3-fold) and *IL-1 β* production. This aligns with recent studies that elevated serum inflammatory markers correlate with cognitive decline and brain atrophy in older adults and experimental animals [25–27].

The relationship between neuroinflammation and the reduced expression of neurotrophic factors observed in our study parallels the findings by Giacobbo et al. [28], who demonstrated that chronic inflammation suppresses *BDNF* mRNA expression in the hippocampus. However, our study uniquely demonstrated that the anti-inflammatory effects of OSM occur through the simultaneous modulation of the *PTGS2* and NF- κ B pathways, leading to the restoration of *BDNF* and *CNTF* mRNA levels. The molecular docking results showing the low binding energies of the Omija and soybean compounds with *PTGS2* provide a molecular basis for these anti-inflammatory effects. This multi-target action was different from those seen in previous studies [29, 30] that typically focused on single pathways. The neuroprotective effects of OSM in preserving CA1 neurons align with previous research showing that reducing hippocampal inflammation can prevent neuronal death [5, 31]. Importantly, our study is the first to demonstrate that combining Omija with soybean provides superior neuroprotection, probably due to their complementary effects on the inflammatory and neurotrophic pathways.

Our network pharmacology analysis identified *SLC6A3* (dopamine transporter) as a key target of soybean,

suggesting potential modulation of dopaminergic signaling. This prediction was validated by our experimental findings showing reduced hippocampal dopamine levels (1.3-fold decrease) in scopolamine-treated rats, which were effectively restored by the OSM treatment. This aligns with previous studies [32, 33] demonstrating that scopolamine administration disrupts not only cholinergic but also dopaminergic neurotransmission in the hippocampus. While Yan et al. [31] reported that Omija treatment could partially restore dopamine levels, in our study, treatment with a combination of Omija and soybean in OSM showed superior efficacy in normalizing dopaminergic signaling.

The marked elevation in hippocampal AChE activity (5.2-fold) observed in our scopolamine model was consistent with findings reporting similar increases in AChE activity correlating with memory deficits [34]. However, our study demonstrated that OSM treatment more effectively reduced AChE activity than Omija treatment alone [5]. This enhanced efficacy may be attributed to the synergistic effects of the direct inhibition of AChE by Omija and the anti-inflammatory properties of soybean, as inflammation has been shown to modulate AChE expression [35]. The simultaneous normalization of both cholinergic and dopaminergic systems by OSM represents a novel therapeutic approach, as most existing treatments target only one neurotransmitter system.

Our findings revealed a novel aspect of the therapeutic effects of OSM through the modulation of the GBA. The GBA represents a bidirectional communication network between the gastrointestinal tract and the central nervous system, operating through multiple pathways including neural (parasympathetic nervous system, especially vagus nerve), hormonal (hypothalamic–pituitary–adrenal axis), immune (cytokines and inflammatory mediators), and metabolic (microbial metabolites such as SCFA) mechanisms [36, 37]. Our analysis of intestinal morphology showed that scopolamine administration significantly disrupted the intestinal structure, characterized by reduced colonic epithelium length, increased width, and diminished crypt height. These structural changes align with findings from previous studies [38, 39]. It reports similar large intestinal morphological

(See figure on next page.)

Fig. 4 Results of memory function tests post-intervention with OSM. **a** Latency time to enter the darkroom in the passive avoidance test ($n = 10$). **b** Latency to locate the zone with the platform, and the period of stay in the platform zone on day 5 during the water maze test ($n = 10$). **c** Right turn percentage in the Y-maze test and recognition index in novel object recognition test ($n = 10$). Bars and error bars represent the means $\pm$ standard deviations ($n = 10$). ^{a,b,c} Different letters on the bars indicate a significant difference among the groups by Tukey's test at $P < 0.05$. The scopolamine-induced amnesic rats were fed high-fat diets with Control (750 mg dextrin/kg bw), Low-OSM (100 mg OSM/kg bw), Medium-OSM (250 mg OSM/kg bw), High-OSM (750 mg OSM/kg bw), and positive-control (Positive-C; 1 mg/kg donepezil). Saline-injected (no scopolamine) rats fed high-fat diets with 750 mg dextrin/kg bw were Normal-C

