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Research Article

Comparison of Neuroprotective and Cognition-Enhancing Properties of Hydrolysates from Soybean, Walnut, and Peanut Protein

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Hydrolysates were prepared from soybean, walnut, and peanut protein by papain, respectively. Their amino acid compositions and molecular weight distributions, the effects of various hydrolysates on H_2O_2-induced injury PC12 cells, and cognition of mice were investigated, respectively. Results showed that the three hydrolysates were dominated by the peptides with 1–3 KDa with large amount of neurotrophic amino acids. All the hydrolysates exhibited much stronger inhibitory activity against H_2O_2-induced toxicity than cerebrolysin, and soy protein hydrolysate showed the highest activity. Moreover, the hydrolysates also could reduce the rate of nonviable apoptotic cells at the concentration of 2 mg/mL. The test of animal's cognition indicated that three hydrolysates could present partly better effect of improving recurred memory ability of normal mice and consolidated memory ability of anisodine-treated mice than piracetam. Therefore, soybean, walnut, and peanut protein hydrolysates were recommended as a potential food raw material for prevention or treatment of neurodegenerative disorders.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease of the brain. The population suffering from AD is currently up to 20 million worldwide and is predicted to be between 30 and 40 million worldwide by 2050 [1]. According to many studies, two factors related to AD progress have received considerable attention, which referred to antioxidative and neuroprotective functions [2]. Therefore, the discovery of new materials with neurotrophic and neuroprotective properties is becoming a new strategy for the treatment of neurodegenerative disorders. Specific daily dietary foods have also been recognized as likely contributors to a greater or lower risk of developing AD. For example, high intake of fish is associated with a lower risk of AD due to the high level of omega-3 fatty acids [3]. Other foods associated with decreased risk of AD include folic acid-containing foods such as green vegetables, citrus fruits, liver, and whole grains [4].

Walnut has been used in traditional medicine for treatment of various diseases owing to its polyphenols or polyunsaturated fat [5]. Meanwhile, its antioxidative and angiotensin-I-converting-enzyme (ACE) inhibitory functions [6] and other biological functions have also been reported. Additionally, the antioxidant activity and ACE inhibitory activity were also discovered in peanut protein hydrolysate [7]. Recently, peptides have been shown to have neurotrophic and neuroprotective potential as demonstrated both *in vivo* and *in vitro*. Liu et al. [8] reported that soybean peptides could protect PC12 cells against H_2O_2-induced lipid peroxidation and cell apoptosis as a neuroprotective agent. It was reported that cerebrolysin is an effective means of treating cerebral vascular insufficiency patients. It could decrease the severity of memory and attention impairments to improve the overall cognitive status of the patients [9]. Thus, peptides derived from food could possess multiple mechanisms of action on alleviating cognitive deficits.

The objective of this study is to compare the antioxidative and neuroprotective properties of hydrolysates from soybean protein, walnut protein, and peanut protein, respectively. To clarify their different effects on alleviating memory deficits, the antioxidative, PC12 cell protection, and behavioral tests were conducted.

2. Experimental

2.1. Materials. Defatted peanut meal was purchased from Shandong Luhua Group Co. Ltd. Defatted soybean meal was purchased from Yuwang Soy Company (Shandong, China). Defatted walnut meal was purchased by Huizhiyuan Food Co. Ltd. (Lincang, China). Papain was obtained from Guangzhou Huaqi Biotechnology Co. (Guangzhou, China). All the other chemicals and solvents were of analytical grade.

2.2. Preparation of Hydrolysates. Fifty grams of protein materials (defatted soybean meal, defatted walnut meal, and defatted peanut meal) was mixed with 100 mL of deionized water, respectively, and homogenized at 10,000 rpm for 1 min using an Ultra Turrax homogenizer (Beijing Jingke Huarui Instrument Co. Ltd., Beijing, China). The three homogenates were all preincubated at 55°C for 20 min prior to enzymatic hydrolysis using papain. All the mixtures were conducted at pH 7.0, respectively. The pH of the slurries was constantly maintained during hydrolysis by addition of 2 M NaOH. When the degree of hydrolysis reached 10%, the enzyme was inactivated at 95°C for 15 min. The hydrolysates were centrifuged in a GL-21M refrigerated centrifuge (Xiangyi Instrument Co. Ltd., Changsha, China) at 5,000 ×g for 20 min at 20°C and the supernatants were collected, lyophilized (R2L-100KPS, Kyowa Vacuum Engineering, Tokyo, Japan), and stored in a desiccator for further analysis. Hydrolysate from soybean protein will be expressed as SPH, from walnut protein as WPH, and from peanut protein as PPH for short, respectively.

