https://doi.org/10.48048/tis.2024.7245

Purple Eggplant (*Solanum melongena* L.) Ameliorates D-Galactose-Induced Cognitive Impairment Through Inhibition of Oxidative Stress and Acetylcholinesterase in the Hippocampus of an Aging Rat Model

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Received: 24 June 2023, Revised: 21 July 2023, Accepted: 25 July 2023, Published: 1 October 2023

Abstract

The induction of neurotoxicity by d-galactose (D-Gal) is a widely recognized model that is utilized for investigating the process of aging and the associated cognitive impairment, as well as oxidative damage and cholinergic dysfunction. Eggplant fruit is a highly consumed vegetable owing to its high phenolic content, which exhibits antioxidant properties. The current investigation aimed to assess the effect of an aqueous extract of Thai purple eggplant (PEP) fruit (*Solanum melongena* L., 'Ma Khuea Muang') on memory impairment and explore the underlying mechanisms. The quantification of total phenolic contents was conducted on the PEP extract, and an analysis of its antioxidant activity was performed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2-Azino-bis (3-ethyl benzothiazoline-6-sufonic acid) (ABTS) scavenging assays. Male Wistar rats were subcutaneously (s.c.) injected (150 mg/kg/day) with D-Gal to generate a model of accelerated aging and administered orally with EP extract (250, 500, and 1,000 mg/kg/day) or donepezil (DNP) (3 mg/kg/day) for 8 weeks. Cognitive performance was evaluated by the Morris water maze (MWM) and novel object recognition (NOR) tests. Malondialdehyde (MDA) level and acetylcholinesterase (AChE) and superoxide dismutase (SOD) activities in the hippocampus were also evaluated after the behavioral studies. The continuous administration of PEP extract at a dosage of 1,000 mg/kg/day or DNP resulted in the amelioration of cognitive impairment and the reversal of the increase in AChE activity caused by D-Gal in the hippocampus. Furthermore, the administration of PEP extract resulted in a significant reduction in MDA levels in the hippocampus. These findings suggest a potential attenuation of oxidative stress. The findings indicate that the PEP extract has a beneficial effect on cognitive decline induced by D-Gal, which is attributed to the restoration of cholinergic function and the reduction of oxidative damage.

Keywords: Aging, Cognitive impairment, Acetylcholinesterase, Oxidative stress, Purple eggplant, *Solanum melongena* L.

Introduction

By 2030, the number of individuals aged 60 and older will reach 1.4 billion, representing one-fifth of the global population [1]. The aging-related physiological changes that occur gradually cause a number of neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, and stroke [2-4]. With the world's aging population increasing, anti-aging has emerged as a critical concern. Increasing evidence supports the idea that oxidative stress plays an important role in aging processes. Oxidative stress is caused by an imbalance between oxidants, specifically reactive oxygen species (ROS) (free radicals), and antioxidants, which causes DNA damage, protein oxidation, and lipid peroxidation and is the leading cause of age-related neurodegeneration and cognitive decline [5-8]. Consequentially, the antioxidant mechanisms are implicated in the protective response against oxidative stress situations. Moreover, research findings indicate that impairments in the cholinergic system have the potential to impact synaptic transmission and are distinguished by synaptic loss. reduction in acetylcholine (ACh), a neurotransmitter, and an elevation in AChE activity in the brain could be caused by aging-associated cognitive dysfunction [9,10].

D-Gal is a monosaccharide that is ubiquitously present in various tissues of the human body. At elevated concentrations, galactose oxidase catalyzes the conversion of galactose to hydrogen peroxide and aldose, leading to the generation of superoxide anion, oxygen-derived free radicals, and consequent cellular injury [11]. According to recent reports, the chronic administration of D-Gal has been related to the acceleration of aging and the influence of age-related alterations, including reduced antioxidant enzyme activities, elevated oxidant levels, and cognitive deficits [9,12,13]. Moreover, chronic administration of D-Gal also affects the cholinergic system, causing increased oxidative stress and elevated AChE activity in rodent' brains, leading to learning and memory impairment [9,12-14].

Eggplant (EP) (*Solanum melongena* L.), a common solanaceous vegetable consumed in Thailand and in many other countries, is available in numerous colors, sizes, and shapes. Purple-colored eggplants with an oblong or elongated shape are used worldwide [15,16]. It has been reported to possess various potential health benefits for diseases such as cancer, diabetes, and cardiovascular disease [17-19]. The high concentration of phenolic compounds with antioxidant properties is a defining feature of EP. In accordance with the findings of Cao *et al.* [20], EP has been among the top 10 vegetables with the highest antioxidant activity. EP is recognized for its elevated concentrations of phenolic acids, particularly chlorogenic acid, in the flesh. Additionally, it is known for its flavonoid content in the peel, with a large amount of the anthocyanin nasunin [21,22]. Previous studies reported that EP extract has been reported to improve memory impairment in streptozotocin-induced diabetic rats [23] and scopolamine-induced amnesic mice [24]. Despite the various benefits of EP, the efficiency of EP against age-related cognitive decline has not been investigated. Therefore, the present study examined the effects of Thai PEP fruit aqueous extract on cognitive impairment, oxidative stress, and cholinergic dysfunction in D-Gal-induced aging rat model.

