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Neuroprotective Effects of Peanut (*Arachis hypogaea*) Skin Extract in a Rat Model of Global Cerebral Ischemia-Reperfusion: Behavioral, Biochemical, and Histological Evaluation

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Abstract

Background & Objectives: Stroke is a major consequence of cerebral ischemia. This study investigated the effects of peanut skin extract (PSE) (or *Arachis hypogaea*) on behavioral, biochemical, and histological parameters of the hippocampus in rats subjected to global ischemia-reperfusion (I/R).

Materials & Methods: In this experimental study, adult male Wistar rats were assigned to the following groups: control (no I/R and no manipulation; received 0.9% saline), sham (no I/R; surgical site opened; received 0.9% saline), PSE (100 mg/kg), I/R, and I/R + PSE (10 and 100 mg/kg). Ischemia was induced by bilateral occlusion of the common carotid arteries for 20 minutes, followed by 24 hours of reperfusion. PSE was extracted using the maceration method and was administered following 24 hours of ischemia induction and then given daily for 1 week. Anxiety-like behavior, pain perception, and recognition memory were assessed after the final injection. Hippocampal microscopic structure and acetylcholinesterase (AChE) activity and serum oxidative stress markers were measured.

Results: PSE improved anxiety-like behavior, pain perception, and recognition memory in I/R rats ($P < 0.05$). I/R decreased superoxide dismutase (SOD) and glutathione reductase (GR) activities (both $P < 0.001$) and increased serum ($P < 0.05$) and hippocampal AChE activity ($P < 0.01$). PSE treatment decreased GSSG levels and AChE activity ($P < 0.01$) and increased catalase activity ($P < 0.01$). PSE treatment reduced the percentage of dead pyramidal cells in the hippocampal CA1 region of I/R rats ($P < 0.05$).

Conclusion: PSE appears to exert neuroprotective effects, likely by reducing oxidative stress markers and attenuating histopathological damage in the hippocampus.

Keywords: Anxiety, Antioxidants, Ischemia-Reperfusion, *Pain, Arachis hypogaea*, Peanut skin.

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Introduction

Stroke is among the leading causes of death and disability worldwide, disrupting neurological function, leading to various disabilities, and significantly reducing survivors' quality of life (1). Stroke triggers rapid glutamate toxicity, oxidative stress, inflammation, and tissue damage (2). There are two main categories of stroke: ischemic and hemorrhagic, which account for approximately 85% and 15% of cases, respectively (3). The extent of neuronal damage resulting from reduced blood flow to the brain is determined by the severity of ischemia and the duration of insufficient perfusion (4).

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Cerebral ischemia can affect the entire brain (*global ischemia*) or be limited to a specific area (*focal ischemia*) (5). During *global ischemia*, various behavioral functions such as anxiety, pain perception, and cognition are impaired. Anxiety is the second most common neuropsychiatric condition after stroke, affecting around 20–30% of survivors (6). Furthermore, approximately 64% of stroke survivors experience hyperalgesia (7). Reactive oxygen species (ROS) generated during the reperfusion period can lead to cerebral vasospasm, often associated with pain. Additionally, inflammatory molecules, including chemokines and cytokines, contribute to pain in affected individuals (8). Cognitive functions, including learning and memory, are adversely affected in stroke patients due to *ischemia–reperfusion* injury, which occurs after blood flow is restored to a previously occluded artery (9).

Current therapeutic options for cerebral ischemia remain limited in efficacy**,** reducing their effectiveness in addressing the condition (10). Medicinal plants are increasingly recognized for their preventive and therapeutic potential in *ischemia–reperfusion* (I/R) injury, as they generally produce fewer side effects than conventional pharmaceuticals (1). Plant-based extracts contain numerous phytochemicals and neuroprotective agents that may play a significant role in research targeting cerebral ischemia (1). Flavonoids such as galangin and tangeretin have demonstrated neuroprotective effects in *global cerebral ischemia*, improving cognitive deficits in animal models (11, 12). Pistachio extract has shown notable protective effects in stroke conditions in ovariectomized female rats (13). Polyphenolic extracts of *Terminalia chebula* Retz have exhibited neuroprotective properties in cerebral I/R injury (14).

Peanuts (*Arachis hypogaea*) are annual herbaceous plants belonging to the *Leguminosae* family. Although botanically classified as legumes, peanuts are often regarded as nuts due to their comparable nutritional profile. They are highly nutritious, rich in fatty acids, vitamins, carbohydrates, calcium, and phosphorus (15, 16). Peanut skin (PS), often discarded as a low-value by-product, is in fact rich in polyphenolic and antioxidant compounds that promote human health. Peanut skin extract (PSE) represents an affordable alternative to costlier herbal medicines owing to its high polyphenol content, including proanthocyanidins and flavonoids, which enhance its antioxidant capacity. This combination not only strengthens its neuroprotective properties but also demonstrates PSE's antioxidant potential comparable to that of established neuroprotective agents, making it a promising cost-effective option for clinical neuroprotection (17, 18). PS has beneficial effects in managing diabetes, lowering blood lipid levels, exhibiting anticancer properties, and inhibiting lipid oxidation (19, 20). PS consumption is associated with various health benefits, as it contains dietary fiber (42–55% of total weight), proteins, minerals, fatty acids, tannins, resveratrol, and proanthocyanidins (17% of total weight) (21, 22). PS contains more procyanidin tetramers than grape seeds, conferring a significant advantage as an antioxidant source for food products (23, 24).

Despite the *gassy* taste of PS, which often leads to its removal, its status as an economical source of potent antioxidants such as catechin and procyanidin (15) and the absence of comprehensive research on its effects in ischemic stroke induced by a *global* I/R model prompted this study. We therefore aimed to evaluate the effects of PSE on behavioral, histological, and biochemical parameters in adult male rats subjected to *global* I/R.

Materials and Methods

Animals and experimental groups

Adult male Wistar rats (200 ± 20 g) were obtained from the Laboratory Animal Breeding Center of Jundishapur University of Medical Sciences, Ahvaz, Iran. Rats were housed under standard laboratory conditions with ad libitum

access to food and water, except during testing periods. Animals were randomly assigned to six groups: control (no manipulation, received 0.9% saline), sham (surgical site opened without carotid artery occlusion, received 0.9% saline), PSE (100 mg/kg for 1 week, without I/R), I/R (received 0.9% saline for 1 week after 24 h reperfusion), and PSE (10 or 100 mg/kg for 1 week with 24 h I/R). All procedures complied with institutional ethical guidelines for animal experimentation and were approved by the Ethics Committee of Shahid Chamran University of Ahvaz, Iran (Code: scu.ec.sc.402.1099). All injections were administered intraperitoneally at 1 ml/kg, and each group consisted of seven animals.

Induction of global I/R model

Rats were anesthetized with intraperitoneal ketamine (60 mg/kg) and xylazine (4 mg/kg), and the *global* I/R model was induced. A midline incision was made in the neck region, and the surrounding tissue and fascia were separated. The right and left carotid arteries were exposed, and the vagus nerve was isolated. Global ischemia was induced via bilateral occlusion of the carotid arteries for 20 minutes. The clamps were then removed, and reperfusion was allowed for 24 hours (25).

Peanut skin extraction

Peanuts were purchased from northern Iran (Astaneh Ashrafieh province). The extraction process was performed under the supervision of plant physiology experts in the Biology Department of Shahid Chamran University of Ahvaz. PSE was prepared using the maceration method: fresh PS was dried at room temperature for 1 week, peeled, and powdered. The powdered PS was soaked in a 70:30 (v/v) ethanol–water solvent at room temperature for 3 days with agitation, then centrifuged and filtered three times through filter paper. The resulting extract was evaporated on plates for one day (26). Dried PSE was stored at 4 °C (Figure 1).