2.3. Molecular Weight Distribution of Peptides in SPH, WPH, and PPH. The molecular weight distribution of peptides in SPH, WPH, and PPH was determined by gel filtration chromatography, at a wavelength of 214 nm using an HPLC system equipped with a TSK gel G2000 SWXL analytical column, respectively. The mobile phase (isocratic elution, 0.02 M sodium phosphate buffer) was at a flow rate of 0.5 mL/min. Six protein and peptide standards, conalbumin (75,000 Da), ovalbumin (43,000 Da), cytochrome C (12,384 Da), aprotinin (6,512 Da), vitamin B12 (1,855 Da), and glutathione (307 Da), were used to establish a reference calibration curve. The fitting line equation, that is, the logarithm of the relative MW versus elution volume, was $y = 0.1547x + 5.6431$ ($R^2 = 0.9957$), where y is the logarithm of standard peptide MW and x is the elution volume.

2.4. Amino Acid Composition of SPH, WPH, and PPH. Amino acid analysis was performed following the method of Fujiwara et al. [10] using an A300 auto-amino acid analyzer (MembraPure, Bodenheim, Germany) based on o-phthalaldehyde derivatives.

2.5. NGF-Differentiated PC12 Cells Culture. PC12 cells were cultured in 25 cm^2 flasks in growth medium composed of Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal calf serum (FCS), 10% horse serum (HS), 10,000 U/mL penicillin, and 100 μg/mL streptomycin. Cells were grown at 37°C, in a humidified atmosphere of 5% CO_2. Actively proliferating PC12 cells (2×10^5 cells/well) were seeded onto 12-well plates precoated with 200 μg/mL collagen type-I and differentiated with 50 ng/mL Nerve Growth Factor (NGF) for 2 days. Fresh medium-containing NGF was changed every three days. In all experiments, only NGF-differentiated PC12 cells were used. The morphologic observation and the detection of microtubule-associated protein 2 (MAP2) expression by immunohistochemistry test were conducted to identify the differentiated neurons.

2.6. MTT Cell Proliferation Assay. For assay performance, the NGF-differentiated PC12 cells were harvested from the flasks using a 0.25% trypsin/EDTA 1x-solution (Gibco) and were counted using a CASY cell counter (Roche) and 5×10^3 cells per well (100 μL) were seeded in coated 96-well-plate. After 72 hours, SPH, WPH, PPH, and cerebrolysin were individually added into the wells with the final concentrations of 5, 2, 1, 0.5, 0.2, 0.1, 0.05, and 0 mg/mL. The 96-well plate was again incubated for 24 hours following MTT assay. MTT (0.5 mg/mL) were added to each well and the plate was incubated for another 4 h at 37°C. Then, the supernatant was removed, and 100 μL of dimethyl sulfoxide was added to each well. MTT metabolism was measured spectrophotometrically at 490 nm in a Biorad microplate reader (Biotek Instruments, Burlington, VT, USA). Results were expressed as the percentage of MTT reduction, taking the absorbance of control cells as 100%.

2.7. Flow Cytometry Analysis of Apoptosis. Flow cytometry analysis of apoptosis was done to detect the possible proapoptotic effects of SPH, WPH, and PPH. The NGF-differentiated PC12 was seeded at a concentration of 5×10^3 cells/well in a 96-well plate and grown in DMEM medium containing 5% FCS and 10% HS for 72 h at 37°C. Then, cells were treated with 0.1 mmol/L of H_2O_2 for 30 min and were individually preincubated with SPH (0, 1, 2, and 5 mg/mL), WPH (0, 1, 2, and 5 mg/mL), PPH (0, 1, 2, and 5 mg/mL), and cerebrolysin (0, 1, 2, and 5 mg/mL) for 48 h. Cells without treatment of H_2O_2 were taken as control. The cell samples were trypsinized and then centrifuged at 1000 rpm for 5 min. The cells were resuspended with 500 μL binding buffer at a concentration of 10^6 cells/mL, after washing two times with PBS at 1000 rpm for 5 min. Then, 5 mL FITC-conjugated annexin V and 5 mL PI were added to the cells and incubated at room temperature for 15 min in the dark. The samples were analyzed by flow cytometry within 1 h after staining. Experiments were repeated three times.

