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Defatted Hickory Meal Hydrolysate's Impact on Memory Impairment Induced by D-Galactose in Mice

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ABSTRACT: Defatted hickory meal (DHM), a by-product of hickory oil production, is a protein source rich in essential amino acids. In this study, the functional properties of DHM hydrolysate (DHMH) were assessed using *in vitro* and *in vivo* assays in context to its antioxidant and memory-enhancing effects. To induce memory impairment, D-galactose (D-gal) was administered to mice at a dose of 120 mg/kg body weight per day, and DHMH was orally administered at doses of 300, 600, and 1000 mg/kg body weight per day for 8 weeks. DHMH treatment led to improved memory performance in D-gal-induced memory-impaired mice, as observed in the Morris water maze test. Furthermore, DHMH mitigated the accumulation of amyloid beta 1-42 triggered by D-gal exposure. Notably, high-dose DHMH significantly reduced the elevation of pro-inflammatory markers, including tumor necrosis factor alpha, interleukin 1β, and interleukin 6. Additionally, DHMH prevented the decline in total superoxide dismutase activity, glutathione peroxidase activity, and glutathione levels, while reducing malondialdehyde content in D-gal-induced mice, indicative of its antioxidant properties. Moreover, DHMH treatment effectively prevented histological alterations in neurons within the hippocampal CA1 area induced by D-gal. Collectively, our findings suggest that DHMH may counteract memory dysfunctions resulting from oxidative stress injury in the brain, positioning it as a potential candidate for use as a functional food.

Keywords: hickory, memory impairment, aging, antioxidant activity, oxidative stress, neuroinflammation, hippocampal nerve

1. Introduction

When it comes to measuring an individual's health and quality of life, aging and memory impairment are two crucial factors. Aging is an inevitable process that leads to the degeneration of certain organs, particularly the brain. Molecular and cellular changes in the hippocampus, cortical density, and neurotransmitter systems characterize brain aging[1]. These changes occur through various pathways, such as oxidative stress-induced damage. Brain aging, caused by reactive oxygen species (ROS) and active nitrogen

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(RNS) containing species, protein oxidation, lipid peroxidation, and mitochondrial DNA (mtDNA) damage, can result in memory impairment[2]. With advancing age, cognitive abilities, memory, and thinking flexibility gradually decline, making memory impairment a common issue among the elderly[3]. Memory impairment can range from mild to severe. Mild memory impairment is typically characterized by forgetfulness and temporary memory problems. Moderate memory impairment may lead to frequent amnesia of recent events and the inability to remember new information, which affects elderly daily life. Severe memory impairment can cause the inability to recall past events, names, or common-sense information, severely impacting daily life and even independence[4].

D-galactose (D-gal) is a reducing sugar that, according to a study, can create a safe rodent model of aging within a short period of time[3]. This model exhibits behavioral, neurological, and biochemical deficits similar to natural aging in humans. Therefore, D-gal can be utilized to screen anti-aging drugs for neurodegenerative diseases, including memory disorders. In addition, excessive levels of D-gal can result in the generation of advanced glycosylation end products (AGEs)[5]. Several hypotheses have been proposed to elucidate the underlying mechanisms of the aging process, which encompass the formation of AGEs and oxidative stress. These mechanisms play pivotal roles in diverse pathological conditions, including Alzheimer's disease (AD)[6]. Empirical investigations have demonstrated that prolonged systemic administration of D-galactose induces oxidative stress by reducing antioxidative enzymes, promoting neuroinflammation, and triggering apoptosis[7]. The alterations observed in rodents exposed to D-galactose parallel those found in the aging brains of humans, evidenced by progressive loss of neurons and cognitive decline.

Food-derived peptides can be utilized in daily dietary supplements to boost health. These peptides exhibit characteristics such as safe consumption, convenient sourcing, a simple production process, and potent functional activities[8]. Chen et al.[9] reported on the efficacy of walnut meal enzymatic hydrolysate in enhancing mice's learning and memory abilities, exhibiting potential efficacy in water maze and dark box avoidance tasks. Hickory (*Carya cathayensis Sarg.*) nuts are esteemed for their high nutritional value, delightful taste, and distinctive flavor[10]. According to the present study, pecans exhibit potential in enhancing memory function. These nuts are abundant in beneficial components such as essential fatty acids, polyphenols, vitamin E, and β-sitosterol[4]. These compounds possess potential antioxidant, anti-inflammatory, and neuroprotective properties, thereby aiding in the improvement of learning and memory functions[11]. YingLi's[12] study demonstrated that pecan oil effectively enhanced rats' learning and memory abilities, with a potential mechanism linked to the reduction in NF-κB expression. Nevertheless, research on hickory's impact on memory primarily focuses on pecan oil, and there is currently insufficient exploration regarding the effects of hickory peptides on memory enhancement and their underlying action mechanisms. Defatted hickory meal (DHM) to encompass a protein content of up to 40% and a rich amino acid profile, is a by-product of walnut oil production. However, during the pressing process, DHM's functional, nutritional, and sensory attributes tend to deteriorate due to denaturation, leading to its

predominant use as animal feed and substantial resource wastage. Therefore, the rational utilization of DHM holds extensive application potential, research and development value, and a significant market opportunity. In recent years, the scientific community has been actively exploring the use of various oil cakes as raw materials for synthesizing functional peptides, with enzymatic development of functional peptides emerging as a notable focus. This method offers an economically and environmentally sustainable means to transform oil cake by-products into valuable bioactive compounds, especially in sectors where these by-products have historically been underutilized or discarded[13].

This study focused on investigating the antioxidant capabilities of DHM hydrolysate (DHMH) and examined the potential protective benefits of DHMH supplementation against behavioral, neuroinflammatory, and oxidative stress effects in D-gal-induced memory impairment in mice. To gain deeper insights into the mechanisms through which nut protease hydrolysate enhances memory, the research also probed DHMH's neuroprotective effects on neurons and synapses.

2. Materials and methods

2.1 Materials and chemicals

Hickory was purchased from Zhejiang Lin'an District Tuankou hickory professional cooperative. The alkaline protease was provided by Hangzhou Shisheng Technology Co., Ltd. Enzyme activity was 46 m U/g,2, 2-DiPhenyl-1-picrylhydrazyl (DPPH). 2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS) was from Shanghai Lingfeng Chemical Co., LTD. Glutathione (GSH) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade. Malondialdehyde (MDA) kit, superoxide dismutase (SOD) kit, GSH kit, GSH peroxidase (GSH-PX) kit, interleukin 6 (IL-6) kit, interleukin 1β (IL-1β) kit, and tumor necrosis factor (TNF-α) kit were from Jiancheng Institute of Bioengineering, Nanjing. KM male mice were from Beijing Vitonglihua Laboratory Animal Technology Co., Ltd. (laboratory animal production license number: SCXK (Zhejiang) 2020-0002). Sterilized mouse maintenance feed was from Beijing Vitonglihua Laboratory Animal Technology Co., Ltd.

2.2 Preparation of DHMH

The preparation method of DHMH was referred to Wang et al.[14] appropriate modifications were made. Combine 750 mL of distilled water in a container with 50 g of DHM. The suspension was heated at 85℃ for 15 min. After cooling, pH was adjusted till 9.3 using 1 mol/L NaOH solution, 4760 U/g alkaline protease was added for enzymolysis. NaOH was used to maintain pH of the reaction system during enzymolysis, and ultrasound was used to promote enzymolysis aided by sonication for 3 h. The hydrolysis reaction was terminated by placing in a boiling water bath, with the pH of the cooled mixture adjusted to 7.0 using the appropriate solution. To separate DHMH from the reaction mixture, it was placed in a centrifuge at 8000 g and a temperature of 4℃ for 15 min. Finally, the resulting DHMH solution was freeze-dried (PVT100 IlShinBioBase, Yangju, Korea) to convert it into a stable and dry powder.

2.3 Determination of molecular weight (MW) distribution

The MW distribution was determined using the method described by Jin et al.[15] using the Waters 2695 High-Performance Liquid Chromatography with 2487 UV detector and Empower workstation GPC Software.

The experimental conditions were configured as follows: a 10 mL injection volume was utilized, with a flow rate set at 0.5 mL/min. The detection temperature was maintained at 30℃, while the detection wavelength was fixed at 220 nm. The mobile phase consisted of acetonitrile, water, and trifluoroacetic acid in a ratio of 40/60/0.1 (V/V/V). Cytochrome C (12384 Da), aprotinin (6500 Da), bacitracin (1422 Da), glycine–glycine–tyrosine–arginine (451 Da), and ethanol–ethanol–ethanine (189 Da) were used as standard curves. Each sample was subjected to chromatographic analysis, and the resulting peaks were segmented into eight intervals based on their MWs: (MW >10000, 1000-5000, 5000-3000, 3000-2000, 2000-1000, 1000-500, 500-180 and <180 Da). The appropriate peak area for each period was used to calculate the relative content of each MW range.