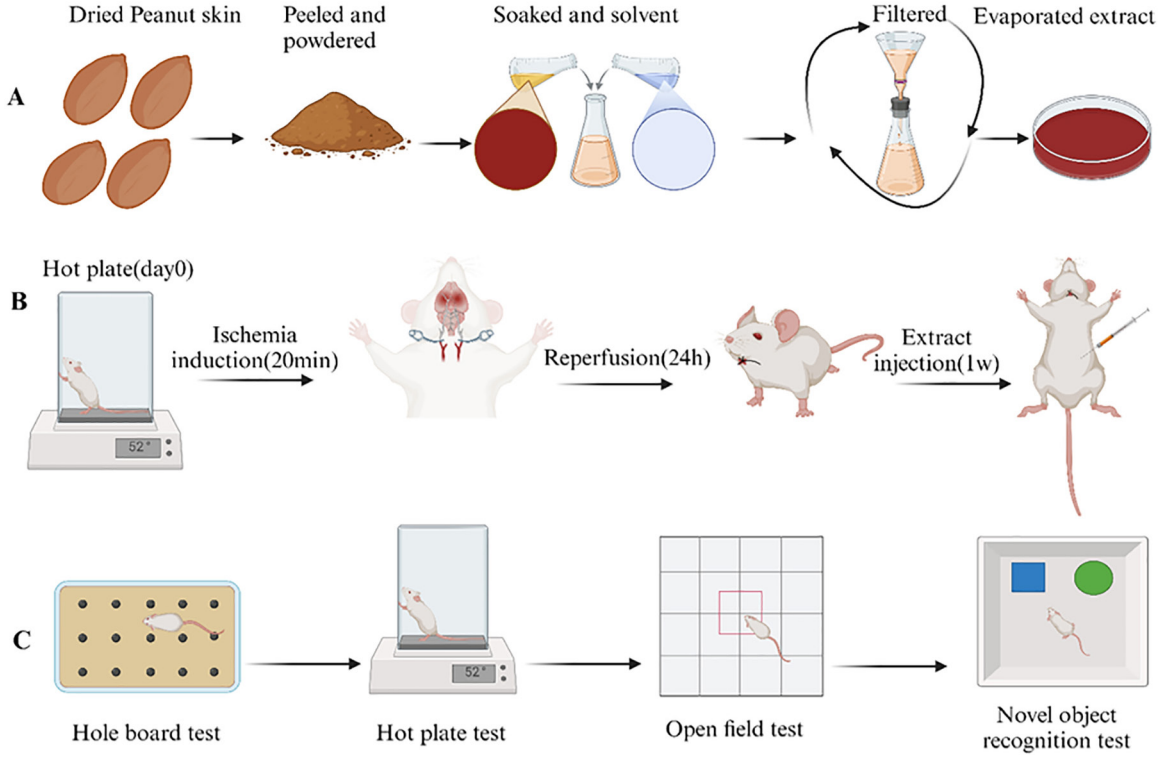

Figure 1. Protocol of this study. A: PSE protocol. B: Ischemia surgery protocol. C: Behavioral tests. PSE: Peanut skin extract. (Designed using Biorender.com)

Studies that have investigated the effects of natural compounds in various plants on reducing cerebral ischemia have used doses of 10-100 mg/kg, such as resveratrol found in peanuts, which has been used at a dose of 100 mg/kg (27).

Behavioral Assessments

Behavioral tests were conducted on day 0 (baseline, before surgery), at 24 hours post-I/R (30 minutes after the first injection), and on day 7 post-I/R (28) (Figure 2). Behavioral impairments can persist or evolve by day 7 post-I/R, making this interval a critical window for assessing cognitive, motor, nociceptive, and anxiety-related outcomes (29).

Hole- Board test

Anxiety-like behavior was assessed using the hole-board test. The apparatus consisted of a Plexiglas box (40 × 40 cm) with a flat floor containing 16 holes arranged in four identical rows. Latency to the first head dip and the total number of head dips during a 5-minute trial served as indices of anxiety-like behavior (30). The test was performed on day 7 post-I/R.

Hot Plate Test

Acute somatic nociception was assessed using the hot-plate test. The plate temperature was maintained at 52 ± 2 °C, and rats were placed on the surface to elicit nocifensive responses. Latency to the first paw lick was recorded as the nociceptive index**.** To prevent tissue injury, a cutoff time of 120 s was imposed, at which point animals were removed from the plate. Nociception was evaluated at baseline (day 0, before surgery), at 24 hours post-I/R (30 minutes after the first injection), and on day 7 post-I/R (31).

Analgesia was expressed as a percentage of maximum possible effect (MPE %) as follows (Eq. 1.):

$$\%MPE = \frac{\text{post_treatment time} - \text{pre_treatment time}}{\text{Cutoff time} - \text{pre_treatment time}} \times 100 \quad (1)$$

Where pre-treatment time is =latency time before surgery, and post-treatment time is =latency time on 24 hours and the 7th day post-I/R.

Open Field Test

Locomotor activity was assessed in the open-field test. The apparatus consisted of a Plexiglas box (40 × 40 × 40 cm) divided into nine equal squares. Each animal was placed in the center and allowed to explore the arena freely for 5 minutes; locomotor activity was quantified as the number of line crossings during the 5-minute session (32). The test was performed on day 7 post-I/R.

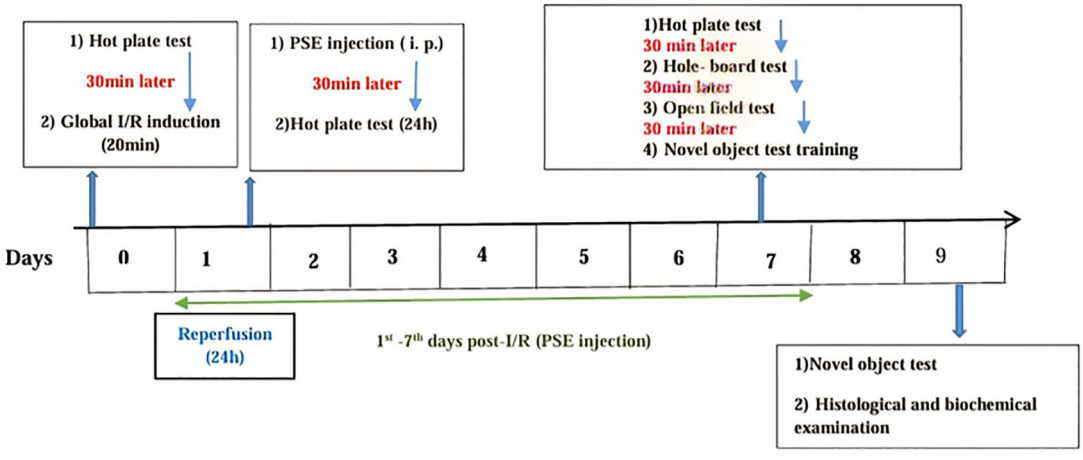

Figure 2: Study timeline. Day 0 (baseline, before surgery): initial evaluation of pain perception with the hot-plate test to determine each subject's baseline response prior to ischemia induction. Reperfusion (24 h): first follow-up to detect the immediate impact of ischemia and reperfusion on nociception. Day 7 post-I/R: final assessment conducted one week later to evaluate longer-term effects. PSE: peanut skin extract; I/R: ischemia/reperfusion.

Novel Object Test

Recognition memory was assessed using the novel object recognition test. Testing spanned five consecutive days, culminating on day 7 post-I/R. Animals were habituated to the arena on day 1; during the three subsequent training days, rats were exposed to two identical objects. On the test day, one familiar object was replaced with a novel object, and animals were allowed to explore both objects; the cumulative time spent interacting with each object was recorded during a 5-minute test session (33).

After the final behavioral assessment (day 9 post-I/R), rats were euthanized by chloroform inhalation. Blood was collected via cardiac puncture and centrifuged to obtain serum. For hippocampal AChE determination, whole brains were removed, and the hippocampus from one hemisphere was isolated and homogenized in phosphate buffer (1:5, w/v). After centrifugation, the supernatant was retained for biochemical assays, while the contralateral hemisphere was fixed in 10% neutral-buffered formalin for histological examination.

Histological and Histometrical Analyses

Brain tissue samples were excised and fixed in 10% neutral-buffered formalin. Paraffin-embedded sections (5 µm) were stained with Hematoxylin and Eosin for histomorphological evaluation under a light microscope (Olympus BH, Tokyo, Japan). For histometric analysis, the mean numbers of dead and intact pyramidal cells were quantified per unit length in the CA1 region of the hippocampus at ×40 magnification, and the extent of damage was estimated (Figure 3). Intact pyramidal cells were identified by a clear nucleus and prominent nucleolus, whereas neurons exhibiting cell shrinkage and hyperchromatic staining were classified as dead.

Biochemical Analysis

The levels of oxidant and antioxidant enzymes were measured in serum, and AChE activity was determined in both serum and hippocampal tissue homogenates (Figure 3).

Malondialdehyde (MDA) Level

MDA was measured by mixing 1.5 mL of serum with 1.5 mL of TBA reagent (prepared by mixing two volumes of 0.8% TBA solution with one volume of 0.7% perchloric acid). The mixture was incubated at 37 °C for 30 min, followed by heating in a boiling water bath for 10 min to promote the reaction between thiobarbituric acid (TBA) and MDA. After cooling, 3.0 mL of a pyridine–butanol mixture and 1.0 mL of NaOH were added, and the absorbance was read at 548 nm (34).