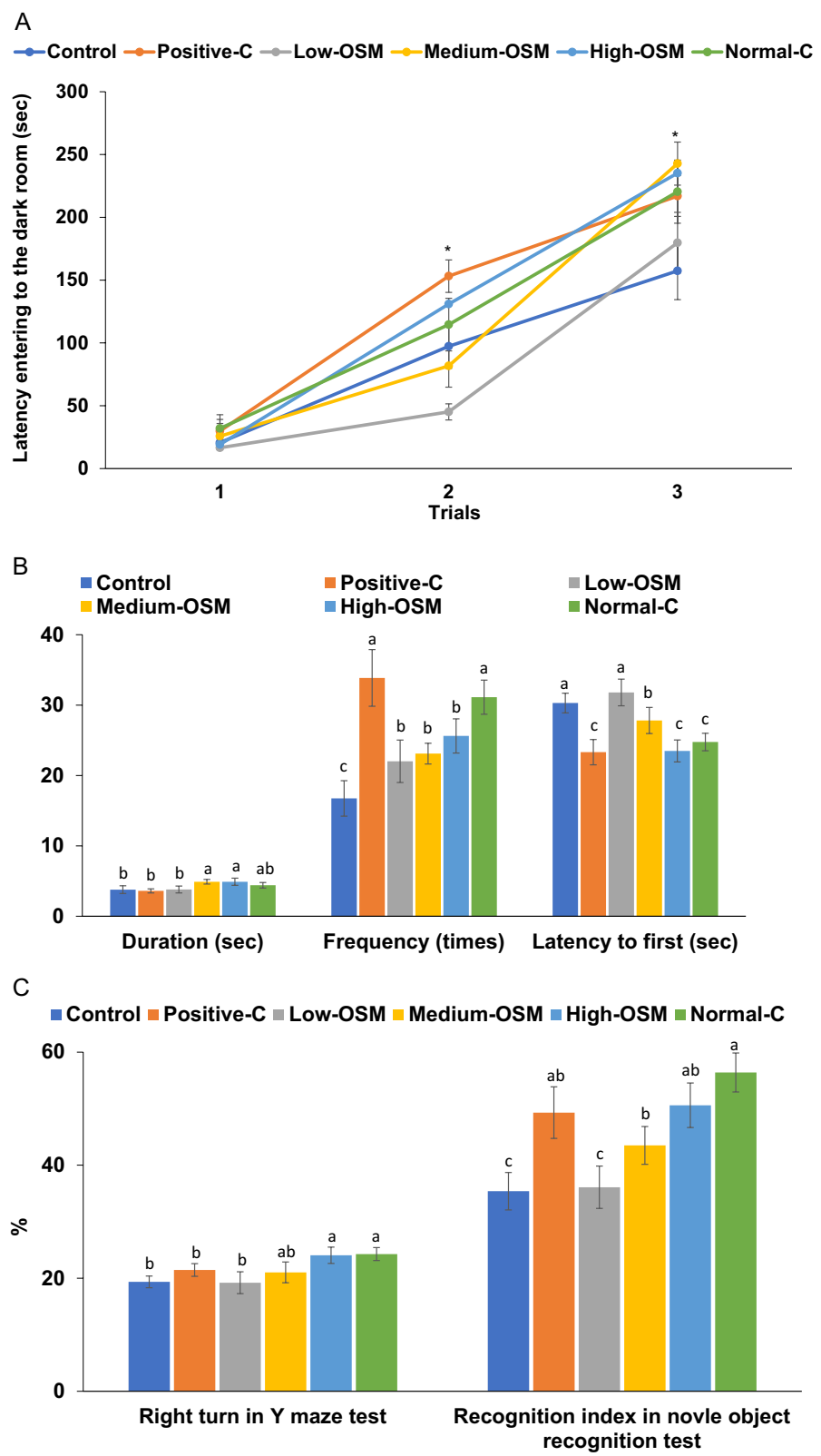


Fig. 4 (See legend on previous page.)