2.4 Amino acid composition analysis

The standard product 50 μL was mixed with 50 μL protein precipitator (including NVL), and then refrigerated for 4 min at 13,200 g. Subsequently, 8 μL of the supernatant was added to 42 μL labeled buffer to mix and then left to disperse. The mixture was supplemented with 20 μL of derivative liquid. Following instantaneous separation, the resulting product was incubated at a temperature of 55℃ for 15 min. After the derived sample was cooled in the refrigerator, it was mixed and left to separate, with 50 μL aliquoted for testing.

2.5 Determination of in vitro antioxidant activities of DHMH

2.5.1 Determination of DPPH radical-scavenging activity

In this assay, 3.0 mL of a solution containing 0.2 mmol/L DPPH and 1.0 mL of various concentrations of tested solutions (0.05, 0.1, 0.2, 0.3, and 0.4 mg/mL) were mixed in a test tube. The DPPH radical-scavenging activity was determined following the method of Rumpf et al.[16]

2.5.2 ABTS scavenging activity assay

In this assay, 1.0 mL of sample solution was mixed with different concentrations (0.05, 0.1, 0.2, 0.3, and 0.4 mg/mL) of tested solutions with 4.0 mL of ABTS solution. The ABTS scavenging activity was determined following the method of Ma and Xiong[17].

2.5.3 Hydroxyl radical (-OH) scavenging activity assay

In this study, sample solutions at concentrations ranging from 2.0 to 6.0 mg/mL were prepared. Each 1.0 mL sample solution was mixed with 1 mL of 9 mmol/L sodium salicylate, 1 mL of 9 mmol/L iron (II) sulfate heptahydrate, and 50 μL of hydrogen peroxide (0.025%, v/v). The hydroxyl radical scavenging activity was determined following the method of Lin et al.[18]

2.5.4 Reducing capacity

Various solutions with different mass concentrations (0.2, 0.4, 0.8, 1.6, and 2.0 mg/mL) were mixed with phosphate buffer (pH 6.6) at a concentration of 0.2 mol/L. Reducing capacity assays were performed according to the method of Onder et al.[19]

2.6 In vivo memory improvement by feeding DHMH

Kunming mice (male, 22–24 g) were purchased from Zhejiang Weitong Lihua Experimental Animal Technology, laboratory animal production license number: SCXK (Zhejiang) 2020-0002. The assay was conducted in accordance with the International Guiding Principles for Animal Biomedical Research. All experimental protocols were approved by the Animal Ethics Committee of Zhejiang Academy of Agricultural Sciences (Committee approval number: 2024ZAASLA001).

After one week of acclimatization, mice were divided into 5 groups: control group, model group, low-dose enzyme hydrolysate group (DHMH-L), medium-dose enzyme hydrolysate group (DHMH-M), and high-dose enzyme hydrolysate group (DHMH-H), with each group to include 10 mice (n = 10). The mice in the model group and enzymolysis group received subcutaneous injections of 120 mg/kg D-gal every day for 8 weeks. The blank control group received the same amount of a normal saline injection. Starting from the 5th week, the blank control group and model group received normal saline administration using gavage, while the low, medium, and high enzyme hydrolysate groups were administered 300 mg/kg, 600 mg/kg, and 1000 mg/kg enzyme hydrolysate solution, respectively, also through gavage. The selected dosage was determined based on previous reported[20]. Specifically, the administered dose of 300 mg/kg DHMH was equivalent to 10 times the dosage typically prescribed for an adult weighing 60 kg. The mice body weight was recorded every 6 days throughout the experiment.

2.6.1 Morris water maze (MWM) evaluation

The experimental method of the water maze was referred to as the method of Yuan[21]. The spatial or long-term memory of mice was assessed using the Morris water test. The water maze utilized in this assay had a height of 0.5 m and a diameter of 1.2 m. A platform, with dimensions of 0.09 m in diameter and 0.3 m in height, was strategically concealed just beneath the water surface at a depth of 1~2 cm. The following parameters were set as follows: swimming time (60 s), stay time on the platform (15 s), mouse experiment mode, and adjusting the red line circle to accurately frame the pool and platform ranges. White markers indicating the four cardinal directions (E, S, W, and N) were conspicuously placed on the pool's wall to serve as cues for facilitating learning and memory processes. The experiment started 8 weeks after the treatment was administered in mice and was divided into two parts: directional navigation and space exploration.

Orientation navigation training of the mice was conducted within 1 h after daily administration to the stomach, and each mouse was trained for 4 times. The mice were placed in water at a particular position, and the time required to enter the water and find the platform to climb on the platform stably (i.e., to escape the incubation period) was immediately recorded by the software. The use time was recorded if the mice could climb on the platform within 60 s and stand steadily for 15 s. If the platform could not be found within 60 s

after entering the water, the incubation period was recorded as 60 s. Following guidance to the platform, the mice were given a 15-second period to maintain a stable standing posture, after which they were provided a resting interval of 30 to 60 seconds before commencing the subsequent training session.

In the space exploration assay, removing the underwater platform was deemed necessary to allow for the observation and recording of two key parameters: the number of times the mice crossed the initial platform within a 60-second timeframe upon entering the water and the duration of exploration within the quadrant where the original underwater platform was situated.

2.6.2 Enzyme-linked immunoassay (ELISA) assay

The expressions of SOD and GSH-PX and levels of MDA, GSH, IL-1β, IL-6, TNF-α, and $A\beta_{1-42}$ in the mouse cerebral tissue lysates, or serum were determined using ELISA kit (Jiancheng Institute of Bioengineering, Nanjing, China) according to the manufacturer's instruction.

2.6.3 Histological analysis

The collected brain samples were fixed in 4% paraformaldehyde solution for 48 h. Subsequently, the brain tissue blocks underwent paraffin embedding, followed by sectioning at a thickness of 4 μm. Sections were stained with hematoxylin and eosin (H&E). An optical microscope was used to observe and capture photographs of the histological sections at 20× magnification.

2.7 Expression of PSD-95 in mouse hippocampus was detected by western blotting

The total protein of the mouse hippocampus was extracted following Gao et al.[22] method, and the protein concentration was adjusted for consistency in each group. The protein concentration was sequentially subjected to SDS-PAGE electrophoresis, constant current flow to PVDF membrane, and 5% skim milk powder sealing buffer at room temperature for 2 h. The primary antibodies, including rabbit anti-mouse PSD-95 and β-actin (1:1000 dilution, Abbkine Scientific Co.,Ltd dizhi, Wuhan, China), were added to the sample and incubated overnight at a temperature of 4℃. Subsequently, the sample was rinsed for 30 min, followed by incubation with the horseradish peroxidase (HRP)-labeled secondary antibody (1:1000 dilution, Abbkine Scientific Co.,Ltd dizhi, Wuhan, China) at room temperature for 1 hour. After rinsing for 30 min, the chemical color rendering and gel image processing systems collected the protein band images and analyzed the absorbance of the target band.

2.8 Immunohistochemistry

Immunohistochemistry is primarily used to detect specific antigens in tissue samples. This process generally involves dewaxing and rehydrating the tissue sections, followed by applying antigen repair methods to restore the immune reactivity of the antigen. Subsequently, the tissue slices were incubated with PSD-95 antibodies (1:1000, Abbkine Scientific Co.,Ltd dizhi, Wuhan, China), and staining techniques were used to visualize the resulting reaction. After the staining was completed, the tissue sections were evaluated and analyzed under a panoramic section scanner (PANNORAMIC DESK/MIDI/250/1000, 3DHISTECH,

Hungary). Finally, the computer software ImageJ (National Institute of Health, USA) was used for quantitative measurement and analysis.

2.9 Statistical analysis

All data were expressed as mean ± standard errors of the mean (SEMs). GraphPad Prism Version 7.0 (GraphPad Software, San Diego, CA, USA) was used to compare all groups using a one-way analysis of variance. Statistical significance was set at $P < 0.05$.

3. Results and discussion

3.1 MW distribution of enzymolysis polypeptides from hickory meal

As depicted in Table 1, the MWs of the enzymatic hydrolysates derived from hickory meal were predominantly less than 3 kDa, constituting approximately 97.2% of the total protein composition. The action of proteases facilitated the release of bioactive peptides embedded within the protein matrix. Ketnawa et al.[23] previously reported that peptides within MWs below 3 kDa exhibited improved antioxidant activities and other beneficial physiological effects compared to larger macromolecular peptides. This heightened efficacy may be ascribed to the heightened affinity of smaller molecules to engage with free radicals, thereby mitigating peroxidation induced by these radicals. These results indicated that alkaline proteases can effectively break down hickory proteins into diminutive peptides or free amino acids, with the resultant hydrolysates displaying notable antioxidant properties.

Table 1 Molecular weight distributions of defatted hickory meal hydrolysate.