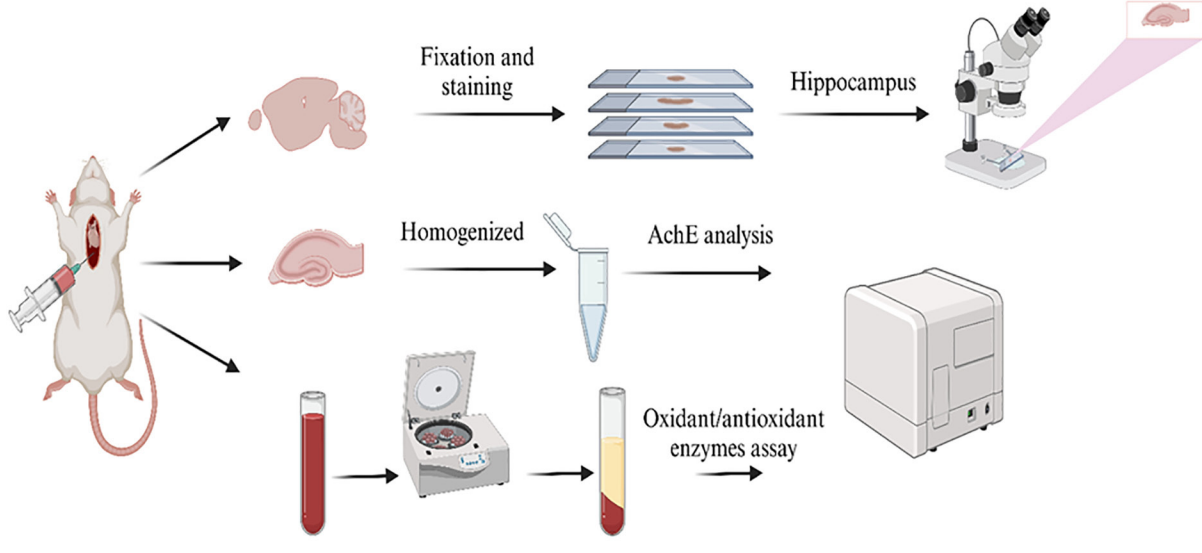

Figure 3. Schematic diagram of Biochemical tests and Histological analysis. (Designed using Biorender.com).

Glutathione (GSH) Activity

Ellman's reagent (50 µL) was mixed with 2.5 mL of phosphate buffer, after which 250 µL of serum was added; for the blank, 250 µL of buffer was used instead of sample. Both mixtures were incubated at room temperature for 15 min, and absorbance was measured at 412 nm. Sulfhydryl content was determined by comparison with a standard curve prepared from cysteine hydrochloride (35).

Glutathione Disulfide (GSSG) Activity

The initial GSH concentration in serum was determined, then sodium borohydride ($NaBH_4$) was used to reduce GSSG to GSH. Total GSH (initial GSH plus GSH generated from reduced GSSG) was measured with Ellman's reagent, and GSSG concentration was calculated by subtracting initial GSH from total GSH (35).

Superoxide Dismutase (SOD) Activity

To assess SOD activity, 150 µL of 50 mM sodium carbonate buffer (pH 10.2) containing 0.1 mM EDTA was placed into each microplate well, followed by 30 µL of 60 µM NBT and 20 µL of serum. Subsequently, 50 µL of hydroxylamine and 20 µL of Triton X-100 were added and the mixtures were incubated at 37 °C for 10 min. Optical density (OD) was recorded at 560 nm twice at 5-minute intervals, and the difference between readings was used to calculate SOD activity (36).

Glutathione reductase (GR) activity

For GR activity, 170 µL of 100 mM phosphate buffer (pH 7.5) was added to each well, followed by 10 µL of 50 mM GSSG and 10 µL of serum; the reaction was initiated by adding 10 µL of 4 mM NADPH. The reaction was monitored at 340 nm for 5 min with readings every 30 s, and GR activity was determined from the rate of change in absorbance (37).

Glutathione Peroxidase (GPx) Activity

GPx activity was assayed by adding 180 µL of 50 mM Tris–HCl buffer (containing EDTA) to each well, then 20 µL of 1 mM GSH, 15 µL of serum, and 20 µL of 0.05% cumene hydroperoxide; the mixture was incubated at 37 °C for 5 min. After incubation, 20 µL of the chromogenic reagent was added, and the reaction was monitored for 5 min by recording absorbance changes at 412 nm (38).

Catalase (CAT) Activity

Catalase activity was measured by adding 50 µL of Tris–HCl buffer (pH 7.8, 0.05 mM) to each well, followed by 100 µL of 10 mM hydrogen peroxide. Then 5 µL of serum was added and the reaction was allowed to proceed for 10 min at room temperature. The reaction was terminated by adding 50 µL of 4% ammonium molybdate, which reacts with residual hydrogen peroxide to form a yellow complex; absorbance was measured at 410 nm and compared with a hydrogen peroxide standard curve to determine the amount of hydrogen peroxide consumed (39).

Acetylcholinesterase Activity

To determine serum AChE activity, 250 µL of 0.1 M phosphate buffer was mixed with 30 µL of serum, then 50 µL of S-butyrylthiocholine iodide (BSCh) was added; the mixture was incubated at 37 °C for 10 min, after which 20 µL of DTNB (0.01 M) was introduced and absorbance was measured at 412 nm (40).

For hippocampal AChE activity, 230 µL of 0.1 M phosphate buffer was mixed with hippocampal homogenate, followed by 20 µL of Triton X-100 and 50 µL of BSCh; the mixture was incubated at 37 °C for 10 min, then 20 µL of DTNB (0.01 M) was added and absorbance was measured at 405 nm (38).

Total Antioxidant Activity (TAC)

For TAC, 8 µL of serum was mixed with 240 µL of working reagent (25 mL acetate buffer + 2.5 mL TPTZ + 2.5 mL $FeCl_3 \cdot 6H_2O$ solution) and incubated at 37 °C for 10 min. After incubation, 248 µL of each reaction was transferred to a microplate and OD was recorded at 593 nm (41).

Statistical Analysis

Data were analyzed using InStat 3 software. One-way ANOVA followed by Tukey's post hoc test was used for multiple-group comparisons, and paired Student's t-tests were applied where

appropriate; normality was assessed for all datasets. Statistical significance was set at $p < 0.05$. Graphs were prepared using GraphPad Prism version 8.4.3 (686), and data are presented as mean ± standard error of the mean (SEM). Graphical figures were created with the BioRender platform.

Results

Effects of PSE on Anxiety-like Behavior

Figure 4 shows no significant difference between the control and sham groups, nor between the sham and I/R groups, in the anxiety index measured by the hole-board test ($p > 0.05$) (Figure 4A, B). Administration of PSE at 100 mg/kg significantly increased latency to the first head dip in I/R rats ($p < 0.05$), and administration of PSE at 10 and 100 mg/kg significantly decreased the number of head dips in I/R rats ($p < 0.05$) (Figure 4A, B). There was no significant difference between the control group and the PSE (100 mg/kg, no I/R) group ($p > 0.05$).

Effects of PSE on pain perception

Figure 5 shows that there was no significant difference between the control and sham groups in MPE% on day 1 and day 7 post-I/R ($p > 0.05$). Similarly, the I/R group did not differ significantly from the sham group in MPE% on day 1 or day 7 post-I/R ($p > 0.05$). In contrast, administration of PSE (10 and 100 mg/kg) produced a significant increase in MPE% on both day 1 and day 7 post-I/R ($p < 0.05$). Treatment with PSE (100 mg/kg) also significantly increased MPE% relative to the control group at 24 h post-reperfusion ($p < 0.05$) (Figure 5).

Effects of PSE on locomotor activity

Figure 6 demonstrates that administration of PSE (10 and 100 mg/kg) to I/R animals significantly reduced locomotor activity compared with untreated I/R rats ($p < 0.05$). Moreover, PSE at 100 mg/kg significantly decreased locomotor activity relative to the control group ($p < 0.05$).

Effects of PSE on recognition memory

Figure 7 illustrates the percentage of time spent exploring the novel object in the novel-object recognition test as an index of recognition memory. Treatment with PSE (100 mg/kg) significantly enhanced recognition memory in I/R rats ($p < 0.05$).

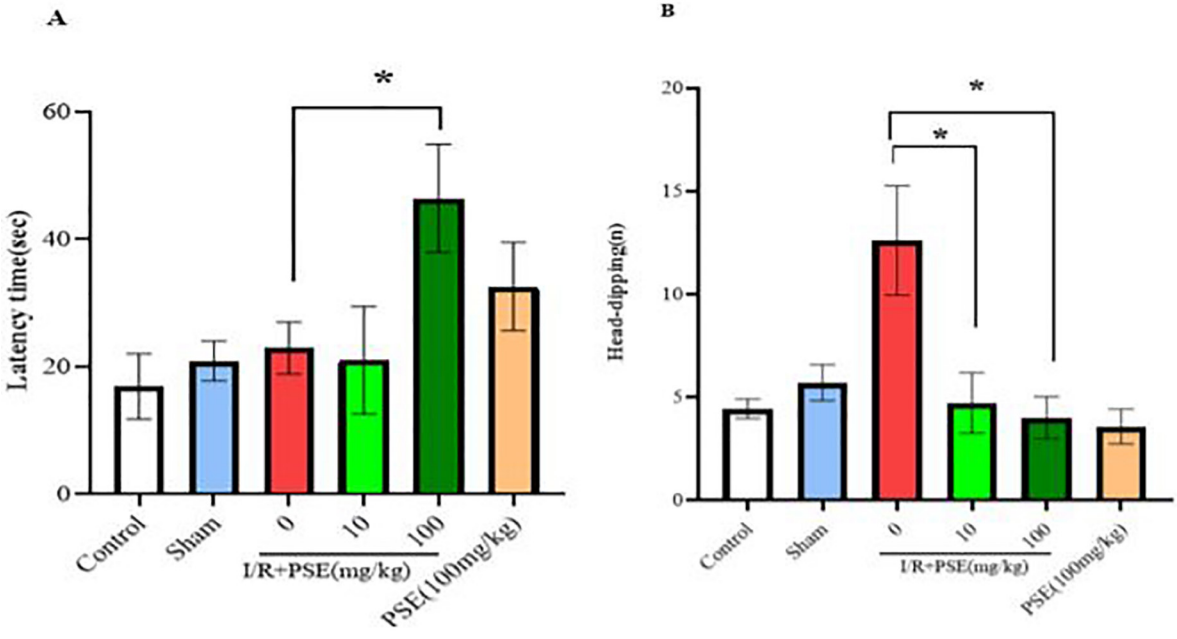

Figure 4. Effects of PSE on anxiety-like behavior in Hole Board test. A: latency time (second), B: Head dipping (number). * shows $p < 0.05$. I/R: Ischemia/reperfusion, PSE: Peanut skin extract.