2.8. Animal Experiments. NIH (National Institutes of Health) mice were used to detect the effects of SPH, WPH, and PPH on cognition. Seventy-five health female mice, 20 ± 2 g from Guangdong Medical Lab Animal Center, were randomly divided into 9 groups (C, H_1, H_2, H_3, HA_1, HA_2, HA_3, CA, and P). Group C is the control group with oral administration of distilled water; Group H_1 with oral administration of SPH; Group H_2 with oral administration of WPH; Group H_3 with oral administration of PPH; Group HA_1 with oral administration of SPH and anisodine in turn; Group HA_2 with oral administration of WPH and anisodine in turn; Group HA_3 with oral administration of PPH and anisodine in turn; Group CA with oral administration of distilled water and anisodine in turn as a negative control; and Group P with oral administration of piracetam as a positive control. After adaption for 1 week in lab room, sample and medicine were orally administrated for 4 weeks as follows: Group H_1 and Group HA_1 with a dose of 333.3 mg/kg SPH, Group H_2 and Group HA_2 with a dose of 333.3 mg/kg WPH, Group H_3 and Group HA_3 with a dose of 333.3 mg/kg WPH, Group C and Group CA with equivalent distilled water, and Group P with a dose of 800 mg/kg piracetam. The doses of SPH, WPH, PPH and piracetam were 10 times that of human adult with 60 kg body weight. Then, all groups were trained and tested in a platform recorder (BW-YLS-3TB, Shanghai Biowill Co. Ltd.). During the experiments, sample and medicine were continuously and individually administrated as usual. But a dose of 10 mg/kg anisodine was administrated to Group HA_1, Group HA_2, Group HA_3, and Group CA after 1 h of the last administration at the 4th week treatment.

The training and testing experiments were performed at the second day of the last administration for Groups C, H_1, H_2, and H_3; at 10 min later of last administrating anisodine for Groups HA_1, HA_2, HA_3, and CA. At the beginning of experiments, all mice were placed in the platform recorder. After adaption for 3 min, 36 V alternating current was used to stimulate them. Given one time of training, the learned score (obtained memory, OM) was then recorded as the latent period of first jumping to platform [11] and times of wrong jumping in 5 min (WT). After 24 h, mice were retested to record the latent time of first jumping to platform, times of wrong jumping in 3 min, and quantity of animals shocked by electric (ESA). Consolidated memory (CM) was calculated as the percentage of wrong reactive animals (WRA, (1)). After 5 days later, memory recessive experiment was performed with the procedure of retesting and recorded as recurred memory (RM):

$$\text{WRA\%} = \frac{\text{the quantity of ESA}}{\text{total animals in one group}} \times 100\%. \quad (1)$$

All experimental protocols were approved by the local Ethical Committee, and all experiments were performed in accordance with the NIH Guide for Care and Use of Laboratory Animals (1985).

2.9. Statistical Analysis. Statistical analysis was performed using the statistical package SPSS 17.0 (SPSS Inc., Chicago, IL) with one-way ANOVA. Duncan's multiple range test and the independent-sample *t*-test were employed for comparing mean values and evaluating significant differences (at $p < 0.05$, 0.01, and 0.001) among treatments. When necessary, data are expressed as mean ± SD, and variance analysis was carried out using Origin 7.5.

Table 1: Amino acid composition (g/kg) in SPH, WPH, and PPH*.