Materials and methods

Preparation of eggplant aqueous extract

PEP was collected from Chiang Mai, Thailand. A voucher specimen (number AI 2267) has been deposited at the herbarium Center of Excellence in Agricultural Innovation for Graduate Entrepreneurs, Maejo University, Chiang Mai, Thailand. To obtain the PEP crude extract, dried powdered plant material (100 g) was soaked in 1,000 mL of boiling hot water for 10 min. The liquid extract was filtered 3 times through filter paper (Whatman, Kent, UK). The filtrate was then concentrated using a rotary evaporator and lyophilized using a lyophilizer (Labconco, MO, USA). The percent yield of the final extract was 40.

Determination of total phenolic contents (TPC)

The TPC of PEP crude extract was assessed by the Folin-Ciocalteu method. Gallic acid was used as the reference standard compound. The PEP crude extract was incubated with Folin-Ciocalteu's phenol reagent and sodium carbonate (7.5 %) at room temperature for 30 min. Absorbance at 750 nm was measured using a microplate reader (BioTek Instruments, Inc., USA) with 3 replicates. The total phenolic content was presented as gallic acid equivalents (GAE) in mg per g of extract.

Measurement of antioxidant activity

ABTS radical scavenging assay

The radical scavenging activity of PEP extract was determined by the ABTS method. In brief, the ABTS cation radical ($ABTS^{\bullet+}$) reagent was generated by a mixture of 7 mM ABTS and 2.45 mM potassium persulfate oxidant, which was then stored in a dark environment at room temperature for a duration of 12 - 16 h. Prior to use, the reagent was diluted with deionized water to achieve an absorbance of 0.70 ± 0.02 at 734 nm. $ABTS^{\bullet+}$ reagent was incubated at 30 °C with 100 µL various concentrations of PEP extract (12.5 - 400 µg/mL) solution at room temperature for 30 min before measuring the absorbance at 734 nm using a microplate reader. The ABTS cation radical capacity was expressed as 50 % of the radical scavenging activity (IC50).

DPPH radical scavenging assay

The radical scavenging activity of PEP extract was evaluated using the DPPH assay. Briefly, a methanolic DPPH solution was added to various concentrations of the PEP extract (1.56 - 50 mg/mL). After 30 min of incubation in the dark at room temperature, the absorbance was measured using a microplate reader at 540 nm. The DPPH radical scavenging activity of the eggplant extracts was expressed as IC50.

Experimental animals

Male Wistar albino rats (6 - 8 weeks old) were purchased from Nomura Siam International Co., Ltd. (Bangkok, Thailand). The rats were housed for a week under control conditions before initiating the experiments with constant room temperature (25 ± 2 °C), relative humidity (60 ± 10 %), and daily lighting for 12 h. The rats were given a standard diet (Feed Food No. 082; C.P. Company, Bangkok, Thailand) and RO water ad libitum throughout the experimental period.

Experimental design

After acclimating for 1 week, the rats were divided randomly into 6 groups (N = 7 per group): Control sham, D-Gal (Sigma-Aldrich, St Louis, MO, USA) (aging model group), D-Gal + DNP (positive control group), D-Gal + PEP250, D-Gal + PEP500, and D-Gal + PEP1000. As shown in **Figure 1**, the D-Gal groups were injected subcutaneously with D-Gal (150 mg/kg daily) into the back of the neck of rats for 8 weeks [25] while the rats in the Control sham group were injected subcutaneously with the same volume of normal saline. The Control sham group and aging model group were orally administered with reverse osmosis (RO) water, and the rats in the D-Gal + DNP or PEP groups were orally administered with DNP at doses of 5 mg/kg or PEP at doses of 250, 500, and 1,000 mg/kg, respectively, after subcutaneous injection of D-Gal. The MWM, probe trial, and NOR were used to test the memory and learning abilities of rats in each group from days 50 - 54 and on days 55 and 56 before being sacrificed, respectively.