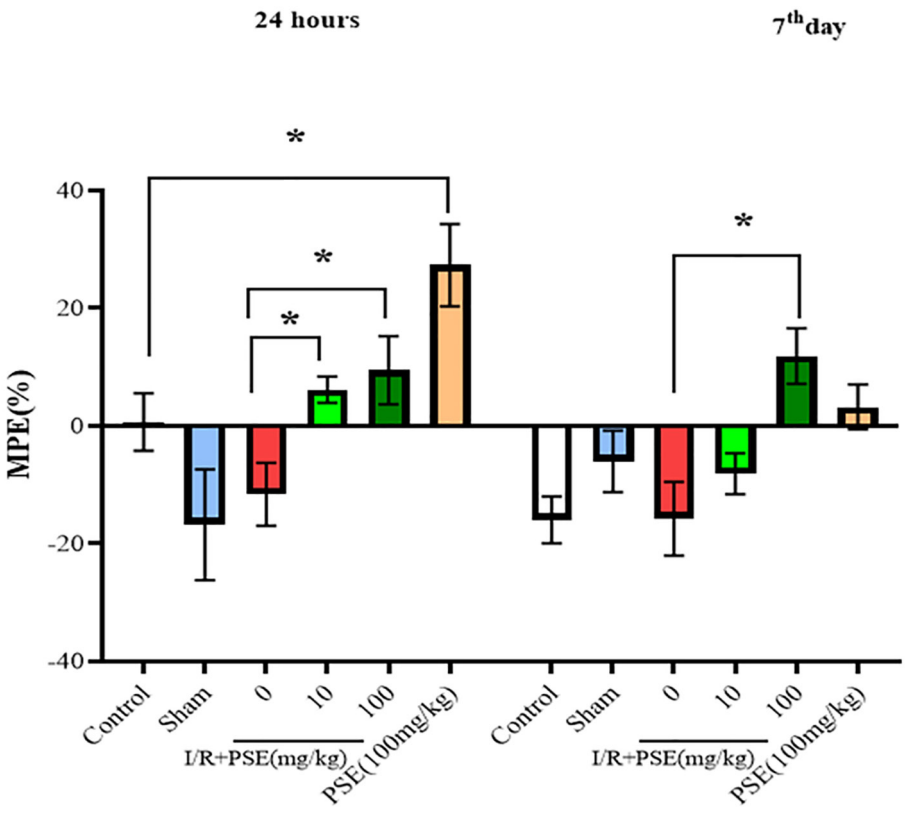

Figure 5. Effects of PSE on pain perception in hot plate test (days of 1st and 7th after I/R induction). * shows p<0.05. Abbreviation; I/R: Ischemia/reperfusion, PSE: Peanut skin extract.

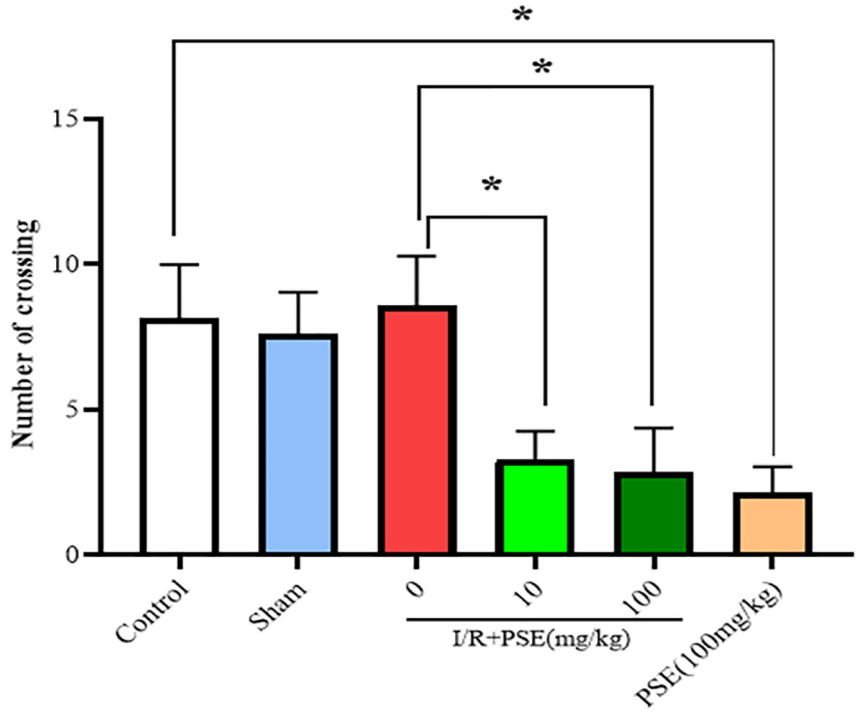

Figure 6. The effects of PSE on locomotor activity in open field test. * shows p <0.05. I/R: Ischemia/reperfusion, PSE: Peanut skin extract.

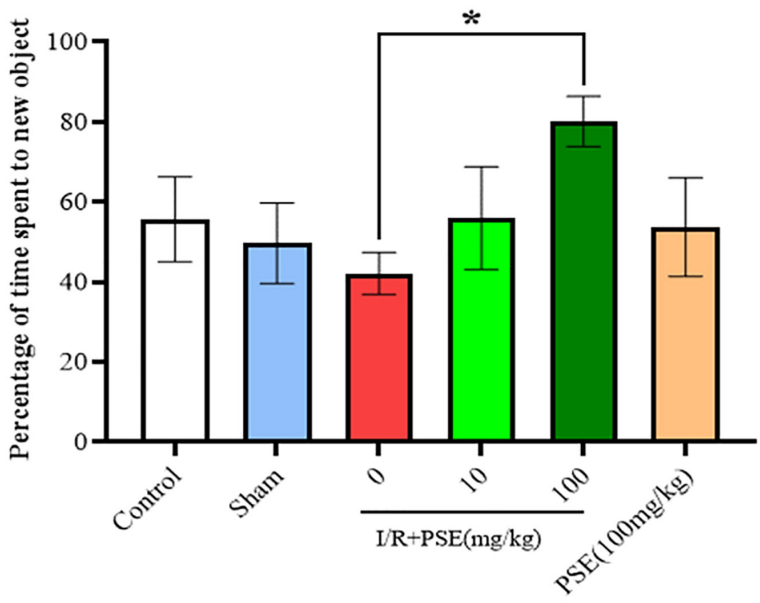

Figure 7. Effects of PSE on recognition memory in novel object test. * shows p<0.05. I/R: Ischemia/reperfusion, PSE: Peanut skin extract.

Histological and histometrical evaluation of the effects of peanut skins extract in the hippocampus CA1 area

Figure 8 depicts the histological architecture of the CA1 region of the hippocampus. Figures 8B and 8C show that, in control and sham animals, the CA1 pyramidal layer comprised compact rows of spheroidal to ovoid pyramidal neurons characterized by basophilic cytoplasm and euchromatic nuclei with prominent nucleoli. Following I/R induction, pyramidal neurons in CA1 exhibited degeneration and cell death; affected cells displayed shrunken cell bodies, acidophilic cytoplasm, and pyknotic nuclei (Figure 8D). Administration of PSE (10 and 100 mg/kg) to I/R rats attenuated structural damage in the CA1 region compared with untreated I/R animals (Figure 8F, G).