	SPH	WPH	PPH
Asp	111.1 ± 3.7^{a}	$103.0 \pm 5.2^{a,b}$	97.2 ± 7.5^{b}
Thr	45.5 ± 0.9^{a}	38.3 ± 1.0^{b}	34.2 ± 2.5^{c}
Ser	67.1 ± 1.8^{a}	43.7 ± 1.4^{b}	44.3 ± 1.9^{b}
Glu	189.3 ± 3.5^{b}	196.7 ± 8.5^{b}	227.8 ± 6.4^{a}
Gly	61.3 ± 0.9^{a}	43.4 ± 1.9^{b}	64.5 ± 2.6^{a}
Ala	71.2 ± 1.1^{b}	82.9 ± 4.1^{a}	40.4 ± 5.1^{c}
$(Cys)_2$	5.3 ± 0.2^{a}	2.1 ± 0.4^{b}	5.0 ± 0.6^{a}
Val	56.3 ± 2.3^{a}	$52.6 \pm 2.6^{a,b}$	46.5 ± 4.4^{b}
Met	16.3 ± 0.5^{b}	31.9 ± 3.1^{a}	17.5 ± 2.0^{b}
Ile	46.4 ± 1.8^{a}	36.6 ± 1.7^{b}	40.8 ± 3.9^{b}
Leu	76.1 ± 2.8^{a}	65.9 ± 2.4^{b}	65.1 ± 3.2^{b}
Tyr	27.3 ± 2.0^{c}	45.4 ± 1.2^{a}	41.2 ± 1.2^{b}
Phe	36.8 ± 0.8^{c}	46.5 ± 1.4^{a}	43.9 ± 1.8^{b}
His	27.1 ± 0.7^{b}	22.4 ± 2.5^{c}	44.5 ± 2.0^{a}
Lys	57.6 ± 2.4^{a}	24.9 ± 1.4^{c}	37.0 ± 1.5^{b}
Arg	66.0 ± 1.6^{c}	128.3 ± 6.5^{a}	107.3 ± 6.8^{b}
Pro	39.4 ± 0.9^{b}	35.4 ± 1.3^{c}	42.8 ± 2.0^{a}

*The values in the same row followed by different letters are significantly different ($p < 0.05$).

3. Results and Discussion

3.1. Amino Acids Composition Analysis. The amino acid composition of proteins and peptides is widely recognized as an important index of their function and bioactivity [12]. The amino acid composition of SPH, WPH, and PPH is shown in Table 1. Obviously higher contents of Glu (189.2–227.8 g/kg) and Asp (97.2–111.1 g/kg) were observed in all the protein hydrolysates, due to their plant-derived protein sources. The highest ($p < 0.05$) content of Asp, Thr, Ser, Gly, Ile, Leu, and Lys was observed in SPH, while WPH had the most Ala, Met, Phe, and Arg and PPH had higher contents of Glu, His, and Pro.

Some amino acids have been reported to have effect on cognitive performance. *In vitro*, amino acids show some influences on neurite outgrowth and synaptic plasticity. Ser was recently shown to promote process outgrowth and differentiation of chick retinal explants [13]. SPH exhibited the highest content of Ser; this might be useful for its neurotrophic function. Arginine, as a biosynthesis precursor, would be transformed to nitric oxides (NO) in a NADPH-dependent reaction, which could modulate learning and memory in cognitive performance [14]. And Paul et al. [15] found that the administration of Arg exhibited strong inhibitory activity against picrotoxin impairing on both learning and memory processes. The order of Arg content was WPH > PPH > SPH. Therefore, the effects of different

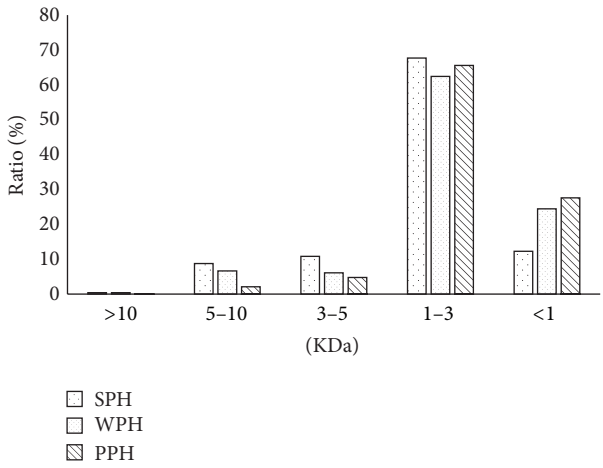

Figure 1: The molecular weight distribution of peptides from SPH, WPH, and PPH.

protein hydrolysates on memory improving might be variety with the content of Arg. Tyrosine/phenylalanine depletion lowers dopamine synthesis which involves reinforcement, motor control, and frontal lobe functions and impairs working memory performance [16]. The highest content of Tyr and Phe was observed in WPH. This might be good for its function on working memory. The contents of above amino acids were relatively high in the three protein hydrolysates, and they would be potentially effective on cognitive performance. However, another study showed that cerebrolysin (peptide/amino acids mixtures) could protect the cell against degeneration and apoptotic effects, while amino acids mixture (the same amino acid ratio of the free amino acids naturally found in cerebrolysin) could not show the corresponding effect [13]. In the results, the relationship between amino acids and efficacy was not clearly direct relationship, suggesting that the function of active peptide may be related to the specific peptide segment.