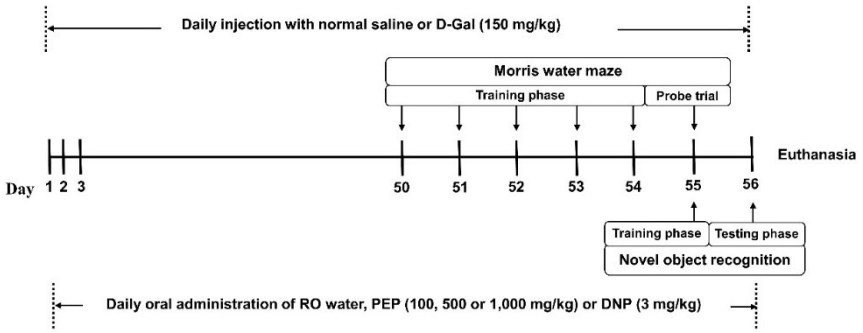

Figure 1 Schematic representation of the experimental design. D-Gal = d-galactose; PEP = purple eggplant; DNP = donepezil; RO = reverse osmosis.

Morris water maze (MWM) test

The MWM test was performed to evaluate spatial learning and memory in rodents. The procedure used was a modification of that described in Kangwan *et al.* [26]. The experimental apparatus consisted of a dark blue circular pool (diameter: 150 cm; height: 45cm) that was divided into 4 quadrants located in a room with numerous extra-maze cues. The pool was filled with cloudy water (starch solution) to a depth of 25 cm, and the water temperature was maintained at approximately 25 °C. A 12.5-cm-diameter platform was fixed at the center of one quadrant and was hidden 2 cm below the water surface. In the training phase, each rat was subjected to 3-trial training per day at a trial of 90 s for 5 consecutive days (days 50 - 54). The experiment started with gently placing each rat on the water surface by facing each sign on the wall of the pool. The location of the hidden platform was placed in the same quadrant every single day. The average time taken to locate the platform, referred to as the escape latency, was utilized as an indicator of spatial learning progress. On day 55, the rats were subjected to a probe trial (spatial memory retrieval test), in which the platform was removed and each rat was deposited in the pool for 90 seconds, beginning in the quadrant opposite the platform quadrant. A video camera was fixed to the ceiling of the room and connected to a computer for recording. The time spent in the target quadrant was recorded [27].

The novel object recognition (NOR) test

The NOR test was used to evaluate recognition memory. The NOR test was performed in a 54×90×50 cm^3 (length × width × height) gray plastic box. On day 55 before the training phase, the rats were allowed to freely explore the apparatus in the absence of stimuli for 5 min for acclimation. Then, the rats were taken back to their cage for 5 min. During the training phase, the 2 similar objects (A and A) were placed in the box, and the rats were allowed to explore the objects freely for 5 min. Exploration was considered when its nose pointed toward the object at a distance ≤ 2 cm. The testing phase was performed 24 h after the training phase. The rats were reintroduced to the same task, but one of the familiar objects

was replaced by a novel object (B). The recognition index (RI) of each rat was calculated using the formula RI = TB/(TA + TB) (TA = the time spent exploring the familiar object; TB = the time spent exploring the novel object) [28].

Preparation of tissue homogenate

At the end of the 56th day, all experimental animals were deeply anesthetized with an intraperitoneal injection (i.p.) of thiopental sodium (100 mg/kg) (Jagsonpal Pharmaceuticals Ltd., India) and transcranial perfused with normal saline. After perfusion, the whole brain was rapidly removed from the skull, and the hippocampi were microdissected on an ice-cold glass dish. The hippocampal homogenate (10%, w/v) was prepared in ice-cold phosphate buffer saline (1 M, pH 7.4) and centrifuged at 14,000 rpm for 15 min at 4 °C. The supernatant was collected and rapidly stored at −80 °C until used.

Determination of the malondialdehyde (MDA) level

The level of MDA was determined according to the method previously described by Pohsa *et al.* [29] with some modifications. Briefly, 40 µL of sample or standard (1,1,3,3 tetraeth oxypropane) was reacted with 80 µL of 8.1 % sodium dodecyl sulphate (SDS), 600 µL of 20 % acetic acid (pH 3.5), and 600 µL of 0.8 % of thiobarbituric acid (TBA). The mixture was incubated at 95 °C for 60 min. After cooling, the mixture was centrifuged at 10,000×g for 5 min at 4 °C. The absorbance was measured at 540 nm by a microplate reader. The results were evaluated using the standard curve and expressed as nmol/mg proteins.

Estimation of the superoxide dismutase (SOD) activity

SOD activity was estimated using a commercial kit (19160, Sigma-Aldrich; Merck KGaA) according to the manufacturer's protocol. The results were expressed as the percentage inhibition of the rate of WST-1 formazan formation.