Table 3 Hippocampal lipids, oxidative stress and neuroinflammation

	Control	Positive-C	Low-OSM	Medium-OSM	High-OSM	Normal-C
Serum TNF- α (ng/mL)	0.84 $\pm$ 0.08 ^a	0.54 $\pm$ 0.07 ^b	0.78 $\pm$ 0.07 ^a	0.57 $\pm$ 0.07 ^b	0.49 $\pm$ 0.06 ^c	0.56 $\pm$ 0.06 ^b
Serum IL-1 β (pg/mL)	16.9 $\pm$ 2.07 ^a	16.6 $\pm$ 1.87 ^a	16.3 $\pm$ 2.02 ^a	13.2 $\pm$ 1.64 ^b	12.8 $\pm$ 1.37 ^b	12.2 $\pm$ 1.35 ^b
Triglyceride (mg/g tissue)	54.6 $\pm$ 2.91	52.5 $\pm$ 1.34	54.5 $\pm$ 1.49	53.4 $\pm$ 4.37	52.4 $\pm$ 1.58	53.3 $\pm$ 2.73
Cholesterol (mg/g tissue)	36.1 $\pm$ 1.62 ^b	40.3 $\pm$ 1.29 ^a	35.1 $\pm$ 1.35 ^{bc}	34 $\pm$ 1.27 ^c	31.6 $\pm$ 1.67 ^d	32.3 $\pm$ 0.78 ^d
Glycogen (mg/g tissue)	14.3 $\pm$ 4.06 ^b	14.2 $\pm$ 3.26 ^b	14.7 $\pm$ 3.29 ^b	17.7 $\pm$ 2.01 ^a	18.6 $\pm$ 3.08 ^a	17.6 $\pm$ 2.77 ^a
Acetylcholinesterase activity (U/mg protein)	0.83 $\pm$ 0.09 ^a	0.48 $\pm$ 0.06 ^c	0.77 $\pm$ 0.09 ^a	0.63 $\pm$ 0.07 ^b	0.52 $\pm$ 0.07 ^c	0.16 $\pm$ 0.03 ^d
Dopamine (pg/mg protein)	5.33 $\pm$ 0.65 ^c	7.34 $\pm$ 0.83 ^a	5.64 $\pm$ 0.67 ^c	6.46 $\pm$ 0.84 ^b	7.06 $\pm$ 0.75 ^b	8.16 $\pm$ 0.83 ^a
Lipid peroxides (MDA μ mol/g tissue)	4.21 $\pm$ 0.58 ^a	3.22 $\pm$ 0.39 ^b	3.94 $\pm$ 0.48 ^a	3.44 $\pm$ 0.43 ^b	2.43 $\pm$ 0.37 ^c	2.52 $\pm$ 0.33 ^c
SOD (U/mg protein)	56.9 $\pm$ 5.34 ^a	48.4 $\pm$ 4.23 ^b	52.4 $\pm$ 5.21 ^{ab}	47.3 $\pm$ 4.35 ^b	41.5 $\pm$ 4.07 ^c	43.5 $\pm$ 4.23 ^c
TNF- α contents (ng/g protein)	5.34 $\pm$ 0.63 ^a	3.72 $\pm$ 0.59 ^b	4.83 $\pm$ 0.66 ^a	3.79 $\pm$ 0.45 ^b	3.38 $\pm$ 0.54 ^b	3.54 $\pm$ 0.43 ^b
Relative <i>PTGS2</i> mRNA (AU)	1 ^a	0.87 $\pm$ 0.10 ^b	1.05 $\pm$ 0.13 ^a	0.79 $\pm$ 0.59 ^b	0.66 $\pm$ 0.83 ^c	0.62 $\pm$ 0.08 ^c
Relative <i>TNF-α</i> mRNA (AU)	1 ^a	0.95 $\pm$ 0.06 ^{ab}	0.96 $\pm$ 0.07 ^{ab}	0.90 $\pm$ 0.07 ^b	0.81 $\pm$ 0.07 ^c	0.77 $\pm$ 0.08 ^c
Relative <i>IL-1β</i> mRNA (AU)	1 ^a	0.96 $\pm$ 0.08 ^{ab}	0.95 $\pm$ 0.08 ^{ab}	0.90 $\pm$ 0.07 ^b	0.84 $\pm$ 0.07 ^{bc}	0.81 $\pm$ 0.08 ^c
Relative <i>IL-17</i> mRNA (AU)	1 ^a	0.94 $\pm$ 0.07 ^a	0.93 $\pm$ 0.08 ^{ab}	0.88 $\pm$ 0.08 ^b	0.78 $\pm$ 0.07 ^c	0.77 $\pm$ 0.07 ^c
Relative <i>BDNF</i> mRNA (AU)	1 ^c	1.53 $\pm$ 0.13 ^a	1.09 $\pm$ 0.10 ^c	1.32 $\pm$ 0.12 ^b	1.49 $\pm$ 0.12 ^a	1.42 $\pm$ 0.12 ^{ab}
Relative <i>CNTF</i> mRNA (AU)	1 ^c	1.81 $\pm$ 0.23 ^b	1.12 $\pm$ 0.17 ^c	1.54 $\pm$ 0.21 ^b	1.68 $\pm$ 0.23 ^b	2.45 $\pm$ 0.32 ^a

PBEH, porcine brain enzyme hydrolysates; MDA, malondialdehyde; TNF- α , tumor necrosis factor- α ; IL-1 β , interleukin-1 β ; BDNF, brain-derived neurotrophic factor; CNTF, ciliary neurotrophic factor. Male rats had intraperitoneal injections of scopolamine (2 mg/kg body weight/day) at 30 min after daily feeding of *Omija* (50% ethanol extract; *Schizandra chinensis* BAALL) plus soybean mixture (OSM) at 100, 250, or 750 mg (Low-OSM, medium-OSM, and high-OSM), or 1.2 mg donepezil (Positive-control) per kg body weight per day through feeding needle for 6 weeks. The normal-control rats had saline injections without scopolamine

Values represent means $\pm$ standard deviations (n = 10)

^{a,b,c} Means on the same row with different superscript letters indicated a significant difference among the groups by Tukey test at P < 0.05

alterations in cognitive decline models. The OSM treatment effectively prevented these morphological changes and restored goblet cell counts, with high-dose treatment showing particular efficacy in maintaining crypt height. Such compromised intestinal barrier function allows increased translocation of bacterial endotoxins and pro-inflammatory molecules into the systemic circulation, which can cross the blood–brain barrier and directly contribute to neuroinflammation and cognitive decline [40, 41].