3.2. Molecular Weight Distribution of Hydrolysates. As shown in Figure 1, all the hydrolysates (SPH, WPH, and PPH) were rich in fractions with molecular weight <10 KDa and were dominated by the fractions with 1–3 KDa. It indicated that the proteins were greatly degraded by papain. It was notable that ratios for 5–10 KDa, 3–5 KDa, and 1–3 KDa fractions were relatively higher (8.77%, 10.82%, and 67.70%, resp.), and only 12.30% of <1 KDa was observed in SPH. Moreover, for WPH, much more large peptides were degraded into small peptides; the percentages for all the three fractions were smaller than that for SPH, whereas PPH was mainly composed of 1–3 KDa and <1 KDa fractions (65.54% and 27.6%). Results revealed that the peanut proteins were greatest hydrolyzed into small peptides by papain, followed by walnut protein and soybean protein.

Proteins were degraded into peptides with various bioactivity, and it is reported that small peptides showed stronger antioxidant activity, especially the peptides with 1–3 KDa [17]. As the results show, it was suggested that the three protein hydrolysates showed strong bioactivity and potential cytoprotective benefits. However, there was little literature on the relationship between peptide molecular weight and the function of improving memory. Arginine vasopressin (AVP), as a octapeptide, showed lower behavioral activity than its hexapeptide ([4–9] AVP) and tetrapeptide ([5–8] AVP) fragments in a radial maze test [18], while both [6–8] AVP and [5–7] AVP demonstrated no activity in the same test. It indicated that the function of active peptide may be related to the specific peptide segment, not direct relationship to molecular weight [19].

3.3. Characterization of the PC12 Cell Model. The models of H_2O_2 damaged PC12 cells and anisodine-treated mice were utilized in some studies [20–24].

As shown in Figure 2(a), initially, PC12 cells grew along the plate wall showing ovoid or polygon shaped with discernible cell boundaries. And no cytoplasmic extensions or neurites are present. Exposure to 50 ng/mL NGF and PC12 line began to extend neurite after 24 h and, by 4–10 days, displayed dense neurite networks (Figure 2(b)).

Microtubule-associated protein 2 (MAP2) is a common nerve cells marker, due to be abundance in dendrites *in vivo* in almost all neurites. Figure 2(c) showed that no brown (pictures were not showing the color) was observed in cytoplasm of PC12 cells. Interestingly, after 7 days of exposure to NGF, cytoplasm and neurite all showed obviously brown (Figure 2(d)). It revealed that the NGF-treated PC12 cells had the characteristic of secreting nerve cells.

In order to analyze the protective effects of the protein hydrolysates (SPH, WPH, and PPH), oxidative stress was induced in PC12 cells by hydrogen peroxide (H_2O_2). A clear dose dependency of viability after H_2O_2 (0.005 mM–4.0 mM) treatment was exhibited by MTT method. And H_2O_2 at concentration of 0.1 mM was used for further experiments because of the resulted viability of 61.76% (data not shown). As shown in Figure 3, in low concentration (0.10–0.20 mg/mL), only SPH exhibited significantly ($p < 0.05$) inhibitory activity against H_2O_2-induced toxicity, while significantly ($p < 0.01$) inhibitory activities were observed in SPH and PPH with 5.00 mg/mL and 1.00 mg/mL, suggesting that they could exhibit various neurotrophic effects results in no significantly harm to PC12 cells.

3.4. Flow Cytometry Results of SPH, WPH, and PPH Pretreatment in H_2O_2-Induced PC12 Cell Apoptosis. Flow cytometry was applied to study the neuroprotective capacity in cultured PC12 cells of SPH, WPH, and PPH. According to Table 2, when the PC12 cells were treated with 0.1 mM H_2O_2, nonviable apoptotic cells and viable apoptotic cells significantly ($p < 0.05$) increased to 24.63% and 63.31%, respectively, while viable cells significantly ($p < 0.05$) decreased to 11.62%. No effects on viability were found by SPH treatment at 1 mg/mL, whereas viable cells significantly ($p < 0.01$) increased to 22.07%, and nonviable apoptotic cells significantly ($p < 0.01$) decreased to 15.60% in the presence of 2 mg/mL SPH. It is notable that inverse results were obtained with the concentration increasing to 5.00 mg/mL, viable apoptotic cells displayed drastic decrease to 7.94% ($p < 0.01$), and