Table 1. Biochemical factors

Factors	SOD (umol/mg pr/min)	Catalase (umol/mg pr/min)	GPx (umol/ mg pr)	GSH (umol/mg pr)	GSSG (umol/mg pr)	TAC (umol/mg pr)	GR (umol/mg pr)	MDA (umol/mg pr)	AchE (serum) (umol/mg pr)	AchE (hip-pocampus (umol/mg pr)
Control	2.493± 0.351	0.8688± 0.1152	0.5864± 0.0634	5.823± 0.83	0.8377± 0.0827	10.637± 0.75	1.678± 0.0758	7.780± 0.704	0.1160± 0.0164	0.04857± 0.0130
Sham	3.570± 0.467	0.8285± 0.09798	0.5794± 0.0398	5.996± 0.817	1.212± 0.154	9.002± 1.30	1.895± 0.188	7.025± 0.839	0.08079± 0.0101	0.03026± 0.00154
I/R (1w)	1.023± 0.215***	0.6869± 0.07361	0.3838± 0.0693	3.627± 0.571	1.775± 0.225	6.841± 0.453	0.8928± 0.109***	10.380 ± 1.45	0.1424± 0.0238*	0.07238± 0.00879**
PSE (100mg/ kg)	2.469± 0.331	0.9737± 0.02882	0.5501± 0.0500	5.636± 0.673	1.434± 0.279	8.361± 0.898	1.583± 0.191	8.540± 0.986	0.09529± 0.0180	0.06218± 0.00683
I/R+ PSE (10 mg/kg)	2.474± 0.542	0.9781± 0.03566$$	0.6325± 0.0064	4.197± 0.810	0.8261± 0.0821$$	7.069± 0.516	0.8154± 0.0686	9.220± 1.30	0.07116± 0.025$	0.05090± 0.0105
I/R+ PSE (100 mg/ kg)	1.753± 0.210	0.9856± 0.04106$$	0.4927± 0.0560	4.849± 0.613	0.8956± 0.0705$$	8.470± 0.702	1.028± 0.103	8.640± 1.07	0.09411± 0.0171$	0.05501± 0.0116

* p < 0.05 **p<0.01 and ***p < 0.001 are in comparison with the sham group. $ p < 0.05 and $$ p < 0.01 are in comparison with I/R group. Abbreviation; I/R: Ischemia/reperfusion, PSE: Peanut skin extract.

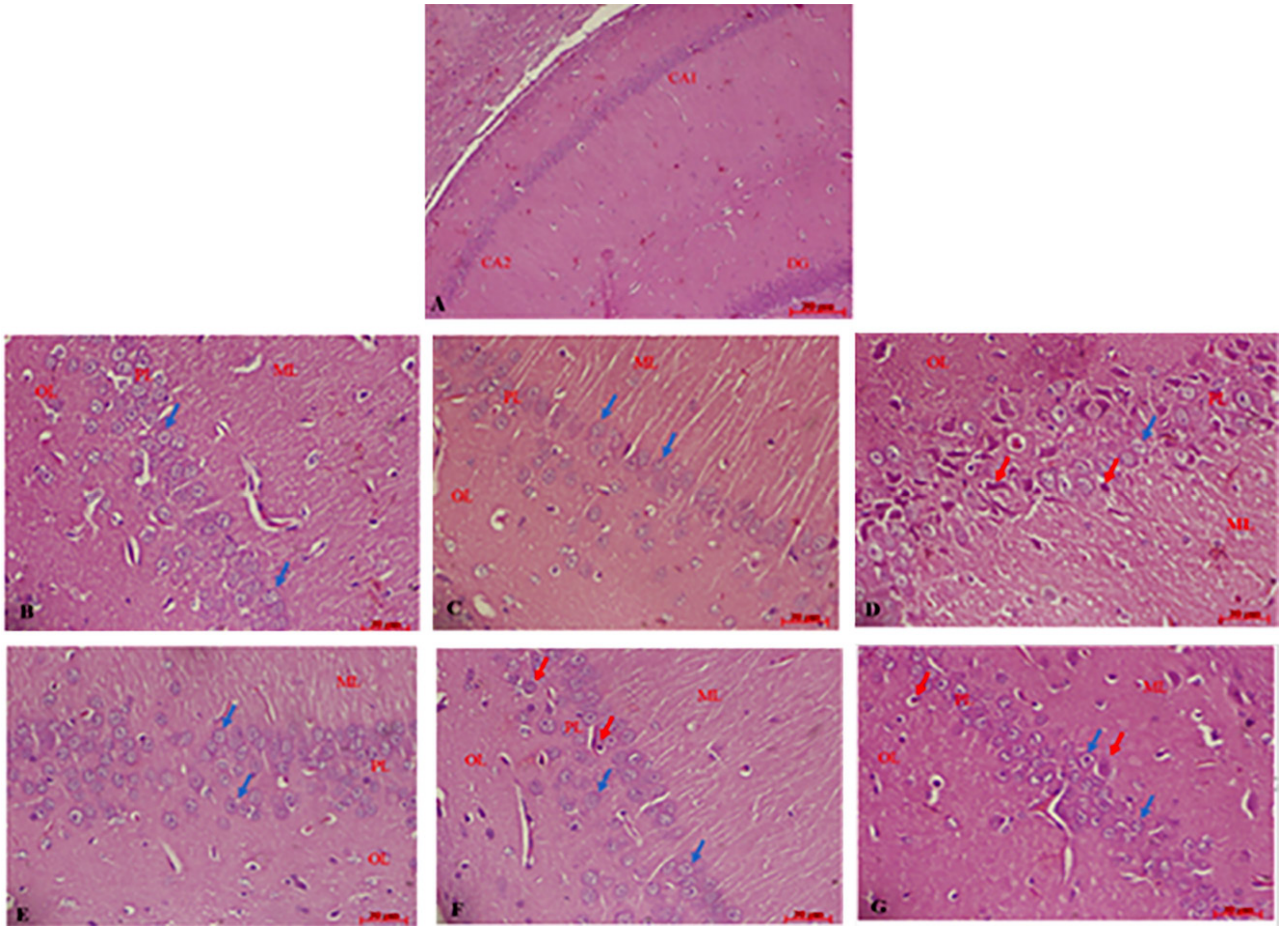

Figure 8. Microscopic sections of the hippocampus CA1 region (A) in control (B), sham(C), I/R (D), PSE (100 mg/kg) (E), I/R treated with PSE (10 mg/kg) (F), and I/R treated with PSE (100 mg/kg) (G) groups (Hematoxylin-Eosin staining, ×10 and ×40). Intact pyramidal cells are spherical to oval cells with a basophilic cytoplasm, a large euchromatin spherical to oval nucleus with a distinct nucleolus (blue arrow). Dead pyramidal cells are cells with a wrinkled cell wall, acidophilic cytoplasm, and a wrinkled or pyknotic nucleus (red arrow). ML: molecular layer, PL: pyramidal layer, OL: outer layer. Abbreviation; I/R: Ischemia/reperfusion, PSE: Peanut skin extract.

Histometric analysis of the CA1 region revealed that the I/R group had a significantly greater percentage of damaged pyramidal cells than the control and sham groups ($p < 0.05$) (Figure 8D). Conversely, the percentage of dead pyramidal cells was significantly lower in I/R rats treated with PSE than in untreated I/R rats.

Effects of PSE on Biochemical Factors

Analysis of biochemical factors showed that I/R induction significantly decreased SOD activity compared to the sham group ($P<0.001$). In the groups receiving PSE (10 and 100 mg/kg) along with I/R induction, CAT activity significantly increased, and GSSG activity significantly decreased compared to the I/R group ($p<0.01$). GR activity significantly decreased in the I/R group compared to the sham group ($p<0.001$) (Table 1).

AchE activity in the blood serum of the I/R group showed a significant increase compared to the sham group ($p<0.05$) (Table 1). Conversely, in the groups receiving PSE (10 and 100 mg/kg) along with I/R induction, AchE activity in the blood serum significantly decreased compared to the I/R group. Also, AchE activity significantly increased in the hippocampus tissue of the I/R group compared to the sham group ($p<0.01$) (Table 1).

Discussion

In the present study, for the first time, we investigated the effects of PSE on behavioral parameters, hippocampal histological structure, and biochemical indices after global I/R injury in rats. The results demonstrated that I/R induction elevated anxiety levels in rats, while PSE treatment decreased these indices (Figure 3). The anxiolytic effect of PSE can be attributed to the anxiolytic properties of polyphenols, which modulate the hypothalamic-pituitary-adrenal (HPA) axis and regulate serotonergic and adrenergic pathways involved in mood and anxiety (42).

Analgesia was observed after PSE injection and persisted for 7 days after I/R induction (Figure 4). This analgesic effect is likely due to the anti-inflammatory properties of peanut-derived bioactive components, which reduce oxidative stress and modulate pro-inflammatory cytokines (43).

Locomotor activity was significantly reduced in groups treated with PSE (Figure 5). These findings highlight the dual role of PSE in modulating both anxiety and motor function. The decrease in locomotor activity in the PSE-supplemented groups could be due to peanut skin phenolic compounds, which, by affecting the nervous system, cause sedation and immobility in the animal (22).

I/R induction also negatively affected cognitive memory (Figure 6). Previous research on I/R injury has identified oxidative stress as a critical factor contributing to neuronal damage (44). Cerebral ischemia decreases access to oxygen and glucose, leading to a significant reduction in mitochondrial adenosine triphosphate (ATP) and triggering the immediate toxicity of glutamate, ultimately resulting in oxidative stress, inflammation, and apoptosis (45).