The analysis of SCFA in the portal vein revealed distinct patterns of alteration that directly impact brain function through multiple mechanisms. SCFA, particularly butyrate, can cross the blood–brain barrier and exert neuroprotective effects by modulating microglial activation, promoting neurogenesis, and enhancing synaptic plasticity [42]. Earlier studies have indicated that butyrate levels are linked to enhancement of the GBA, improving

cognitive function through both peripheral anti-inflammatory effects and direct central nervous system actions [8, 43]. While acetate and propionate showed variable responses, butyrate levels were markedly reduced in the scopolamine-treated rats and significantly increased by the OSM treatment, particularly in the medium-dose group. These findings parallel previous works showing that reduced circulating butyrate levels are associated with cognitive impairment [37, 44].

The restoration of butyrate levels by OSM is particularly significant given butyrate's established role in reducing neuroinflammation, strengthening the blood–brain barrier, and supporting cognitive function through direct neural signaling pathways. Additionally, butyrate can activate the vagus nerve, which transmits signals directly to brain regions involved in memory formation, including the hippocampus [45]. While we did not directly assess microbiota composition, the observed improvements in

(See figure on next page.)

Fig. 5 Large intestinal morphology after OSM intervention. **a** Damaged cells and goblet cell count (n = 5). **b** Alcian Blue-Periodic Acid Schiff (AB-PAS) staining (The scale bar indicated 10 μ m and magnification was X100). The violet color indicates mucin (n = 5). **c** Serum short-chain fatty acid levels in the portal vein (n = 10). Bars and error bars represent the means $\pm$ standard deviations. ^{a,b,c} Different letters on the bars indicate a significant difference among the groups by Tukey's test at P < 0.05. *Omija* (50% ethanol extract; *Schizandra chinensis* BAALL) plus soybean mixture (OSM). The scopolamine-induced amnesic rats were fed high-fat diets with Control (750 mg dextrin/kg bw), Low-OSM (100 mg OSM/kg bw), Medium-OSM (250 mg OSM/kg bw), High-OSM (750 mg OSM/kg bw), and positive-control (Positive-C; 1 mg/kg donepezil). Saline-injected (no scopolamine) rats fed high-fat diets with 750 mg dextrin/kg bw were Normal-C