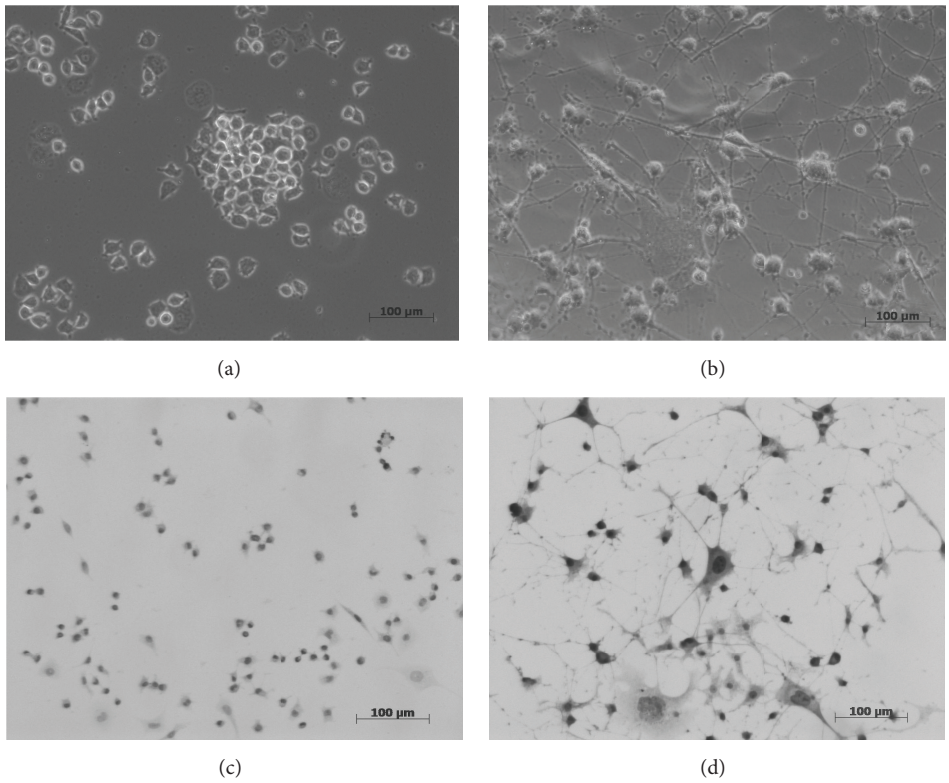

Figure 2: The characterization of the PC12 cell model. (a)/(b) The morphologies of PC12 cells before/after exposure to NGF. (c)/(d) The staining results of microtubule-associated protein 2 (MAP2) before/after exposure to NGF. (Scale bar = 100 μm.)

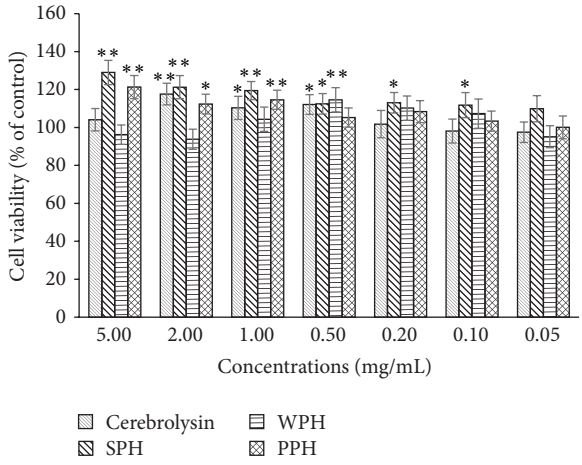

Figure 3: Promoting effect of different hydrolysates in cultured PC12 cells. Each value indicates a mean ± SD ($n = 3$). $^{*}p < 0.05$ and $^{**}p < 0.01$, compared with control group.

nonviable apoptotic cells significantly ($p < 0.01$) increased to 75.69%.

Similar dose-response characteristics for WPH and PPH first showed effects in all cells at 1 mg/mL, whereas higher concentration (2 mg/mL) resulted in an increase in viable cells and a decline in nonviable apoptotic cells. Conversely, viable apoptotic cells decreased and nonviable apoptotic cells increased, when the concentration increased to 5 mg/mL.