Molecular weight range (KDa)	Peak area percentage (%，λ220 nm)	Number-average molecular weight	Weight-average molecular weight
>10	0.05	17366	17991
10~5	0.34	5653	5718
5~3	2.39	3653	3725
3~2	3.68	2402	2435
2～1	9.32	1352	1407
1~0.5	15.70	666	691
0.5～0.18	30.22	281	301
＜0.18	38.30	36	74

3.2 Amino acid composition determination

Distinct amino acids exert varying influences on the growth and functionality of the nervous system and brain. Arginine, for instance, regulates nicotinamide adenine dinucleotide phosphate-dependent reactions and is intricately linked to the modulation of cognitive learning and memory process[24]. Lysine, as the foremost essential amino acid, plays a crucial role in the regeneration of brain nerve cells, bolstering immunity, supporting growth and development, and enhancing the overall performance and stability of the central nervous system[25].

In this study, the measured levels of hydrolyzed amino acids, free amino acids, and additional amino acids were detected at (2107.97 ± 760.03) μg/g, (775.56 ± 27.87) μg/g, and 1332.41 μg/g, respectively. Notably, the hickory meal enzyme lysate exhibited the highest levels of glutamic acid and tyrosine at

(133.59 ± 3.09) μg/g and (130.08 ± 8.23) μg/g, as detailed in Table 2. Glutamic acid, the most abundant amino acid in the mammalian brain, functions as an excitatory neurotransmitter[26]. Additionally, it plays critical roles in cerebral energy metabolism, GABA and glutathione synthesis, as well as ammonia detoxification. Glutamine, formed through the combination of glutamic acid and ammonia, can cross the blood-brain barrier[27]. Within the brain, the enzyme glutamine synthetase (GS) is responsible for the majority of endogenous glutamine production[27]. In a study conducted by Chen et al.[26], glutamine supplementation was administered to two animal models of Alzheimer's disease. The study revealed that this supplementation led to a reduction in various biochemical markers associated with brain dysfunctions. Specifically, there was a decrease observed in inflammation-induced neuronal cell cycle activation, tau phosphorylation, and ATM-activation. These results suggested that glutamine supplementation may exert potential benefits in alleviating dysfunction related to Alzheimer's disease, and suggestive that amino acids likewise enriched in DHMH can traverse the blood-brain barrier, contributing to memory enhancement.

Table 2. Amino acid composition and content of defatted hickory meal hydrolysate.

Amino	Free amino acid (μg/g)	Hydrolysis amino acid (μg/g)
Ala	10.76 ± 0.92	212.75 ± 63.84
Arg	95.54 ± 1.85	28.65 ± 11.60
Asn	0	69.71 ± 25.15
Asp	114.82 ± 1.90	37.20 ± 15.31
Cit	0.27 ± 0.01	24.97 ± 7.65
Cys	2.34 ± 0.04	0.71 ± 0.09
Glu	133.59 ± 3.09	757.57 ± 346.74
Gln	0	3.47 ± 1.03
Lys	73.74 ± 1.04	25.05 ± 12.78
Gly	4.34 ± 0.30	30.94 ± 12.88
His	26.43 ± 5.09	36.68 ± 9.21
Ile	4.82 ± 0.08	94.30 ± 25.87
Leu	28.25 ± 1.22	140.29 ± 45.21
Phe	31.26 ± 1.25	76.27 ± 26.15
Met	18.84 ± 0.20	83.00 ± 13.46
Pro	0.40 ± 0.32	19.77 ± 2.28
Ser	13.46 ± 0.52	60.11 ± 21.91
Thr	27.78 ± 0.37	80.09 ± 24.41
Trp	0	57.57 ± 20.78
Tyr	130.08 ± 8.23	119.41 ± 63.50
Val	58.84 ± 1.44	149.46 ± 10.18
Total	775.56 ± 27.87	2107.97 ± 760.03

3.3 DHMH In vitro antioxidant activity

The specific causes of memory loss are still unidentified, as indicated by a prior studies[7]. Nonetheless, the generation of harmful antioxidant compounds triggered by oxidative stress is regarded as a significant factor in memory impairment[28].

DPPH, which is centered around nitrogen, serves as a relatively stable and comprehensive method for rapidly assessing the antioxidant activity of substances[29]. As illustrated in Fig. 1a, the rates of DPPH radical scavenging activity for DHMH, DHM, and GSH increased with the following order of rates: DHMH > DHM > GSH. These findings highlighted DHMH's exceptional effectiveness in quenching free

radicals posing it as a promising natural antioxidant. Furthermore, DHMH exhibited remarkable scavenging capabilities against DPPH free radicals.

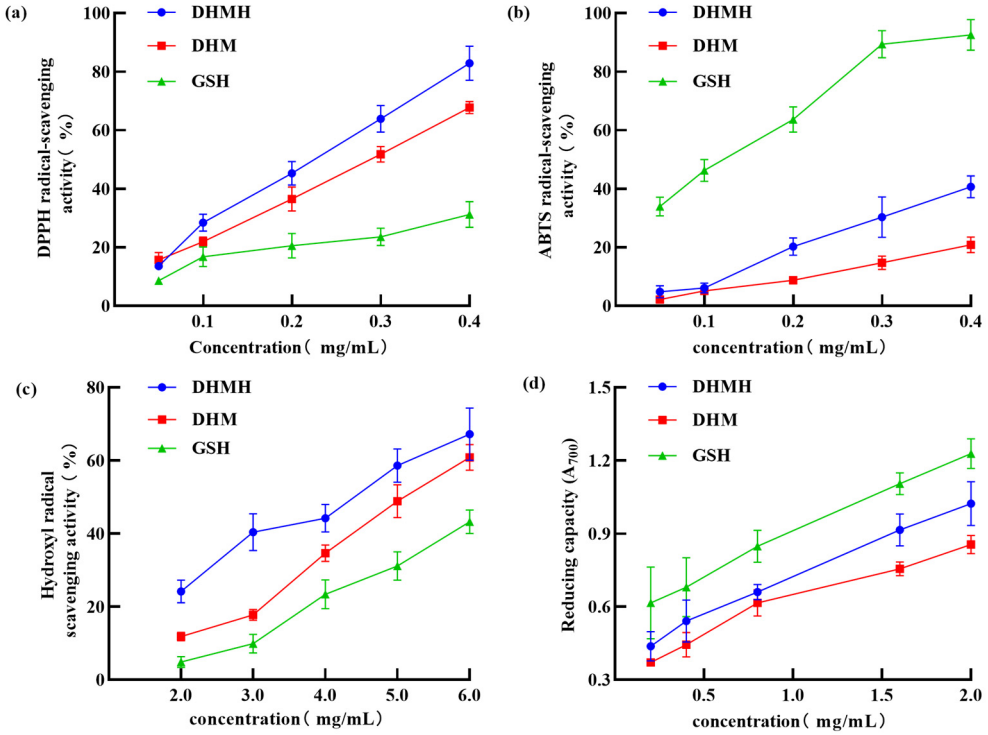

Figure 1. Antioxidant activities of DHMH *in vitro*. DPPH radical-scavenging activity (a), ABTS radical-scavenging activity (b), Hydroxyl radical scavenging activity (c), and Reducing capacity (d) of the DHMH, DHM, and GSH. DPPH, 2-DiPhenyl-1-picrylhydrazyl; ABTS, 2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulfonate; DHMH, defatted hickory meal hydrolysate; DHM, defatted hickory meal; GSH, glutathione.

The evaluation of the antioxidant activity in hydrogen-donating and chain-breaking compounds can be efficiently carried out through the conventional ABTS radical test, which serves as a valuable method for assessing these compounds[30]. As depicted in Fig. 1b, a discernible dose-effect relationship was observed, with the clearance rate of the toxic ABTS peptide increasing as concentrations rose within a specific range. Notably, at a concentration of 0.4 mg/mL, DHMH exhibited a clearance effect on ABTS that surpassed GSH by over 40% and was twice as effective as DHM.

The ability to transfer electrons can be evidenced by the speed at which hydroxyl radicals are neutralized, as well as by the reducing power, both of which serve as indicators of antioxidant efficacy[31]. The aptitude of antioxidants to furnish electrons is mirrored in their reducing power. The antioxidant potential of hickory meal enzymatic hydrolysates was effectively demonstrated through electron transfer and hydrogen transfer assessments. Notably, the strongest free radical scavenging capability was observed in DHMH, followed by DHM. Within the same concentration range, glutathione exhibited significantly superior antioxidant capacity in comparison to the other two chemicals.

3.4 Effects of DHMH on brain functions

3.4.1 Effects of DHMH on body weight gain and organ index in mice

Based on the recorded mice body weight during the feeding period and the recorded organ weights during the dissection process, the experimental results in Table 3 were calculated. The results showed that

the mice weight change in the model group was significantly lower than that of the control group ($P < 0.001$). This indicates that D-gal has a certain inhibitory effect on mice weight gain. However, in the mice treated with orally administered DHMH, there was no significant difference in weight change compared to the control group ($P > 0.05$). Furthermore, in terms of organ index measurement, there was also no significant difference in the liver, kidney, and spleen organ indices of the mice treated with orally administered DHMH compared to the control group ($P > 0.05$). This suggests that orally administered DHMH does not have a significant impact on the organs of the mice. Taking into account, these results of both weight changes and organ indices inferred that DHMH is a safe product with minimal side effects.