Oxidative stress and inflammation after I/R, resulting from cell death in the hippocampal CA1 region of the rat, impair cognitive memory as well (2, 46).

Cognitive memory was significantly improved in the I/R group treated with PSE (Figure 6). Bioactive compounds in peanuts, such as flavonoids and polyphenols, can enhance neuroprotection and cognitive function by combating oxidative stress and neuroinflammation (47). Chunguo et al. (2023) suggested that Scutellarin, a flavonoid present in several plants, improves neuronal dysfunction in Sprague-Dawley rats by inhibiting apoptosis, focal death, and necrosis during I/R injury, thereby protecting neurons from acute ischemic injury through the regulation of glutamatergic signaling (48). It has been shown that PSE combined with fish oil could enhance cognitive performance in mice through antioxidant pathways and activation of brain-derived neurotrophic factor/extracellular signal-regulated kinase/cAMP response element-binding protein (BDNF/ERK/CREB) signaling (49). Notably, in non-I/R rats, PSE did not affect cognitive memory (Figure 6), suggesting that the observed effects are condition-specific and particularly relevant in the context of I/R injury.

Research indicates that neuroprotective agents can positively impact recovery not only during the acute phase of ischemia but also in the subsequent period. Evidence suggests that administering natural compounds after the initial ischemic event can significantly improve recovery by promoting neuroplasticity and reducing secondary injury processes that develop in the days following the event (29). A study found that behavioral assessments conducted between 7 and 90 days following ischemia can reveal deficits that short-term evaluations might miss (29).

Histological evaluation revealed that I/R induction caused significant neuronal damage in the CA1 region of the hippocampus, characterized by degenerated pyramidal cells with shrunken nuclei and acidophilic cytoplasm (Figure 7D). It has been confirmed that global ischemia can induce neuronal apoptosis, particularly in the

hippocampal CA1 and CA2 regions of mice (50).

Treatment with PSE significantly mitigated this damage and preserved the structural integrity of pyramidal cells (Figure 7F & G). In addition, histometrical analysis confirmed a reduction in the percentage of dead pyramidal cells in groups treated with PSE.

This neuroprotection can be attributed to the antioxidative bioactive compounds found in peanuts (27). Mahyar et al. (2025) reported that Quercetin, a natural flavonoid found in some fruits and vegetables, improves the regeneration of pyramidal cells in the CA1 region of the hippocampus in rats after I/R injury (51). They suggested that this effect results from several mechanisms, including a reduction in the levels of pro-inflammatory cytokines such as interleukin-1β (IL-1β), an increase in anti-inflammatory cytokines such as interleukin-10 (IL-10), and inhibition of apoptotic pathways including the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which helps regulate inflammation and cell survival (51). Quercetin is one of the compounds found in peanut skin (22). As a result, the neuroprotective effects of PSE on hippocampal pyramidal cells are likely mediated through a combination of antioxidant activity, anti-inflammatory effects, modulation of critical signaling pathways, and inhibition of apoptosis.

Biochemical analysis showed that I/R induction significantly decreased antioxidant enzyme activities (SOD and GR) and increased oxidative stress markers, including GSSG levels (Table 1). PSE significantly increased CAT activity (Table 1). SOD converts superoxide radicals (O_2^-) into hydrogen peroxide and molecular oxygen, thereby mitigating oxidative stress. This enzyme is vital for protecting cells from oxidative damage, especially in the brain, where high levels of ROS can lead to neuronal injury (52).

CAT is a critical enzyme in detoxifying hydrogen peroxide (53). Catalase decomposes hydrogen peroxide, a byproduct of SOD activity, into water and oxygen. This enzyme is essential for preventing oxidative damage by reducing hydrogen peroxide levels, which can be harmful if they accumulate (52). GPx utilizes glutathione to convert hydrogen peroxide and lipid peroxides into non-toxic substances, contributing to cellular antioxidant defense. It plays a crucial role in maintaining redox balance within cells and protecting against oxidative stress-induced cell death (52).

Furthermore, the significant reduction in serum AChE activity in PSE-treated groups suggests improved cholinergic signaling, which is crucial for memory and cognitive function (54). In the groups receiving PSE, GSSG levels significantly decreased compared to the I/R group (Table 1). Previous research indicates that Crocin, a carotenoid isolated from *Crocus sativus* L. (saffron), increased ACh levels in the posterior hippocampus of ischemic male Wistar rats (55). This elevation in ACh may result from enhanced choline acetyltransferase activity or reduced acetylcholinesterase activity in the brain (56). Samah Elsayed et al. (2021) demonstrated that oxidative stress in male rats with right common carotid artery occlusion (RCCAO) significantly increased MDA levels and decreased SOD activity, whereas pretreatment with red beetroot (*Beta vulgaris* L.) extract reduced reperfusion injury and MDA levels and increased SOD activity (56).

Mahyar et al. (2025) have shown that the administration of Quercetin in the MCAO model of rats increased CAT activity, SOD, and GPx levels, thereby enhancing endogenous antioxidant activity (51).

PSE exhibits hepatoprotective effects. Specifically, it has been demonstrated that PSE can decrease fat accumulation in the liver and enhance liver function in animal models, implying a protective rather than harmful effect (57).

Study Limitations

Some of asudy limitations have been

mentioned here:

1. Lack of CBF (Cereberal blood flow) monitoring: In this study, the cerebral blood flow monitoring technique was not used due to laboratory limitations, but this technique can be used in future studies.

2. Use of cardiac perfusion fixation: For histological analysis, according to previous sources, the postmortem immersion fixation method was used. However, in future studies, we will use the cardiac perfusion fixation method to better preserve the neural tissue, which will yield better results.

3. Lack of phytochemical profile peanut skin: according to previous articles referred to in this study which contain valid and reliable data, we gained a better understanding of the phytochemical properties of peanut skin. Direct analysis of peanut skin extract using HPLC-MS can help us to better understand these properties. Therefore, we plan to directly analyze this extract in future research by improving laboratory conditions and providing financial resources.

4. Carbonated taste of PSE: The carbonated taste of peanut skin extract may hinder its acceptance and use in human food products. Detailed investigation of the sensory and taste characteristics of these extracts requires advanced and time-consuming equipment that is not currently available to us. Therefore, in future plans, we plan to investigate this sensory characteristic in more detail by improving laboratory conditions and securing financial resources.

Conclusion

In conclusion, our findings supposed neuroprotective potential of PSE against global cerebral I/R, as evidenced by reductions in oxidative stress markers and attenuation of hippocampal histopathology. However, further comprehensive studies addressing safety, pharmacokinetics, dose–response relationships, and long-term efficacy are required before clinical application can be contemplated.

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Conflict of Interests

The authors declare no conflicts of interest.

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Ethical Consideration

The study was approved by the Local Ethical Committee at the Shahid Chamran University of Ahvaz, Ahvaz, Iran under ethical approval code: scu.ec.sc.402.1099.

Code of Ethices

Scu.ec.sc.402.1099

Authors' Contribution

L.K.: investigation and original draft preparation; M.K.: conceptualization, supervision, project administration, validation, funding acquisition, and manuscript editing; A.Sh.: software, data curation, and formal analysis; M.D.: software, data curation, formal analysis, and critical review and editing of the manuscript; M.T.: data curation, formal analysis, original draft preparation, and manuscript review and editing.

Abbreviations

AchE: Acetylcholinesterase
Akt: Protein kinase B
ATP: Adenosine triphosphate
BDNF: Brain-derived neurotrophic factor
BSCh: S-butyrylthiocholine iodide

CAT: Catalase
CBF: Cerebral Blood Flow
CREB: cAMP responsive element-binding protein
ERK: Extracellular signal-regulated kinase
GPx: Glutathione peroxidase
GR: Glutathione reductase
GSH: Glutathione
GSSG: Glutathione disulfide
HPA: hypothalamic-pituitary-adrenal axis
I/R: Ischemia/reperfusion
IL-10: Interleukin-10
IL-1β: Interleukin-1β
MDA: Malondialdehyde
MPE: Maximum possible effect
NF-κB: Nuclear factor kappa light chain enhancer from activated B lymphocytes
OD: Optimal density
PAC: Proanthocyanidins
PI3K: Phosphoinositide 3 –kinase
PS: Peanut skin
PSE: Peanut skin extract
RCCAO: Right common carotid artery occlusion
ROS: Reactive oxygen species
SOD: Superoxide dismutase
TAC: Total Antioxidant activity
TBA: Thiobarbituric Acid