It was discovered that SPH showed the highest activity of enhancing normal PC12 cells viability (Figure 3) followed by PPH, WPH, and cerebrolysin. And all hydrolysates could significantly ($p < 0.05$) decrease the rate of mechanical damage in PC12 cells.

3.5. Effects of SPH, WPH, and PPH on Animal's Cognition Trials. To study the effects of various protein hydrolysates on memory-impairment *in vivo*, animal cognition trials were conducted. As shown in Table 3(a), during the acquisition session, the control group showed 73.3% error response rate. After oral administration of samples in four weeks, the positive-, SPH-, and WPH-treated groups exhibited significantly ($p < 0.05$) decreased wrong time and error response rate and increased latent period, compared to control group. However, in the consolidation model, piracetam and protein hydrolysates except for SPH would significantly ($p < 0.05$) decreased wrong time and error response rate, whereas they did not affect latent period.

In order to investigate whether the protein hydrolysates could improve impaired memory, anisodine treatment mice model was applied. Anisodine, as a new ganglion blocking agent, could result in acetylcholine decrease and memory deficits in the mouse brain [25]. Piracetam that has positive effects on the cognitive performance of Alzheimer's disease patients [26, 27] was taken as a positive control. It is notable

Table 2: Flow cytometry analysis for the neuroprotective effects of SPH, WPH, and PPH on H_2O_2-induced apoptosis in cultured PC12 cells.

Sample	Added amount (mg/mL)	Mechanically damaged cells (%)	Nonviable apoptotic cells (%)	Viable cells (%)	Viable apoptotic cells (%)
Control	—	7.75 ± 0.68	4.37 ± 0.32	65.91 ± 4.79	21.97 ± 2.15
H_2O_2	—	0.44 ± 0.12	24.63 ± 3.25	11.62 ± 2.61	63.31 ± 7.05
H_2O_2 + cerebrolysin	1	0.32 ± 0.11	23.29 ± 4.18	12.08 ± 1.49	64.31 ± 6.74
	2	0.32 ± 0.19	17.59 ± 1.02*	18.08 ± 1.83	64.01 ± 10.08
	5	6.49 ± 0.76	70.08 ± 8.70**	12.54 ± 2.04	10.89 ± 1.73**
H_2O_2 + SPH	1	0.14 ± 0.05	25.74 ± 2.56	11.69 ± 1.87	63.43 ± 6.24
	2	0.12 ± 0.04	15.60 ± 3.01*	22.07 ± 3.47*	62.21 ± 7.22
	5	3.46 ± 0.95*	75.69 ± 9.36**	12.91 ± 2.26	7.94 ± 1.88**
H_2O_2 + WPH	1	0.18 ± 0.06*	25.25 ± 5.06	12.32 ± 1.69	62.25 ± 6.54
	2	0.25 ± 0.07*	16.14 ± 1.89*	19.02 ± 2.03*	64.59 ± 6.01
	5	5.87 ± 0.84**	76.57 ± 6.98**	11.57 ± 1.21	5.99 ± 0.79**
H_2O_2 + PPH	1	1.09 ± 0.42	22.87 ± 3.13	12.91 ± 2.07	63.13 ± 6.43
	2	0.16 ± 0.04*	14.25 ± 2.78*	15.14 ± 2.69	70.18 ± 7.22
	5	0.70 ± 0.10	62.66 ± 6.42**	15.04 ± 3.02	21.60 ± 4.56

Each value indicates mean ± SD ($n = 3$). *$p < 0.01$ and **$p < 0.001$, compared with the H_2O_2-treated group.

Table 3: Effects of SPH, WPH, and PPH on memory acquisition, consolidation, and retrieval in normal mice in step-down test in normal and anisodine-treated mice.

(a)

Sample	OM			CM			RM		
	WT in 5 min	LP (min)	Percentage of WRA (%)	WT in 3 min	LP (min)	Percentage of WRA (%)	WT in 3 min	LP (min)	Percentage of WRA (%)
Control	1.1 ± 1.1	2.10 ± 1.25	73.3	0.8 ± 1.1	2.11 ± 1.05	46.7	1.1 ± 1.1	1.63 ± 1.31	60.0
Piracetam	1.0 ± 1.1*	3.20 ± 1.81*	53.3*	0.5 ± 0.7	2.33 ± 0.86	40.0	0.2 ± 0.4*	2.85 ± 0.33**	20.0**
SPH	0.8 ± 1.0*	3.08 ± 2.13*	46.7*	0.8 ± 0.9	2.01 ± 1.04	53.3	0.3 ± 0.8*	2.76 ± 0.66**	20.0**
WPH	0.5 ± 0.6**	3.15 ± 2.07*	46.7*	0.5 ± 0.7*	2.30 ± 1.03	33.3*	0.1 ± 0.4**	2.72 ± 0.78**	13.3**
PPH	1.3 ± 1.0	2.20 ± 1.51	73.3	0.4 ± 0.6*	2.38 ± 0.91	33.3*	0.2 ± 0.4**	2.85 ± 0.33**	20.0**