Table 3. The amount of body weight change and organ index of the mice. The values are presented as the mean ± SD (n= 10). The Model group was treated only with D-gal. $^{###}P < 0.001$ vs control, $^{**}P < 0.01$ vs model. DHMH, defatted hickory meal hydrolysate.

Group	Weight change (g)	Visceral index (%)		
		Liver	kidney	spleen
Control	25.68 ± 3.56	4.34 ± 0.51	1.37 ± 0.13	0.24 ± 0.06
Model	20.35 ± 2.17$^{###}$	4.63 ± 0.37	1.39 ± 0.14	0.23 ± 0.05
DHMH-L	21.62 ± 2.37	4.41 ± 0.37	1.38 ± 0.10	0.25 ± 0.06
DHMH-M	22.51 ± 2.07	4.41 ± 0.59	1.39 ± 0.11	0.23 ± 0.04
DHMH-H	24.95 ± 2.71**	4.35 ± 0.38	1.38 ± 0.11	0.24 ± 0.04

3.4.2 Effect of DHMH on spatial memory in the MWM

The MWM is an experimental paradigm involving the training of rodents, such as rats and mice, to navigate a pool of water and locate submerged platforms[32]. Its primary objective is to evaluate spatial orientation, as well as learning and memory capabilities associated with spatial and directional perception. D-gal can induce cognitive impairment in learning and memory. It is a monosaccharide commonly employed as an energy source and is implicated in energy metabolism. However, excessive D-gal intake can lead to the generation of free radicals and other oxidative by-products, disrupting cellular functions, compromising the activity of antioxidant enzymes within cells, and impeding cognitive functions[33].

The outcomes from the water maze test unveiled a significant extension in the incubation period of mice in the D-gal-treated group (58.27 ± 5.48 s) compared to the control group (12.18 ± 4.52 s) by 46.01 sec ($P < 0.001$, Fig. 2a). Additionally, the total distance swum in the water was significantly greater ($P < 0.001$, Fig. 2b), indicative of accelerated aging and substantial deterioration in learning and memory capacity due to the daily intraperitoneal injection of D-gal. These findings confirm the successful establishment of the aging model. In contrast to the model group, the mice administered with hickory meal hydrolysate exhibited an increased frequency of successful platform landing. The high-dose group, in particular, experienced a remarkable reduction in escape latency (35.13 ± 17.18 s), resulting in a 37.9% decrease ($P < 0.001$). In the high-dose group, the overall distance to the platform, which decreased from (917 ± 161) mm to (525 ± 172) mm, was substantially shorter ($P < 0.01$). These results suggest that hickory meal hydrolysate can decelerate the aging process in mice and enhance their learning and memory performance in water maze tasks.

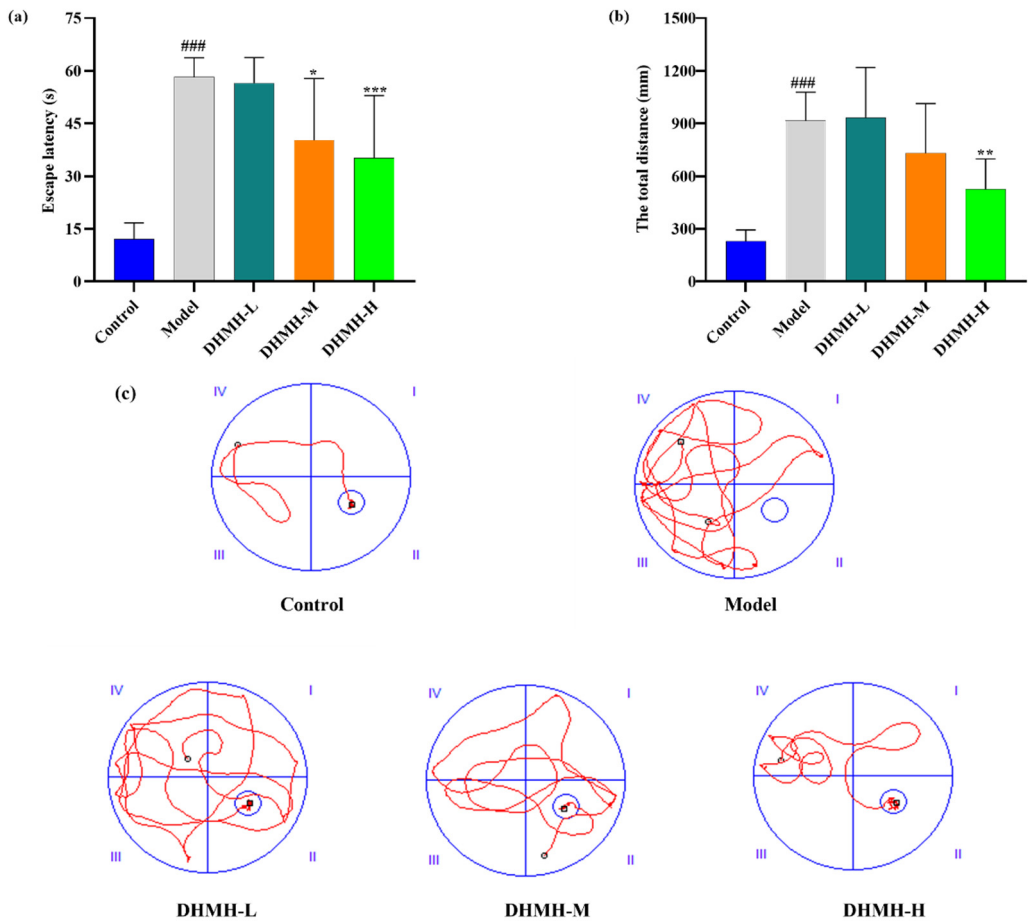

Figure 2. Effects of DHMH feeding on escape latency (a), total swimming distance (b), and represent tracks (c) on day 5 during the Morris water maze test in D-gal-treated mice. The model group was treated only with D-gal. Data are presented as means ± SEMs, n = 10 / group; $^{###}P < 0.001$ vs control, $^{*}P < 0.05$ vs model, $^{**}P < 0.01$ vs model, $^{***}P < 0.001$ vs model. DHMH, defatted hickory meal hydrolysate; SEM, standard error of the mean.

3.4.3. Spatial probe test

Figure 3 illustrates the mouse spatial exploration test, revealing that the treated group mice (12.28% ± 7.42%) exhibited a significantly lower ratio of activity time in the target quadrant compared to the control group mice (28.52% ± 14.90%) ($P < 0.001$, Fig. 3a) leading to a 16.24% reduction in the percentage of time spent in the target quadrant in comparison to the control group. In contrast, the high-dose DHMH group mice demonstrated an extended residence time (34.57% ± 11.67%) in the target quadrant, signifying a 22.3% increase compared to the D-gal-treated group ($P < 0.001$). The results from the spatial exploration and location navigation tests were consistent, supporting the notion that DHMH enhances memory in mice, with a more pronounced effect observed at higher doses. The findings from the MWM assay indicated a notable decrease in the learning and memory capacity of mice in the D-gal treatment group compared to the control group. This aligns with previous studies that have reported long-term D-gal treatment leading to adverse effects on learning and memory function in mice[3]. Importantly, DHMH alleviated D-gal's detrimental impact on learning and memory abilities.

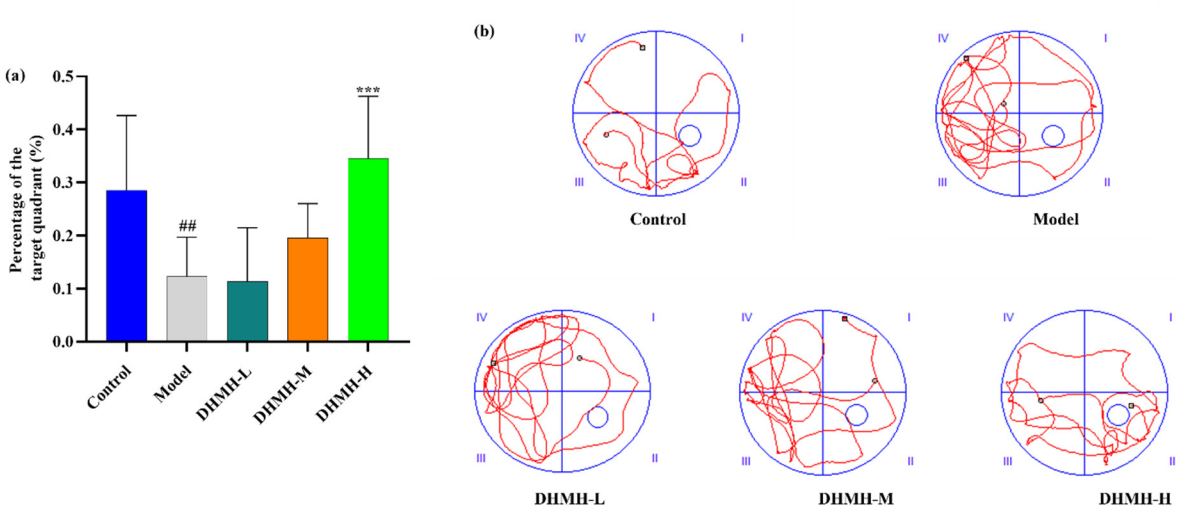

Figure 3. Effects of DHMH feeding on the percentage of the target quadrant (a) and represent tracks (b) on day 5 during the Morris water maze test in D-gal-treated mice. The model group was treated only with D-gal. Data are presented as means ± SEMs, n = 10 / group; $^{\#\#}P < 0.01$ vs control, $^{***}P < 0.001$ vs model.
DHMH, defatted hickory meal hydrolysate; SEM, standard error of the mean.