References

1 Ezzelarab N.M, Saleh N, Khalil E.A, Abdellatif A. Experimental models and plant-based therapy for experimental cerebral ischemia. Int J Fun Nut 2020; 1 (1) : 5. https://doi.org/10.3892/ijfn.2020.5.
2 Tian X, Fan T, Zhao W, Abbas G, Han B, Zhang K, et al. Recent advances in the development of nanomedicines for the treatment of ischemic stroke. Bioact Mater .2021; 6 (9) : 2854-2869. https://doi.org/10.1016/j.bioactmat.2021.01.023.
3 Zhang G, Wang Q, Jiang B, Yao L, Wu W, Zhang X, et al. Progress of medicinal plants and their active metabolites in ischemia-reperfusion injury of stroke: a novel therapeutic strategy based on regulation of crosstalk between mitophagy and ferroptosis. Front Pharmacol. 2024; 15: 1374445. https://doi.org/10.3389/fphar.2024.1374445.
4 Singh S, Shafi Sh, Shrivastav A, Khan Sh, Ahmad T, Khan N.A. A Review on Herbal Plants Used in Brain Ischemic and Reperfusion Injury. J Drug Deliv Ther. 2020; 10 (5) : 376-379. http://dx.doi.org/10.22270/jddt.v10i5.4415.
5 Sarkar S, Chakraborty D, Bhowmik A, Ghosh M.K. Cerebral ischemic stroke: cellular fate and therapeutic opportunities. Front Biosci. (Landmark Ed). 2019; 24 (3) : 435-450. https://doi.org/10.2741/4727.
6 Nelsone L, Rafsten L, Abzhandadze T, Sunnerhagen K.S. A cohort study on anxiety and perceived recovery 3 and 12 months after mild to moderate stroke. Front Neurol. 2023; 14: 1273864. https://doi.org/10.3389/fneur.2023.1273864.
7 Haslam B.S, Butler D.S, Kim A.S, Carey L.M. Chronic pain following stroke: Current treatment and perceived effect. Disabil. Health J. 2021; 14 (1) : 100971. https://doi.org/10.1016/j.dhjo.2020.100971.
8 Wu L, Xiong X, Wu X, Ye Y, Jian Z, Zhi Z, et al. Targeting oxidative stress and inflammation to prevent ischemia-reperfusion injury. Front Mol Neurosci. 2020; 13: 1-13. https://doi.org/10.3389/fnmol.2020.00028.
9 Elendu Ch, Amaechi D.C, Elendu T.C, Ibhiedu J.O, Egbunu E.O, Ndam A.R, et al. Stroke and cognitive impairment: understanding the connection and managing symptoms. Ann Med Surg. 2023; 85 (12) : 6057-6066. http://dx.doi.org/10.1097/MS9.0000000000001441.
10 Huang P, Wan H, Shao C, Li C, Zhang L, He Y. Recent advances in Chinese herbal medicine for cerebral ischemic reperfusion injury. Front Pharmacol. 2022; 12: 688596. https://doi.org/10.3389/fphar.2021.688596.
11 Guan X, Li Z, Zhu S, Cheng M, Ju Y, Ren L, et al.Galangin attenuated cerebral ischemia-reperfusion injury by inhibition of ferroptosis through activating the SLC7A11/GPX4 axis in gerbils. Life Sci. 2021; 264: 118660. https://doi.org/10.1016/j.lfs.2020.118660.
12 Alla N, Palatheeya S, Challa S.R, Kakarla R. Tangeretin confers neuroprotection, cognitive and memory enhancement in global cerebral ischemia in rats. 3Biotech. 2024; 14 (1) : 1-11. https://doi.org/10.1007/s13205-023-03854-y.
13 Hakimizadeh E, Moghadam-Ahmadi A, Shamsizadeh A, Ehsani V, Amin F, Allahtavakoli M. The effects of pistachio hydroalcoholic extract on stroke in permanent middle cerebral artery occlusion in ovariectomized female rats. Pistachio. Health J. 2021; 4 (2) : 52-64. https://doi.org/10.22123/PHJ.2021.290603.1104.
14 Lin K, Zhou M, Leng C, Tao X, Zhou R, Li Y, et al.Neuroprotective effect of polyphenol extracts

from Terminalia chebula Retz. against cerebral ischemia-reperfusion injury. Molecules. 2022; 27 (19) : 6449. https://doi.org/10.3390/molecules27196449.
15 Ibrahim A.K, AL-Azawi A.H. Nephro-protective effect of (Arachis hypogaea L.) peanut skin extracts on CCl4 induced kidney damage in mice. Int J Nat Eng Sci. 2019; 13 (1) : 07-14. https://ijnes.org/index.php/ijnes/article/view/530.
16 Parilli-Moser I, Domínguez-López I, Vallverdú-Queralt A, Hurtado-Barroso S, Lamuela-Raventós R.M. Urinary phenolic metabolites associated with peanut consumption may have a beneficial impact on vascular health biomarkers. Antioxidants. 2023;. 12 (3) : 698. https://doi.org/10.3390/ antiox12030698.
17 Christman L.M, Dean L.L, Bueno Almeida C, Weissburg J.R. Acceptability of peanut skins as a natural antioxidant in flavored coated peanuts. J Food Sci. 2018; 83 (10) : 2571-7. https://doi.org/10.1111/1750-3841.14323.
18 Huang J, Zhou Y, Xu H, Wang M. Neuroprotective Effects of Peanut Skin Extract Against Oxidative Injury in HT-22 Neuronal Cells. Pharmaceuticals. 2025; 18: 544. https:// doi.org/10.3390/ph18040544.
19 Bonyadlou E, Jafarpour A, Mansouripour S. The effect of alkaline salts and peanut skin extract on the qualitative attributes of fried coated peanuts and the frying oil. J Food Sci. Technol. 2023; 20 (139): 165-179. https://doi.org/ 10.22034/FSCT.20.139.165.
20 Cordeiro-Massironi K, Soares-Freitas R.A, Sampaio G.R, Pinaffi-Langley A.C, Bridi R, de Camargo A.C, et al.In Vitro Digestion of Peanut Skin Releases Bioactive Compounds and Increases Cancer Cell Toxicity. Antioxidants. 2023; 12 (7) : 1356. https://doi.org/10.3390/antiox12071356.
21 Muñoz-Arrieta R, Esquivel-Alvarado D, Alfaro-Viquez E, Alvarez-Valverde V, Krueger C.G, Reed J.D. Nutritional and bioactive composition of Spanish, Valencia, and Virginia type peanut skins. J Food Compost Anal. 2021; 98: 103816. https://doi.org/10.1016/j.jfca.2021.103816.
22 Dean L.L. Extracts of peanut skins as a source of bioactive compounds: methodology and applications. Appl Sci. 2020; 10 (23) : 8546. https://doi.org/10.3390/ app10238546.
23 Lorenzo J.M, Munekata P.E.S, Sant'Ana A.S, Carvalho R.B, Barba F.J, Toldra F, et al. Main characteristics of peanut skin and its role for the preservation of meat products. Trends Food Sci Technol. 2018; 77: 1-10. https://doi.org/10.1016/j.tifs.2018.04.007.
24 Chang M, Sun X, Guo X, Bai H, Liu R, Jin Q, et al. Composition and antioxidant study of procyanidins from peanut skins. J Food Meas Charact. 2020; 14: 2781-2789. https://doi.org/10.1007/s11694-020-00523-z.
25 Farhadi Moghadam B, Fereidoni M. Neuroprotective effect of menaquinone-4 (MK-4) on transient global cerebral ischemia/reperfusion injury in rat. PLoS One. 2020; 15 (3): e 0229769. https://doi.org/10.1371/journal.pone.0229769.
26 Hussain A.I, Chatha S.A, Noor S, Khan Z.A, Arshad M.U, Rathore H.A, et al. Effect of extraction techniques and solvent systems on the extraction of antioxidant components from peanut (Arachis hypogaea L.) hulls. Food Anal Methods. 2012; 5: 890-896. https://doi.org/10.1007/s12161-011-9325-y.
27 Chen S, Chen H, Du Q, Shen J. Targeting myeloperoxidase (MPO) mediated oxidative stress and inflammation for reducing brain ischemia injury: Potential application of natural compounds. Front Physiol. 2020; 11: 433. https://doi.org/10.3389/fphys.2020.00433.
28 Polopalli S, Yetukuri A.R, Danduga R.C, Kola P.K. A prognostic study on the effect of post-traumatic stress disorder on cerebral ischaemia reperfusion-induced stroke. World J Biol Psychiatry. 2022; 23 (2) : 136-150. https://doi.org/10.1080/15622975.2021.1935318.
29 Fréchou M, Margaill I, Marchand-Leroux C, Beray-Berthat V. Behavioral tests that reveal long-term deficits after permanent focal cerebral ischemia in mouse. Behav Brain res. 2019;360:69-80. https://doi.org/10.1016/j.bbr.2018.11.040.
30 Casarrubea M, Di Giovanni G, Crescimanno G. Effects of different anxiety levels on the behavioral patternings investigated through T-pattern analysis in wistar rats tested in the hole-board apparatus. Brain Sci. 2021; 11 (6) : 714. https://doi.org/10.3390/brainsci11060714.
31 Torabi M, Kesmati M, Galehdari H, Varzi H.N, Pourreza N. MgO and ZnO nanoparticles anti-nociceptive effect modulated by glutamate level and NMDA receptor expression in the hippocampus of stressed and non-stressed rats. Physiol Behav. 2019; 214: 112727. https://doi.org/10.1016/j.physbeh.2019.112727.
32 Torabi M, Kesmati M, Varzi H.N, Pourreza N. Effects of Magnesium Oxide and Zinc Oxide Nanoparticles on Motor Activity, Electrocardiographic Parameters, and Oxidative Stress in an Acute Restraint Stress Model of Rats. Jentashapir J Cell Mol Biol. 2022; 13 (1) : e122462. https://doi.org/10.5812/jjcmb-122462.
33 Lueptow L.M. Novel object recognition test for the investigation of learning and memory in mice. J Vis