(b)

Sample	OM			CM			RM		
	WT in 5 min	LP (min)	Percentage of WRA (%)	WT in 3 min	LP (min)	Percentage of WRA (%)	WT in 3 min	LP (min)	Percentage of WRA (%)
Control	3.4 ± 2.8	2.22 ± 1.52	80.0	1.2 ± 1.1	1.79 ± 1.22	60.0	0.9 ± 1.7	2.38 ± 0.94	33.3
Piracetam	2.8 ± 3.5	2.34 ± 1.94	80.0	0.6 ± 1.1*	3.83 ± 1.83**	33.3*	0.3 ± 0.6**	4.22 ± 1.36**	26.7*
SPH	1.9 ± 2.6*	2.32 ± 2.08	60.0*	0.3 ± 0.6**	2.74 ± 0.72*	20.0**	0.5 ± 0.7*	2.69 ± 0.73*	33.3
WPH	3.7 ± 3.6	1.51 ± 1.74*	56.7*	0.5 ± 1.1**	2.73 ± 0.59*	20.0**	0.8 ± 1.1	2.45 ± 0.80	53.3
PPH	3.5 ± 2.8	1.32 ± 1.54*	53.3*	0.5 ± 0.6**	2.65 ± 0.48*	40.0*	0.3 ± 0.7**	2.81 ± 0.55*	20.0*

Note: step-down test results (a) in normal mice trial and (b) in anisodine-treated mice trials. OM, obtained memory group; CM, consolidated memory group; RM, recurred memory group; WT, wrong times of jumping out of the platform; ESA, electric shocked animals; WRA, wrong reactive animals; LP, the latent period of first jumping. Each value indicates mean ± SD ($n = 3$). *$p < 0.05$ and **$p < 0.01$, compared with control (t-test).

that anisodine could significantly ($p < 0.05$) deteriorate learning and memory ability of mice (Table 3(b)) resulting in much more wrong time and error response rate and less latent period. Pretreatment with piracetam and SPH would decrease the wrong time and error response rate and increase latent period of anisodine-treated mice compared to control group. In the consolidation session, piracetam and three protein hydrolysates could improve memory ability of memory-impairment mice. However, except for WPH, the other two protein hydrolysates could also significantly ($p < 0.05$) affect the retrieval memory.

4. Conclusion

The neuroprotective activity has been discovered in some hydrolysates from animal and plant proteins. Cerebrolysin is the famous one which might offer small improvements to symptoms of Alzheimer's disease and vascular dementia [28]. And similar activity was observed in other protein hydrolysates, such as glutathione [29], wheat germ protein hydrolysate [20], and hempseed protein hydrolysate [21]. Jiang et al. [30] also reported that the papain hydrolysate from walnut protein contained peptides with high ACE inhibitory activity. Thus, it is possible that the hydrolysates from soybean protein, walnut protein, and peanut have the neuroprotective activity. In the present study, all the three hydrolysates (SPH, WPH, and PPH) showed large amount of neurotrophic amino acids and were dominated by peptides with 1–3 KDa. The three hydrolysates could reduce the rate of nonviable apoptotic cells at the concentration of 2 mg/mL. And SPH showed higher activity against H_2O_2-induced toxicity than the other hydrolysates (WPH, PPH) and cerebrolysin. In addition, the results from animal's cognition test indicated that three hydrolysates could present partly better effect of improving recurred memory ability of normal mice and consolidated memory ability of anisodine-treated mice. Therefore, soybean, walnut, and peanut protein hydrolysates were proved to be potential food raw materials for ameliorating neurodegenerative disorders. However, the key peptides contributing to special function characteristics of them are still not clear. Further work on purification and identification of soy and walnut protein peptides is worthy to be carried out.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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