3.4.4. DHMH inhibited D-gal induced accumulation of Aβ1-42 in mice

The aging process disrupts the endosomal/lysosomal transport pathway critical for amyloid β (Aβ) metabolism by disrupting the interaction between dynein and dynactin[34], leading to intracellular accumulation of Aβ. This destruction is mainly affected by factors such as oxidative stress and neuroinflammation[35]. In addition, Aβ1-42 is now thought to be a major component of extracellular amyloidosis and to be associated with lesions within neurons[35]. As a common subtype of Aβ, Aβ1-42 has the characteristics of strong toxicity and easy aggregation, which can trigger the deposition of Aβ plaques, neurotoxicity, synaptic damage and neuronal death, thereby leading to the occurrence of AD[36]. According to the data presented in Fig. 4 significant difference in the Aβ1-42 content was detected between the model group and the control group in the hippocampus of mice ($P < 0.001$). The Aβ1-42 content was found highest in the model group (10.00 ng/ml), showing a 37.9% increase compared to the control group, further validating the successful construction of the model. After treatment with hickory residue enzyme hydrolysate by gavage, there was a certain degree of decrease in Aβ1-42 level. With regards to the groups to administer hickory residue enzyme hydrolysate by gavage, Aβ1-42 content reached 9.26 ng/ml, 8.83 ng/ml, and 8.31 ng/ml, respectively. Particularly, there was a significant difference ($P < 0.01$) between the high-dose group and the model group, indicating that the high-dose hickory residue enzyme hydrolysate had a significant effect in preventing the aggregation of Aβ1-42 protein.

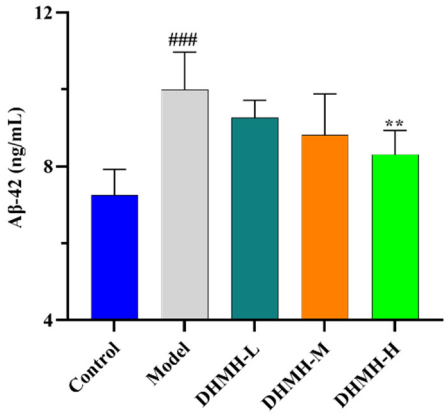

Figure 4. Effects of DHMH feeding on levels of $A\beta_{1\text{-}42}$ in the brains of D-gal-treated mice. The Model group was treated only with D-gal. $^{###}P < 0.001$ vs control, $^{**}P < 0.01$ vs model.
DHMH, defatted hickory meal hydrolysate.

3.4.5. Effects of DHMH on oxidative stress

Aging is mainly induced by oxidative stress, which refers to the excessive production of reactive oxygen species (ROS) within cells. When ROS interacts with cellular molecular structures, it leads to cell damage and impaired function[37]. The body's ability to respond to oxidative stress gradually diminishes with age, primarily as the antioxidant defenses ameliorated[38]. The antioxidant defense system encompasses a range of enzymes and molecules, including superoxide dismutase, glutathione peroxidase, and glutathione, which are responsible for eliminating free radicals from cells and maintaining cellular REDOX balance. Aging is mainly induced by oxidative stress, which refers to the excessive production of reactive oxygen species (ROS) within cells[37]. When ROS interacts with cellular molecular structures, it leads to cell damage and impaired function. The body's ability to respond to oxidative stress gradually diminishes with age, primarily because the antioxidant defenses weaken or degrade[2]. The antioxidant defense system encompasses a range of enzymes and molecules, including superoxide dismutase, glutathione peroxidase, and glutathione, which are responsible for eliminating free radicals from cells and maintaining cellular REDOX balance.

The cognitive decline associated with aging is closely linked to the impact of oxidative stress[3]. Therefore, we investigated the effect of DHMH on the D-gal-induced REDOX imbalance in mice. As a biomarker of lipid peroxidation, a significantly higher MDA concentration in the serum was observed in the D-gal-treated group ((15.35 ± 2.72) nmol/mL, $P < 0.01$, Fig. 5a). Meanwhile, the main components of cellular antioxidant system, GSH, GSH-PX, and SOD, were decreased simultaneously ((4.38 ± 0.21) mgGSH/L, (1224.73 ± 29.48) U/mL, and (306.60 ± 40.48) U/mL, respectively) ($P < 0.01$, $P < 0.001$, and $P < 0.001$, respectively) (Fig. 5b–5d). Consistent with prior results[39], the brains of mice exhibiting cognitive deficits demonstrated decreased SOD and GSH-Px activities, along with an elevated level of MDA. However, high-dose DHMH supplementation effectively alleviated D-gal induced increase of MDA and reduction of GSH, GSH-Px, and SOD levels ((9.78 ± 1.73) nmol/mL, (4.97 ± 0.27) mgGSH/L, (1303.35 ± 20.31) U/mL, and (409.39 ± 33.4) U/mL, respectively) ($P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.001$, respectively) (Fig. 5) in the serum. The activities of superoxide dismutase (SOD) and the content of malondialdehyde (MDA) can respectively reflect the antioxidant capacity and neuronal damage degree in

mice. The stronger the antioxidant capacity, the greater the protective effect on cells, indirectly reflecting the impact on learning and memory in mice. Likewise, Zhao et al.[40] reported that post treatment with sea cucumber peptide, the SOD activity in mouse serum increased while the MDA level decreased, and the mice performed well in brain behavior tests. Combined with the results of this study, DHMH effectively mitigated oxidative stress induced by D-gal, showcasing its potential as a preventive measure.

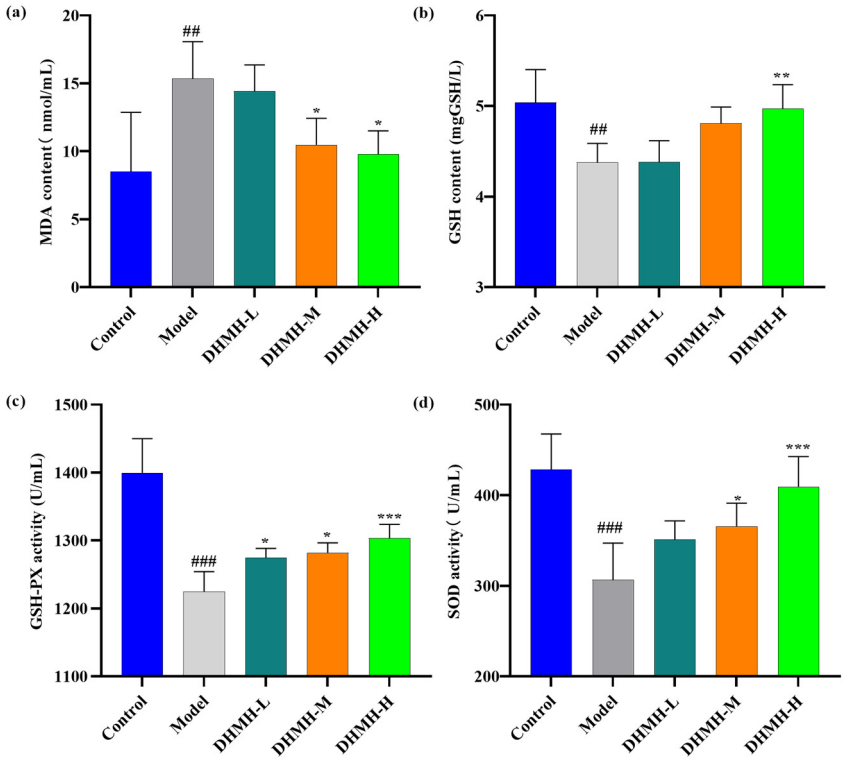

Figure 5. Effects of DHMH feeding on levels of MDA (a) and GSH (b), the activity of GSH-PX (c), and SOD (d) in from serum of D-gal-treated mice. The Model group was treated only with D-gal. $^{\#\#}P < 0.01$ vs control, $^{\#\#\#}P < 0.001$ vs control, $^{*}P < 0.05$ vs model, $^{**}P < 0.01$ vs model, $^{***}P < 0.001$ vs model.
DHMH, defatted hickory meal hydrolysate; MDA, malondialdehyde; GSH, glutathione; GSH-PX, glutathione peroxidase superoxide dismutase; SOD, superoxide dismutase.