Exp. 2017; 126: 55718. https://doi.org/10.3791/55718.
34 Savaram V.R.R, Mantena V.L.N.R, Paul S.S, Devanaboyina N, Thota S, Bhukya P, et al. Enhancement of performance and anti-oxidant variables in broiler chicken fed diets containing sub-optimal methionine level with graded concentrations of sulphur and folic acid. Anim Bio sci. 2022; 35 (5) : 721-729. https://doi.org/10.5713/ab.21.0258.
35 Asadi M, Mohammadi M, Mohammadian B, Shahriari A, Foruozandeh H. The protective effect of Cichorium intybus L. hydroalcoholic extract against methotrexate-induced oxidative stress in rats. J Nat. Pharm Prod. 2018; 13 (4) : e59556. https://doi.org 10.5812/jjnpp.59556.
36 AnushaS, Anilkumar C, Gangaprasad A. Desiccation induced physiological and biochemical changes of Gymnacranthera canarica (King) Warb. seeds in the Myristica swamp forests, Southern Western Ghats, India. J Plant Sci Today. 2022; 9 (4) : 1110–1121. https://doi.org/ 10.14719/pst.1887.
37 Hsu J.Y, Lin H.H, Wang Z.H, Chen J.H. Aqueous extract from Pepino (Solanum muricatum Ait.) leaves ameliorated insulin resistance, hyperlipidemia, and hyperglycemia in mice with metabolic syndrome. J Food Biochem. 2020; e13518. https://doi.org/10.1111/jfbc.13518.
38 El-Saadany A.S, El-Barbary A.M, Shreif E.Y, Elkomy A, Khalifah A.M, El-Sabrout K. Pumpkin and garden cress seed oils as feed additives to improve the physiological and productive traits of laying hens. Ital J Anim Sci. 2022; 21 (1) : 1047-1057. https://doi.org/10.1080/1828051X.2022.2090288.
39 Taghvimi S, Kesmati M, Shahriari A, Tabatabaei S.R.F. Antioxidative Effects of Hydro-Alcoholic Extract and Essential Oil of Quercus brantii Exerts Anti-Depressant-Like Effect and Improvement of Memory Defect in Progesterone-Induced Postpartum Depression in Rats. Zahedan J Res Med Sci. 2024; 26 (2) : e143020. https://doi.org/10.5812/zjrms-143020.
40 Jamadagni S.B, Ghadge P.M, Tambe M.S, Srinivasan M, Prasad G.P, Jamadagni P.S, et al. Amelioration of AlCl3-induced memory loss in the rats by an aqueous extract of Guduchi, a Medhya Rasayana. Pharma cogn Mag. 2023; 19 (2) : 231-243. https://doi.org / 10.1177/09731296221145063.
41 Houshmand F, Heidarian E, Faradonbeh A.S, Chaleshtori S.N. Protective effect of carvacrol against hepato-renal toxicity induced by azathioprine in rats. J Shahrekord Univ Med Sci. 2021; 23 (4) : 148-153. https://doi.org / 10.34172/jsums.2021.25.
42 Pizarro Melendez G.P, Valero-Jara V, Acevedo-Hernandez P, Thomas-Valdes S. Impact of polyphenols on stress and anxiety: a systematic review of molecular mechanisms and clinical evidence. Crit Rev Food Sci Nutr. 2024; 64 (8) : 2340-2357. https://doi.org/10.1080/10408398.2022.2122925.
43 El-Hashash S.A, Gaballah W.A, Faramawy A.A, Rizk N.I, Alsuwat M.A, Alshehri, M.A, et al. Mitigating Acetaminophen-Induced Kidney Injury: The Protective Role of Grape Seed and Peanut Skin Extracts through the iNOS/CYP2E1 Pathway. ACS Omega. 2024; 9 (32) : 35154-35169. https://doi.org/10.1021/acsomega.4c05534.
44 Lee K.H, Cha M, Lee B.H. Neuroprotective effect of antioxidants in the brain. Int J Mol Sci. 2020; 21 (19) : 7152. https://doi.org/10.3390/ijms21197152.
45 Gonzalo-Gobernado R, Ayuso M.I, Sansone L, Bernal-Jiménez J.J, Ramos-Herrero V.D, Sánchez-García E, et al.Neuroprotective effects of diets containing olive oil and DHA/EPA in a mouse model of cerebral ischemia. Nutrients. 2019; 11 (5) : 1109. https://doi.org/10.3390/nu11051109.
46 Salehpour F, Farajdokht F, Mahmoudi J, Erfani M, Farhoudi M, Karimi P, et al. Photobiomodulation and coenzyme Q10 treatments attenuate cognitive impairment associated with model of transient global brain ischemia in artificially aged mice. Front Cell Neurosci. 2019; 13: 74. https://doi.org/10.3389/fncel.2019.00074.
47 Naderi Y, Sabetkasaei M, Parvardeh S, Zanjani T.M. Neuroprotective effect of minocycline on cognitive impairments induced by transient cerebral ischemia/reperfusion through its anti-inflammatory and anti-oxidant properties in male rat. Brain Res Bull. 2017; 131: 207-213. http://dx.doi.org/10.1016/j.brainresbull.2017.04.010.
48 Wang C, Liu Y, Liu X, Zhang Y, Yan X, Deng X, et al. Scutellarin alleviates ischemic brain injury in the acute phase by affecting the activity of neurotransmitters in neurons. Molecules. 2023; 28 (7) : 3181. https:// doi.org/10.3390/molecules28073181.
49 Xiang L, Cao X.L, Xing T.Y, Mori D, Tang R.Q, Li J, et al. Mixture of peanut skin extract and fish oil improves memory in mice via modulation of anti-oxidative stress and regulation of BDNF/ERK/CREB signaling pathways. Nutrients. 2016; 8 (5) : 256. https://doi.org/10.3390/nu8050256.
50 Baranova K, Nalivaeva N, Rybnikova E. Neuroadaptive Biochemical Mechanisms of Remote Ischemic Conditioning. Int J Mol Sci. 2023; 24 (23) : 17032. https://doi.org/ 10.3390/ijms242317032.
51 Mahyar M, Ghadirzadeh E, Nezhadnaderi P, Moayedi Z, Maboud P, Ebrahimi A, et al. Neuroprotective

effects of quercetin on hippocampal CA1 neurons following middle cerebral artery ischemia–reperfusion in male rats: a behavioral, biochemical, and histological study. BMC Neurol. 2025; 25 (1) : 9. https:// doi.org/10.1186/s12883-024-04017-z.

52 Jomova K, Alomar S.Y, Alwasel S.H, Nepovimova E, Kuca K, Valko M. Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. Arch Toxicol. 2024;98:1323-67. https:// doi.org/10.1007/s00204-024-03696-4.

53 Anwar S, Alrumaihi F, Sarwar T, Babiker A.Y, Khan A.A, Prabhu S.V, et al. Exploring Therapeutic Potential of Catalase: Strategies in Disease Prevention and Management. Biomolecules. 2024; 14 (6) : 697. https:// doi.org/10.3390/biom14060697.

54 Yuan Y, Shan X, Men W, Zhai H, Qiao X, Geng L, et al. The effect of crocin on memory, hippocampal acetylcholine level, and apoptosis in a rat model of cerebral ischemia. Biomed Pharmacother. 2020; 130: 110543. https://doi.org/10.1016/j. biopha.2020.110543.

55 Elsayed S, El-Habeby M, El-Sherif N, Al-Gholam M. Influence of global cerebral ischemia/reperfusion injury on rat dentate gyrus and the possible protective effect of beetroot (Beta vulgaris L.) extract. Eur J Anat. 2021; 25 (4) : 493-503. https://eurjanat.com/ articles /influence-of-global-cerebral-ischemia-reperfusion-injury-on-rat-dentate-gyrus-and-the-possible-protective-effect-of-beetroot-beta-vulgaris-l-extract/.

56 Jiang M, Lai X, Zhang Y, Shen M, Ma H, Liu A, et al. Artemisinin Alleviates Cerebral Ischemia/ Reperfusion-Induced Oxidative Damage via Regulating PHB2-Mediated Autophagy in the Human Neuroblastoma SH-SY5Y Cell Line. Oxid Med Cell Longev. 2022; 2022: 6568748. https://doi.org/10.1155/2022/6568748.

57 Sun X.M, Ye H.Q, Liu J.B, Wu L, Lin D.B, Yu Y.L, et al. Assessment of anti-diabetic activity of peanut shell polyphenol extracts. J Biomed Biotechnol. 2018;19(10):764-75. https://orcid.org/0000-0001-9680-5783.