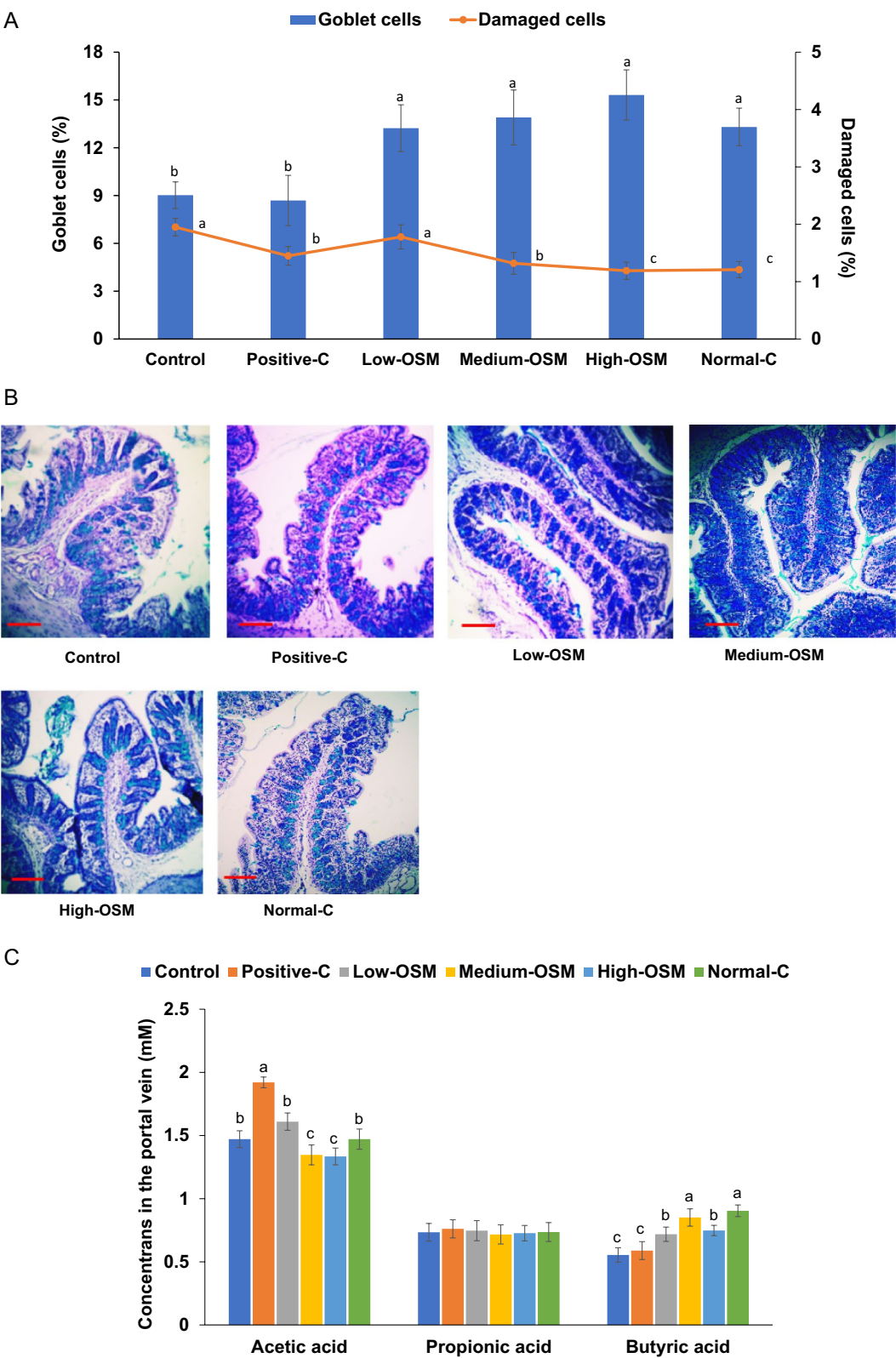


Fig. 5 (See legend on previous page.)

both intestinal structure and SCFA profiles suggest that OSM may enhance cognitive function partly through modulation of the gut environment and its metabolites, which subsequently influence brain function through the established GBA communication pathways.

In conclusion, this study validates network pharmacology predictions through in vivo experimentation, demonstrating OSM's therapeutic potential in memory improvement through complementary mechanisms, namely cholinergic function (AChE inhibition), dopaminergic signaling (SLC6A3), and neuroinflammation (PTGS2/NF- κ B pathways). The anti-inflammatory effects coupled with enhanced expression of neurotrophic factors (*BDNF/CNTF*) improved neuronal survival in the hippocampal CA1 region. Both medium (250 mg/kg bw) and high dose OSM (750 mg/kg bw) showed similar efficacy in improving memory function by directly inhibiting neuroinflammation and indirectly modulating the GBA through enhanced intestinal morphology and butyrate production. This multi-target approach, combining neuroprotective effects with GBA modulation, suggests that OSM could be a promising therapeutic option for treating memory deficits. The effectiveness of the medium dose of OSM suggests a potential human equivalent dose of 40 mg/kg bw/day. Future clinical trials are warranted to validate these findings in human populations and establish optimal dosing regimens.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13765-025-01019-4>.

Additional file 1 (Figure S1. Network pharmacology of Omija (*Schisandra chinensis* (Turcz.) Baill) and soybean bioactive compounds and memory deficit targets. Figure S2. High-Performance Liquid Chromatography (HPLC) chromatogram of Omija and soybean mixture. Figure S3. The area under the curve of serum glucose and insulin concentrations after Omija and soybean mixture (OSM) treatments during oral glucose tolerance tests (OGTT) and intraperitoneal insulin tolerance test (IPITT). Fig. S4. Large intestinal morphology after the intervention)

Author contributions

S.P.: Conceptualization, Supervision, Methodology, Writing—original draft. Y.Y.: Conceptualization, Formal analysis, Visualization, and Visualization. L.C., H.J.Y., M.J.K., M.S.K.: Formal analysis, and Methodology. D.Y.J.: Conceptualization, Supervision, and Writing—review and editing.

Funding

This study was funded by the “Food Functionality Evaluation program” under the Ministry of Agriculture, Food and Rural Affairs.

Data availability

The data included in the manuscript and supplementary materials.

Declarations

Ethics approval and consent to participate

All procedures were approved by the Institutional Animal Care and Use Committee (HSUIACUC-24-009) and followed the guidelines published in the

Guide for the Care and Use of Laboratory Animals, National Institutes of Health (NIH).

Completing interests

The authors do not have any financial or personal interest in the work incorporated in this manuscript.

Author details

¹Department of Bioconvergence, Hoseo University, Asan 31499, Korea.

²Research Group of Precision Nutrition, Korea Food Research Institute, Wanju, Jeonbuk-do 55365, Republic of Korea. ³Department of Food and Nutrition, Obesity/Diabetes Research Center, Hoseo University, 165 Sechul-Ri, BaeBang-Yup Asan-Si, ChungNam-Do, Asan 31499, Korea.