3.4.6. Effects of DHMH on neuroinflammation factors

Representative inflammatory markers in cognitive impairment caused by aging include IL-1β, IL-6, and TNF-α, highlighting the significant role of neuroinflammation in this process[41]. More evidence shows that inflammation is one of the main causes of Aβ neurotoxicity and AD. Compared with the control group, D-gal induced a marked increase in expressions of IL-1β, IL-6, and TNF-α in the brain ((259.56 ± 10.18) pg/mL, (279.13 ± 45.54) pg/mL, (1196.77 ± 57.89) pg/mL, respectively) ($P < 0.01$, $P < 0.01$, and $P < 0.001$, respectively) (Fig. 6), which were significantly reversed by high-dose DHWH administration ((193.56 ± 35.67) pg/mL, (180.71 ± 28.46) pg/mL, and (934.60 ± 57.25) pg/mL, respectively) ($P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively) (Fig. 6). These results indicated that DHWH supplementation decreased D-gal-induced neuroinflammation in the hippocampus and cortex of mice. The observed outcome is consistent with the findings of Tian et al.[42], revealing that WKPH could effectively inhibit the expression of proinflammatory cytokines in aging mice, thus leading to a reduction in the inflammatory response. Experimental evidence suggests that DHMH may potentially ameliorate the effects of D-gal-induced aging

in mice by suppressing the secretion of inflammatory factors such as TNF-α, IL-16, and IL-1B by microglial cells.

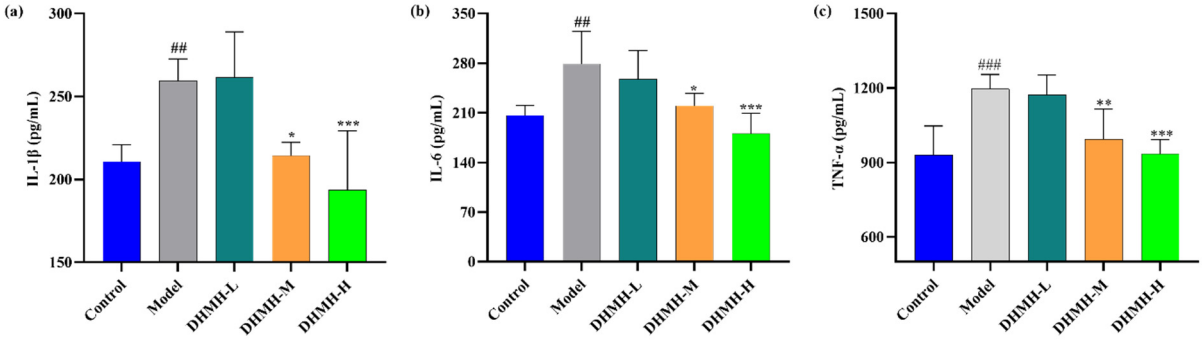

Figure 6. Effects of DHMH feeding on levels of IL-1β (a), IL-6 (b), and TNF-α (c) in the brain from D-gal-treated mice. The Model group was treated only with D-gal. $^{\#\#}P < 0.01$ vs control, $^{\#\#\#}P < 0.001$ vs control, $^{*}P < 0.05$ vs model, $^{**}P < 0.01$ vs model, $^{***}P < 0.001$ vs model.
DHMH, defatted hickory meal hydrolysate; IL-1β, interleukin 1β; IL-6, interleukin 6, and TNF-α, tumor necrosis factor alpha.

3.4.7. Effects of DHMH on hippocampal neurons

H&E staining is the most fundamental and widely used histological and pathological technique to reveal for organ status. Numerous brain illnesses are caused by neuronal and synaptic defects in the cerebral cortex and hippocampus[43]. In the control group, the neurons in the hippocampus CA1 (Fig. 7a) exhibited a well-organized arrangement with intact cellular structures, clear nucleus, and cytoplasm. However, D-gal administration resulted in a disorganized and loosely arranged CA1 region of the hippocampus (Fig. 7b). However, resolution of the lesion in the hippocampal CA1 region was observed after gavage of a high dose of pecan meal enzyme hydrolysate (Fig. 7c). A study has shown that memory loss is associated with disruption of hippocampal pyramidal cells and nuclear atrophy[44]. The hippocampal CA1 region is specifically involved in spatial orientation-related training-specific memory[45]. Based on behavioral experiments, biochemical indicators, and H&E observations of the mouse hippocampus, DHMH undeniably effectively alleviated hippocampal cell damage induced by D-gal, thereby maintaining cell vitality and integrity as much as possible, and improving mouse memory capabilities.

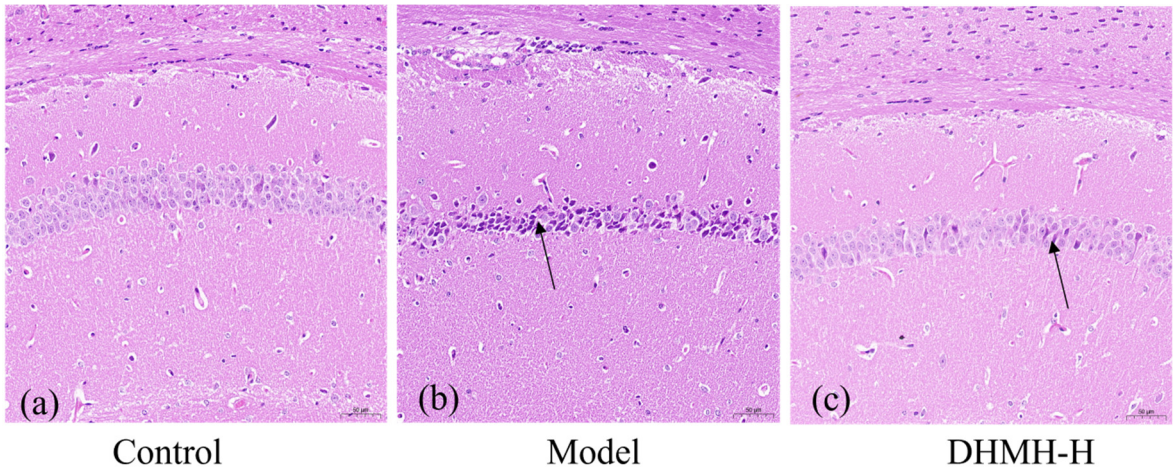

Figure 7. Effects of DHMH feeding on hippocampal neurons in D-gal-treated mice (20×). The Model group was treated only with D-gal. The black arrows indicate the cell body crinkling and hyperstaining..

3.4.8. Effect of DHMH on D-gal induced synaptic structural damage

PSD-95 is a scaffold protein that maintains synaptic connections and influences synaptic plasticity[46]. Decreased PSD-95 expression may result in synaptic dysfunction and deterioration of learning and memory. Synaptic dysfunction during neurodevelopment is closely associated with the onset of neurological illnesses[47]. D-gal exposure is harmful to the synaptic structure in the mouse hippocampus, leading to decreased postsynaptic density[48].

The impact of DHMH intervention on PSD-95 expression in mouse brain tissue was determined by Western blotting (WB) and immunohistochemistry analyses. Fig. 8a represents a WB of PSD-95. WB results revealed that compared with the control group (87.36% ± 4.52%), the relative expression of PSD-95 in the hippocampus of the D-gal-treated group (59.05% ± 6.61%) decreased by 28.31% ($P < 0.01$) (Fig. 8b). Interestingly, high-dose DHMH effectively increased the relative content of the synaptic functional protein PSD-95 in mice with memory impairment (102.34% ± 6.62%) ($P < 0.001$) (Fig. 8b).

In immunohistochemistry, cells positive for PSD-95 were designated as brown in granular form (Fig. 8c) at a mean density in the D-gal treated group (0.0036 ± 0.00184) was significantly lower than that in the control group (0.0507 ± 0.00934) ($P < 0.001$) (Fig. 8d), indicating that D-gal could inhibit the expression of PSD-95 protein. After DHMH intervention, the mean density of PSD-95 staining increased significantly (0.0239 ± 0.00591) ($P < 0.05$) (Fig. 8d). These results indicated that DHMH diet can effectively correct D-gal-induced damage and restore synaptic function.

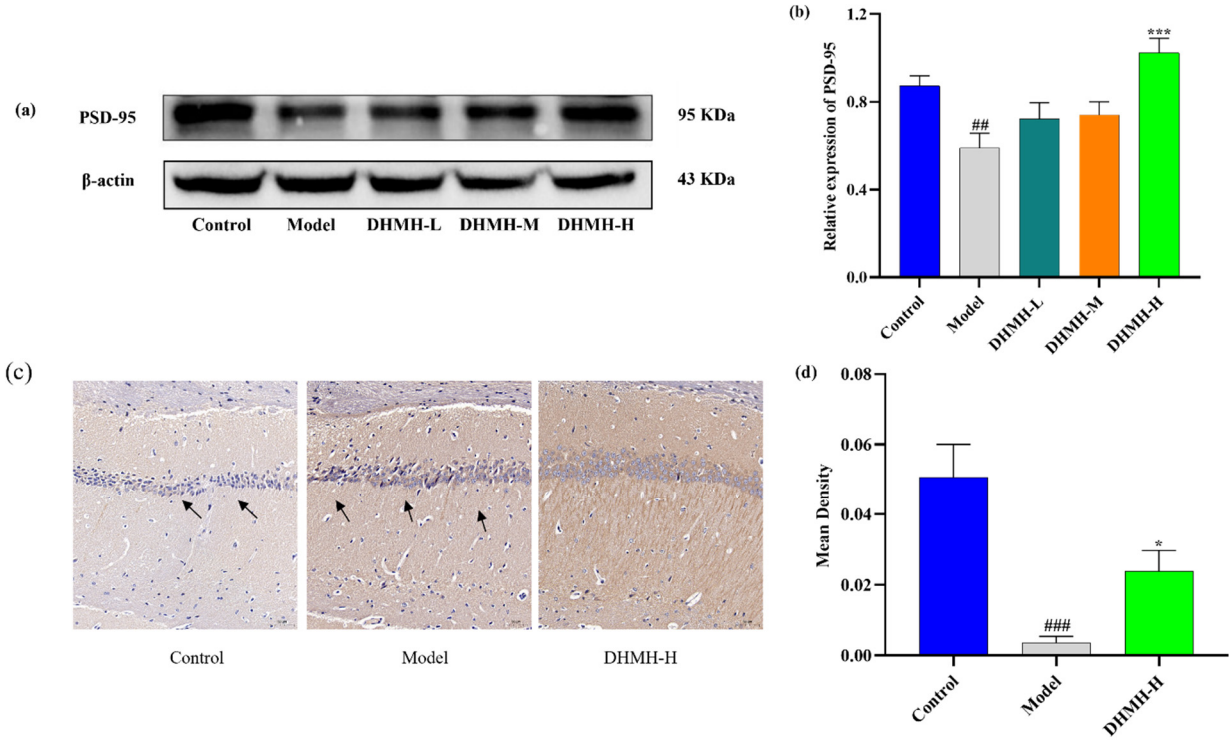

Figure 8. Effects of DHMH on D-gal induced synaptic structural damage in D-gal-treated mice. Western blot of PSD-95 (a), relative expression of PSD-95/β-actin in the hippocampus (b), the expression of PSD-95 positive cells in each group by immunohistochemical staining (20×), the black arrows represent a positive signal (c), immunohistochemical mean optical density of PSD-95 (d), The Model group was treated only with D-gal. $^{\#\#}P < 0.01$ vs control, $^{\#\#\#}P < 0.001$ vs control, $^{*}P < 0.05$ vs model, $^{***}P < 0.001$ vs model.

4. Conclusion

This study investigated the neuroprotective effects of DHMH in a D-gal-induced mise model of aging. DHMH, derived from defatted hickory meal through enzymatic hydrolysis, displayed strong antioxidant and free radical scavenging properties. In assay on mice with D-gal-induced memory impairment, DHMH significantly improved memory deficits. Morris Water Maze tests revealed enhanced learning and memory effects in DHMH-treated mice, as evidenced by reduced escape latency and total swimming distance. Further, DHMH also increased antioxidant enzyme activity, reducing oxidative damage caused by D-gal, and concurrent withalleviated accumulation of $A\beta_{1\text{-}42}$ and inflammatory cytokines, both associated with Alzheimer's disease. Immunohistochemical examination demonstrated that DHMH promoted hippocampal neurogenesis, while protein assay revealed prevention of reduced PSD-95 expression in the hippocampus as the underlying action mechanism. Overall, DHMH demonstrated promise in mitigating cognitive impairments and has the potential to be further develop in nutraceuticals targeting cognitive dysfunctions.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] A. Grimm and A. Eckert, Brain aging and neurodegeneration: from a mitochondrial point of view, J Neurochem. (2017) http://dx.doi/10.1111/jnc.14037.

[2] J.-S. Lee, H.-G. Kim, H.-W. Lee, et al., Hippocampal memory enhancing activity of pine needle extract against scopolamine-induced amnesia in a mouse model, Sci Rep. 5 (2015) 9651. http://dx.doi/10.1038/srep09651.

[3] J. Zhang, Y. Chen, Z. Wang, et al., The protective effect and mechanism of mangiferin on D-galactose-induced oxidative stress and cognitive impairment in aging mice by an integrated network pharmacology and experimental validation strategy, Food Frontiers. 4 (2023) 1927-1945. http://dx.doi/10.1002/fft2.306..

[4] W. Wu, B. Niu, L. Peng, et al., Recent advances on the effect of nut consumption on cognitive improvement, Food Frontiers. (2023) http://dx.doi/10.1002/fft2.298.

[5] S. I. Yamagishi, Role of Advanced Glycation Endproduct (AGE)-Receptor for Advanced Glycation Endproduct (RAGE) Axis in Cardiovascular Disease and Its Therapeutic Intervention, Circ J. 83 (2019) 1822-1828. http://dx.doi/10.1253/circj.CJ-19-0618.

[6] A. Remigante, R. Morabito, S. Spinelli, et al., d-Galactose Decreases Anion Exchange Capability through Band 3 Protein in Human Erythrocytes, Antioxidants. 9 (2020) 1-17. http://dx.doi/10.3390/antiox9080689.

[7] Y. Wen, L. Zhang, N. Li, et al., Nutritional assessment models for Alzheimer's disease: Advances and perspectives, Food Frontiers. 4 (2023) 624-640. http://dx.doi/10.1002/fft2.216.

[8] L. Lin, C. Li, T. Li, et al., Plant-derived peptides for the improvement of Alzheimer's disease: Production, functions, and mechanisms, Food Frontiers. 4 (2023) 677-699. http://dx.doi/10.1002/fft2.210.

[9] H. Chen, M. Zhao, L. Lin, et al., Identification of antioxidative peptides from defatted walnut meal hydrolysate with potential for improving learning and memory, Food Research International. 78 (2015) 216-223. http://dx.doi/10.1016/j.foodres.2015.10.008.

[10] Q. Xu, J. Wu, Y. Cao, et al., Photosynthetic characteristics of leaves and fruits of hickory (Carya cathayensis Sarg.) and pecan (Carya illinoensis K. Koch) during fruit development stages, Trees. 30 (2016) 1523-1534. http://dx.doi/10.1007/s00468-016-1386-5.

[11] E. Ros, A. Singh and J. H. O'Keefe, Nuts: Natural Pleiotropic Nutraceuticals, Nutrients. 13 (2021) http://dx.doi/10.3390/nu13093269.

[12] Y. Li, X. Y. Zhao, X. L. Jin, et al., Pecan Oil Influences Learning and Memory and NF-κB Levels in the Hippocampus of Rats after MWM Test, Advanced Materials Research. 912-914 (2014) 1957-1960. http://dx.doi/10.4028/www.scientific.net/AMR.912-914.1957.

[13] H. Chen, M. Zhao, L. Lin, et al., Identification of antioxidative peptides from defatted walnut meal hydrolysate with potential for improving learning and memory, Food Res Int. 78 (2015) 216-223. http://dx.doi/10.1016/j.foodres.2015.10.008.

[14] M. Wang, W. K. Amakye, L. Guo, et al., Walnut-Derived Peptide PW5 Ameliorates Cognitive Impairments and Alters Gut Microbiota in APP/PS1 Transgenic Mice, Mol Nutr Food Res. 63 (2019) e1900326. http://dx.doi/10.1002/mnfr.201900326.

[15] J. Jin, H. Ma, C. Zhou, et al., Effect of degree of hydrolysis on the bioavailability of corn gluten meal hydrolysates, J Sci Food Agric. 95 (2015) 2501-2509. http://dx.doi/10.1002/jsfa.6982.

[16] J. Rumpf, R. Burger and M. Schulze, Statistical evaluation of DPPH, ABTS, FRAP, and Folin-Ciocalteu assays to assess the antioxidant capacity of lignins, Int J Biol Macromol. 233 (2023) 123470. http://dx.doi/10.1016/j.ijbiomac.2023.123470.

[17] Y. Ma and Y. L. Xiong, Antioxidant and bile acid binding activity of buckwheat protein in vitro digests, J Agric Food Chem. 57 (2009) 4372-4380. http://dx.doi/10.1021/jf803670u.

[18] T.-Y. Lin, Y.-T. Wu, H.-J. Chang, et al., Anti-Inflammatory and Anti-Oxidative Effects of Polysaccharides Extracted from Unripe Carica papaya L. Fruit, Antioxidants. 12 (2023) 1506. http://dx.doi/10.3390/antiox12081506.

[19] D. Onder, Variation in antioxidant capacity, antioxidant activity and mineral composition during flower development of oil-bearing rose (Rosa damascena Mill.), Sci Rep. 13 (2023) 17255. http://dx.doi/10.1038/s41598-023-44461-4.