Received: 14 May 2025 Accepted: 14 July 2025

Published online: 30 July 2025

References

- Committee AD (2024) 2024 Alzheimer's disease facts and figures. *Alzheimers Dement* 20(5):3708–3821. <https://doi.org/10.1002/alz.13809>
- Kim Y, Kang DW, Kim GH, Kim KW, Kim H-J, Na S, Park KH, Park YH, Byeon G, Suh J, Shin JH, Shim Y, Yang Y, Um YH, Oh S-i, Wang S-M, Yoon B, Lee SM, Lee J, San Lee J, Lim J-S, Jung YH, Chin J, Jang H, Choi M, Hong YJ, Rhee HY, Jang J-W (2025) Clinical practice guidelines for dementia: recommendations for cholinesterase inhibitors and memantine. *Dement Neurocogn Disord* 24(1):1–23
- Chin E, Jaqua E, Safaeipour M, Ladue T (2022) Conventional versus new treatment: comparing the effects of acetylcholinesterase inhibitors and N-methyl-D-aspartate receptor antagonist with aducanumab. *Cureus* 14(11):e31065. <https://doi.org/10.7759/cureus.31065>
- Kara B, Gordon MN, Gifani M, Dorrance AM, Counts SE (2023) Vascular and nonvascular mechanisms of cognitive impairment and dementia. *Clin Geriatr Med* 39(1):109–122. <https://doi.org/10.1016/j.cger.2022.07.006>
- Yang H-J, Zhang T, Kim M-J, Hur H-J, Wu X, Jang D-J, Park S (2024) Efficacy and mechanism of *Schisandra chinensis* fructus water extract in Alzheimer's disease: insights from network pharmacology and validation in an amyloid- β infused animal model. *Nutrients* 16(21):3751
- Essawy AE, Abdou HM, Ibrahim HM, Bouthahab NM (2019) Soybean isoflavone ameliorates cognitive impairment, neuroinflammation, and amyloid β accumulation in a rat model of Alzheimer's disease. *Environ Sci Pollut Res* 26(25):26060–26070. <https://doi.org/10.1007/s11356-019-05862-z>
- Atanasov AG, Zotchev SB, Dirsch VM, Orhan IE, Banach M, Rollinger JM, Barreca D, Weckwerth W, Bauer R, Bayer EA, Majeed M, Bishayee A, Bochkov V, Bonn GK, Braid N, Bucar F, Cifuentes A, D'Onofrio G, Bodkin M, Diederich M, Supuran CT, the International Natural Product Sciences T (2021) Natural products in drug discovery: advances and opportunities. *Nat Rev Drug Discov* 20(3):200–216. <https://doi.org/10.1038/s41573-020-00114-z>
- Zheng Y, Bonfili L, Wei T, Eleuteri AM (2023) Understanding the gut-brain axis and its therapeutic implications for neurodegenerative disorders. *Nutrients* 15(21):4631. <https://doi.org/10.3390/nu15214631>
- Carabotti M, Scirocco A, Maselli MA, Severi C (2015) The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Ann Gastroenterol* 28(2):203–209
- Lu S, Zhao Q, Guan Y, Sun Z, Li W, Guo S, Zhang A (2024) The communication mechanism of the gut-brain axis and its effect on central nervous system diseases: a systematic review. *Biomed Pharmacother* 178:117207. <https://doi.org/10.1016/j.biopha.2024.117207>
- Yadang FSA, Nguzeze Y, Kom CW, Betote PHD, Mamat A, Tchokouaha LRY, Taiwé GS, Agbor GA, Bum EN (2020) Scopolamine-Induced Memory Impairment in Mice: neuroprotective Effects of *Carissa edulis* (Forssk.) Valh (Apocynaceae) Aqueous Extract. *Int J Alzheimers Dis* 2020:6372059. <https://doi.org/10.1155/2020/6372059>
- Eshaghi Ghalibaf MH, Rajabian A, Parviz M, Akbarian M, Amirahmadi S, Vafaee F, Hosseini M (2023) Minocycline alleviated scopolamine-induced amnesia by regulating antioxidant and cholinergic function. *Heliyon* 9(2):e13452. <https://doi.org/10.1016/j.heliyon.2023.e13452>
- Zhang T, Kim MJ, Kim MJ, Wu X, Yang HJ, Yuan H, Huang S, Yoon SM, Kim KN, Park S (2022) Long-term effect of porcine brain enzyme hydrolysate