[20] W. Li, T. Zhao, J. Zhang, et al., Comparison of Neuroprotective and Cognition-Enhancing Properties of Hydrolysates from Soybean, Walnut, and Peanut Protein, Journal of Chemistry. 2016 (2016) 1-8. http://dx.doi/10.1155/2016/9358285.

[21] Z. Yuan, H. Zhou, N. Zhou, et al., Dynamic Evaluation Indices in Spatial Learning and Memory of Rat Vascular Dementia in the Morris Water Maze, Sci Rep. 9 (2019) 7224. http://dx.doi/10.1038/s41598-019-43738-x.

[22] Y. Gao, Z. Zong, W. Xia, et al., Hepatoprotective effect of water bamboo shoot (Zizania latifolia) extracts against acute alcoholic liver injury in a mice model and screening of bioactive phytochemicals, Food Frontiers. (2023) http://dx.doi/10.1002/fft2.217.

[23] S. Ketnawa, M. Wickramathilaka and A. M. Liceaga, Changes on antioxidant activity of microwave-treated protein hydrolysates after simulated gastrointestinal digestion: Purification and identification, Food Chem. 254 (2018) 36-46. http://dx.doi/10.1016/j.foodchem.2018.01.133.

[24] L. Zhang, Y.-Y. Bai, Z.-S. Hong, et al., Isolation, Identification, Activity Evaluation, and Mechanism of Action of Neuroprotective Peptides from Walnuts: A Review, Nutrients. 15 (2023) http://dx.doi/10.3390/nu15184085.

[25] Y. Li, Y. Zhao, X. Li, et al., Biological and therapeutic role of LSD1 in Alzheimer's diseases, Front Pharmacol. 13 (2022) 1020556. http://dx.doi/10.3389/fphar.2022.1020556.

[26] J. Chen and K. Herrup, Glutamine acts as a neuroprotectant against DNA damage, beta-amyloid and H2O2-induced stress, PLoS One. 7 (2012) e33177. http://dx.doi/10.1371/journal.pone.0033177.

[27] F. M. Issoglio, N. Campolo, A. Zeida, et al., Exploring the Catalytic Mechanism of Human Glutamine Synthetase by Computer Simulations, Biochemistry. 55 (2016) 5907-5916. http://dx.doi/10.1021/acs.biochem.6b00822.

[28] D. Ren, F. Zhao, C. Liu, et al., Antioxidant hydrolyzed peptides from Manchurian walnut (Juglans mandshurica Maxim.) attenuate scopolamine-induced memory impairment in mice,J Sci Food Agric. 98 (2018) 5142-5152. http://dx.doi/10.1002/jsfa.9060.

[29] D. Liu, Y. Guo, P. Wu, et al., The necessity of walnut proteolysis based on evaluation after in vitro simulated digestion: ACE inhibition and DPPH radical-scavenging activities, Food Chem. 311 (2020) 125960. http://dx.doi/10.1016/j.foodchem.2019.125960.

[30] R. M. Ibrahim, P. M. Abdel-Baki, G. F. Elmasry, et al., Combinative effects of akarkara root-derived metabolites on anti-inflammatory and anti-alzheimer key enzymes: integrating bioassay-guided fractionation, GC-MS analysis, and in silico studies, BMC Complement Med Ther. 23 (2023) 413. http://dx.doi/10.1186/s12906-023-04210-6.

[31] R. a. Apak, M. Özyürek, K. Güçlü, et al., Antioxidant activity/capacity measurement. 1. Classification, physicochemical principles, mechanisms, and electron transfer (ET)-based assays, J Agric Food Chem. 64 (2016) 997-1027. http://dx.doi/10.1021/acs.jafc.5b04739.

[32] B. H. Chen, J. H. Park, D. W. Kim, et al., Melatonin Improves Cognitive Deficits via Restoration of Cholinergic Dysfunction in a Mouse Model of Scopolamine-Induced Amnesia, ACS Chem Neurosci. 9 (2018) 2016-2024. http://dx.doi/10.1021/acschemneuro.7b00278.

[33] Y. Wei, D. Liu, Y. Zheng, et al., Protective effects of kinetin against aluminum chloride and D-galactose induced cognitive impairment and oxidative damage in mouse, Brain Res Bull. 134 (2017) 262-272. http://dx.doi/10.1016/j.brainresbull.2017.08.014.

[34] S. Koinuma, N. Shimozawa, Y. Yasutomi, et al., Aging induces abnormal accumulation of Aβ in extracellular vesicle and/or intraluminal membrane vesicle-rich fractions in nonhuman primate brain, Neurobiol Aging. 106 (2021) 268-281. http://dx.doi/10.1016/j.neurobiolaging.2021.06.022.

[35] R. Coronel, A. Bernabeu-Zornoza, C. Palmer, et al., Role of amyloid precursor protein (APP) and its derivatives in the biology and cell fate specification of neural stem cells, Molecular neurobiology. 55 (2018) 7107-7117. http://dx.doi/10.1007/s12035-018-0914-2.

[36] C. Song, T. Zhang and Y. Zhang, Conformational Essentials Responsible for Neurotoxicity of Abeta42 Aggregates Revealed by Antibodies against Oligomeric Abeta42, Molecules. 27 (2022) http://dx.doi/10.3390/molecules27196751.

[37] J. A. Bolduc, J. A. Collins and R. F. Loeser, Reactive Oxygen Species, Aging and Articular Cartilage Homeostasis, Free Radic Biol Med. 132 (2018) S0891584918315004-. http://dx.doi/10.1016/j.freeradbiomed.2018.08.038.

[38] U. E. Warraich, F. Hussain and H. U. R. Kayani, Aging - Oxidative stress, antioxidants and computational modeling, Heliyon. 6 (2020) e04107. http://dx.doi/10.1016/j.heliyon.2020.e04107.

[39] D. P. Wu, W. Yi, Y. D. Zhao, et al., Gliclazide Ameliorates Neuronal Injury by Attenuating Oxidative Stress in D-gal-Induced Senescent Cells and Aging Mice, Mol Neurobiol. (2023) http://dx.doi/10.1007/s12035-023-03850-6.

[40] J. Zhao, X. Liu, D. Chang, et al., Low-Dose Ketamine Improves LPS-Induced Depression-like Behavior in Rats by Activating Cholinergic Anti-inflammatory Pathways, ACS Chem Neurosci. 11 (2020) 752-762. http://dx.doi/10.1021/acschemneuro.9b00669.

[41] C. Zong, R. Hasegawa, M. Urushitani, et al., Role of microglial activation and neuroinflammation in neurotoxicity of acrylamide in vivo and in vitro, Arch Toxikol. 93 (2019) 2007-2019. http://dx.doi/10.1007/s00204-019-02471-0.

[42] J. Zhang, J. Wu, F. Liu, et al., Neuroprotective effects of anthocyanins and its major component cyanidin-3-O-glucoside (C3G) in the central nervous system: An outlined review,Eur J Pharmacol. 858 (2019) 172500. http://dx.doi/10.1016/j.ejphar.2019.172500.

[43] W. Tian, B. Wu, L. Sun, et al., Protective effect against d-gal-induced aging mice and components of polypeptides and polyphenols in defatted walnut kernel during simulated gastrointestinal digestion, J Food Sci. 86 (2021) 2736-2752. http://dx.doi/10.1111/1750-3841.15744.

[44] W. Tan, Q. Zhang, Z. Dong, et al., Phosphatidylcholine Ameliorates LPS-Induced Systemic Inflammation and Cognitive Impairments via Mediating the Gut-Brain Axis Balance, J Agric Food Chem. 68 (2020) 14884-14895. http://dx.doi/10.1021/acs.jafc.0c06383.

[45] Q. Hou, X. Gao, X. Zhang, et al., SNAP-25 in hippocampal CA1 region is involved in memory consolidation, Eur J Neurosci. 20 (2004) 1593-1603. http://dx.doi/10.1111/j.1460-9568.2004.03600.x.

[46] D. T. Proctor, E. J. Coulson and P. R. Dodd, Reduction in post-synaptic scaffolding PSD-95 and SAP-102 protein levels in the Alzheimer inferior temporal cortex is correlated with disease pathology, Journal of Alzheimer's Disease. 21 (2010) 795-811. http://dx.doi/10.3233/JAD-2010-100090.

[47] K. Stachowicz, P. Panczyszyn-Trzewik, M. Sowa-Kucma, et al., Changes in working memory induced by lipopolysaccharide administration in mice are associated with metabotropic glutamate receptors 5 and contrast with changes induced by cyclooxygenase-2: Involvement of postsynaptic density protein 95 and down syndrome cell adhesion molecule, Neuropeptides. 100 (2023) 102347. http://dx.doi/10.1016/j.npep.2023.102347.

[48] H. Buchanan, M. Mackay, K. Palmer, et al., Synaptic Loss, ER Stress and Neuro-Inflammation Emerge Late in the Lateral Temporal Cortex and Associate with Progressive Tau Pathology in Alzheimer's Disease, Mol Neurobiol. 57 (2020) 3258-3272. http://dx.doi/10.1007/s12035-020-01950-1.