- intake on scopolamine-induced memory impairment in rats. *Int J Mol Sci* 23(6):3361. <https://doi.org/10.3390/ijms23063361>
14. Butler MJ, Muscat SM, Caetano-Silva ME, Shrestha A, Olmo BMG, Mackey-Alfonso SE, Massa N, Alvarez BD, Blackwell JA, Bettes MN, DeMarsh JW, McCusker RH, Allen JM, Barrientos RM (2025) Obesity-associated memory impairment and neuroinflammation precede widespread peripheral perturbations in aged rats. *Immun Ageing* 22(1):2. <https://doi.org/10.1186/s12979-024-00496-3>
 15. Gannon OJ, Robison LS, Salinero AE, Abi-Ghanem C, Mansour FM, Kelly RD, Tyagi A, Brawley RR, Ogg JD, Zuloaga KL (2022) High-fat diet exacerbates cognitive decline in mouse models of Alzheimer's disease and mixed dementia in a sex-dependent manner. *J Neuroinflammation* 19(1):110. <https://doi.org/10.1186/s12974-022-02466-2>
 16. Freeman LR, Haley-Zitlin V, Rosenberger DS, Granholm AC (2014) Damaging effects of a high-fat diet to the brain and cognition: a review of proposed mechanisms. *Nutr Neurosci* 17(6):241–251. <https://doi.org/10.1179/1476830513y.0000000092>
 17. Batool S, Javed MR, Aslam S, Noor F, Javed HMF, Seemab R, Rehman A, Aslam MF, Paray BA, Gulnaz A (2022) Network pharmacology and bioinformatics approach reveals the multi-target pharmacological mechanism of *Fumaria indica* in the treatment of liver cancer. *Pharmaceuticals* 15(6):654
 18. Zhou J, Kim YK, Li C, Park S (2025) Natural compounds for Alzheimer's prevention and treatment: integrating SELFormer-based computational screening with experimental validation. *Comput Biol Med* 185:109523. <https://doi.org/10.1016/j.combiomed.2024.109523>
 19. Liu M, Park S (2024) The role of PNPLA3_rs738409 gene variant, lifestyle factors, and bioactive compounds in nonalcoholic fatty liver disease: a population-based and molecular approach towards healthy nutrition. *Nutrients*. <https://doi.org/10.3390/nu16081239>
 20. Noor F, Tahir ul Qamar M, Ashfaq UA, Albutti A, Alwashmi ASS, Aljasir MA (2022) Network pharmacology approach for medicinal plants: review and assessment. *Pharmaceuticals* 15(5):572
 21. Chen J, Hao W, Zhang C, Cui M, Sun Y, Zhang Y, Wang J, Mou T, Gu S, Zhao T, Wei B (2022) Explore the therapeutic composition and mechanism of *Schisandra chinensis*-*Acorus tatarinowii* Schott on Alzheimer's disease by using an integrated approach on chemical profile, network pharmacology, and UPLC-QTOF/MS-based metabolomics analysis. *Oxid Med Cell Longev* 2022:6362617. <https://doi.org/10.1155/2022/6362617>
 22. Yang HJ, Zhang T, Kim MJ, Hur HJ, Wu X, Jang DJ, Park S (2024) Efficacy and mechanism of *Schisandra chinensis* fructus water extract in Alzheimer's disease: insights from network pharmacology and validation in an amyloid- β infused animal model. *Nutrients* 16(21):3751. <https://doi.org/10.3390/nu16213751>
 23. Rezaadegan M, Mirjalili F, Clark CCT, Rouhani MH (2021) The effect of soya consumption on inflammatory biomarkers: a systematic review and meta-analysis of clinical trials. *Brit J Nutr* 125(7):780–791. <https://doi.org/10.1017/S0007114520003268>
 24. Lee G-Y, Lee C, Park GH, Jang J-H (2017) Amelioration of scopolamine-induced learning and memory impairment by α -pinene in C57BL/6 mice. *Evid Based Complement Alternat Med* 2017(1):4926815. <https://doi.org/10.1155/2017/4926815>
 25. Warren KN, Beason-Held LL, Carlson O, Egan JM, An Y, Doshi J, Davatzikos C, Ferrucci L, Resnick SM (2018) Elevated markers of inflammation are associated with longitudinal changes in brain function in older adults. *J Gerontol A Biol Sci Med Sci* 73(6):770–778. <https://doi.org/10.1093/gerona/glx199>
 26. Luo J, le Cessie S, Blauw GJ, Franceschi C, Noordam R, van Heemst D (2022) Systemic inflammatory markers in relation to cognitive function and measures of brain atrophy: a Mendelian randomization study. *Geroscience* 44(4):2259–2270. <https://doi.org/10.1007/s11357-022-00602-7>
 27. Mekhora C, Lampert DJ, Spencer JPE (2024) An overview of the relationship between inflammation and cognitive function in humans, molecular pathways and the impact of nutraceuticals. *Neurochem Int* 181:105900. <https://doi.org/10.1016/j.neuint.2024.105900>
 28. Lima Giacobbo B, Doorduyn J, Klein HC, Dierckx R, Bromberg E, de Vries EFJ (2019) Brain-Derived Neurotrophic Factor in Brain Disorders: focus on Neuroinflammation. *Mol Neurobiol* 56(5):3295–3312. <https://doi.org/10.1007/s12035-018-1283-6>
 29. Wahl D, Risen SJ, Osburn SC, Emge T, Sharma S, Gilberto VS, Chatterjee A, Nagpal P, Moreno JA, LaRocca TJ (2024) Nanoligomers targeting NF- κ B and NLRP3 reduce neuroinflammation and improve cognitive function with aging and tauopathy. *J Neuroinflammation* 21(1):182. <https://doi.org/10.1186/s12974-024-03182-9>
 30. Peter J, Mayer I, Kammer T, Minkova L, Lahr J, Klöppel S, Grothe MJ, Orth M (2021) The relationship between cholinergic system brain structure and function in healthy adults and patients with mild cognitive impairment. *Sci Rep* 11(1):16080. <https://doi.org/10.1038/s41598-021-95573-8>
 31. Yan T, Mao Q, Zhang X, Wu B, Bi K, He B, Jia Y (2021) *Schisandra chinensis* protects against dopaminergic neuronal oxidative stress, neuroinflammation, and apoptosis via the BDNF/Nrf2/NF- κ B pathway in 6-OHDA-induced Parkinson's disease mice. *Food Funct* 12(9):4079–4091. <https://doi.org/10.1039/d0fo02836c>
 32. Memo M, Missale C, Trivelli L, Spano PF (1988) Acute scopolamine treatment decreases dopamine metabolism in rat hippocampus and frontal cortex. *Eur J Pharmacol* 149(3):367–370. [https://doi.org/10.1016/0014-2999\(88\)90670-X](https://doi.org/10.1016/0014-2999(88)90670-X)
 33. Yeomans JS (1995) Role of tegmental cholinergic neurons in dopaminergic activation, antimuscarinic psychosis and schizophrenia. *Neuropsychopharmacology* 12(1):3–16. <https://doi.org/10.1038/sj.npp.1380235>
 34. Balakrishnan R, Park JY, Cho DY, Ahn JY, Yoo DS, Seol SH, Yoon SH, Choi DK (2023) AD-1 small molecule improves learning and memory function in scopolamine-induced amnesic mice model through regulation of CREB/BDNF and NF- κ B/MAPK signaling pathway. *Antioxidants* 12(3):648. <https://doi.org/10.3390/antiox12030648>
 35. Haam J, Yakel JL (2017) Cholinergic modulation of the hippocampal region and memory function. *J Neurochem* 142(Suppl 2):111–121. <https://doi.org/10.1111/jnc.14052>
 36. Zhang T, Yue Y, Li C, Wu X, Park S (2024) Vagus nerve suppression in ischemic stroke by carotid artery occlusion: implications for metabolic regulation, cognitive function, and gut microbiome in a gerbil model. *Int J Mol Sci* 25(14):7831. <https://doi.org/10.3390/ijms25147831>
 37. Daily JW, Kang S, Park S (2021) Protection against Alzheimer's disease by luteolin: role of brain glucose regulation, anti-inflammatory activity, and the gut microbiota-liver-brain axis. *BioFactors* 47(2):218–231. <https://doi.org/10.1002/biof.1703>
 38. Liu P, Zhou X, Zhang H, Wang R, Wu X, Jian W, Li W, Yuan D, Wang Q, Zhao W (2022) Danggui-shaoyao-san attenuates cognitive impairment via the microbiota-gut-brain axis with regulation of lipid metabolism in scopolamine-induced amnesia. *Front Immunol* 13:796542. <https://doi.org/10.3389/fimmu.2022.796542>
 39. Ryu MS, Yue Y, Li C, Yang H-J, Zhang T, Wu X, Jeong DY, Park S (2024) Moderate capsaicin-containing kochujang alleviates memory impairment through the gut-brain axis in rats with scopolamine-induced amnesia. *Biomed Pharmacother* 178:117091. <https://doi.org/10.1016/j.biopha.2024.117091>
 40. Pellegrini C, Fornai M, D'Antongiovanni V, Antonioli L, Bernardini N, Derkinderen P (2023) The intestinal barrier in disorders of the central nervous system. *Lancet Gastroenterol Hepatol* 8(1):66–80. [https://doi.org/10.1016/S2468-1253\(22\)00241-2](https://doi.org/10.1016/S2468-1253(22)00241-2)
 41. Loh JS, Mak WQ, Tan LKS, Ng CX, Chan HH, Yeow SH, Foo JB, Ong YS, How CW, Khaw KY (2024) Microbiota-gut-brain axis and its therapeutic applications in neurodegenerative diseases. *Signal Transduct Target Ther* 9(1):37. <https://doi.org/10.1038/s41392-024-01743-1>
 42. Cao Q, Shen M, Li R, Liu Y, Zeng Z, Zhou J, Niu D, Zhang Q, Wang R, Yao J, Zhang G (2025) Elucidating the specific mechanisms of the gut-brain axis: the short-chain fatty acids-microglia pathway. *J Neuroinflammation* 22(1):133. <https://doi.org/10.1186/s12974-025-03454-y>
 43. Kearns R (2024) Gut-brain axis and neuroinflammation: the role of gut permeability and the kynurenine pathway in neurological disorders. *Cell Mol Neurobiol* 44(1):64. <https://doi.org/10.1007/s10571-024-01496-z>
 44. Park S, Wu X (2022) Modulation of the gut microbiota in memory impairment and Alzheimer's disease via the inhibition of the parasympathetic nervous system. *Int J Mol Sci* 23(21):13574
 45. Mansuy-Aubert V, Ravussin Y (2023) Short chain fatty acids: the messengers from down below. *Front Neurosci* 17:1197759. <https://doi.org/10.3389/fnins.2023.1197759>

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.