Evaluation of acetylcholinesterase (AChE) activity

The measurement of AChE activity was conducted using the methodology as previously outlined by Nakdook *et al.* [30], with certain adaptations. In brief, the 0.225 mL reaction mixture contained 0.1 M phosphate buffer pH 7.4, 1 mM acetylthiocholine-iodide (ATCI), and 1 mM 5,5′-dithiobis-(2 nitrobenzoic acid) (DTNB). The absorbance was measured at 405 nm every 30 s for 3 min using the microplate reader. AChE activity was expressed as nmole of ATCI hydrolyzed/min/mg of protein.

Statistical analysis

Statistical analysis was performed using the statistical functions of GraphPad Prism version 9.0. Results were expressed as mean ± standard error of mean (SEM) Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons. A *p*-value less than 0.05 was considered statistically significant.

Results and discussion

Total phenolic content and antioxidant activity

As demonstrated in **Table 1**, the total phenolic content of the PEP extract was 793.11 ± 6.12 GAE mg/g extract. The representation of the antioxidant activity of DPPH and ABTS is expressed in terms of IC50 values. The IC50 values of ABTS and DPPH radical scavenging activity of PEP extract were 4,190 ± 1.08 µg/mL and 203.90 ± 1.04 µg/mL, respectively. The radical-scavenging activity of PEP extract suggests that it is an efficient antioxidant.

Table 1 Total phenolic content and antioxidant capacity of PEP extract.

Samples	TPC (mg GAE/g extract)	DPPH assay IC_{50} (µg/mL)	ABTS assay IC_{50} (µg/mL)
PEP extract	793.11 ± 6.12	4,190 ± 1.08	203.90 ± 1.04
Trolox	-	87.79 ± 1.04	5.59 ± 1.04

Values are expressed as the average of triplicate samples with a mean ± SEM; TPC = total phenolic content; ABTS = 2,2′-Azino-bis (3-Ethylbenzothiazoline-6-Sulfonic Acid); DPPH = Diphenyl-1-Picrylhydrazyl; PEP= purple eggplant.

Effects of eggplant extract on learning and memory performance

D-Gal is a reducing sugar and is typically present in the body at normal physiological concentrations. However, when present at high levels, it has the potential to induce the accumulation of ROS, leading to oxidative stress and subsequent cellular damage [31]. Many studies have reported that chronic administration of D-Gal cause cognitive deficits, which are mediated through various mechanisms in the brain, including oxidative stress [32-35], inflammation [32,33,35], cholinergic dysfunction [35], mitochondrial dysfunction [34,36], AGEs accumulation [33]. The hippocampus is a complex structure within the temporal lobe. It is important to learning and memory.

The MWM test was performed to assess spatial learning and memory performances. The rats in each group were trained for 5 consecutive days. The escape latency had decreased in all groups. There were no significant differences groups between groups in the time to reach the platform. (**Figure 2A**). However, compared with the Control sham group, the D-Gal group showed a longer latency, indicating that the ability of spatial learning and memory was impaired in the rats stimulated with D-gal. To examine spatial memory retention, we performed probe trials on day 55. The results showed that the rats in the D-Gal group (39.90 ± 0.03 s) presented worse memory ability spent less time in the platform quadrant than that of the Control sham group (58.31 ± 0.03 s) ($p < 0.001$). This result accords with previous studies [37,38]. Although the escape latency time during the training phase showed only a slight decrease that had no statistically significant difference, we found that the treatment of PEP 1,000 mg/kg and DNP 3 mg/kg (56.05 ± 0.03 and 51.55 ± 0.03 s, respectively) significantly restored the time of rats spending in the target quadrant ($p < 0.001$ and $p < 0.01$, respectively) in the probe trial test (**Figure 2B**). Collectively, the present results suggest that PEP may improve spatial learning and memory performance in D-Gal-induced aging rats. Recognition memory was measured in all rats using the NOR test. As shown in **Figure 3**, the rats were allowed to explore a novel object with a retention time of 24 h. RI in the Control sham rats (0.73 ± 0.03) was significantly higher than ($p < 0.05$) that observed in the aging model group (0.61 ± 0.01). Interestingly, in the D-Gal + PEP1000 group and the D-Gal + DNP group, the RI (0.75 ± 0.04 and 0.74 ± 0.03, respectively) was significantly increased ($p < 0.01$ and $p < 0.05$, respectively) when compared to the aging model group. The increase in the RI was also observed in the D-Gal-induced aging rats-treated with PEP extract at doses of 250 and 500 mg/kg when compared with the aging model group; however, these differences did not show statistical significance ($p > 0.05$). Consistent with other studies [36,37], we found that the impairment of recognition memory was induced by D-Gal, resulting in a reduction of the RI as observed in the NOR test, while PEP extract (500 mg/kg) as well as DNP treatment abolished the amnestic effect of D-Gal.

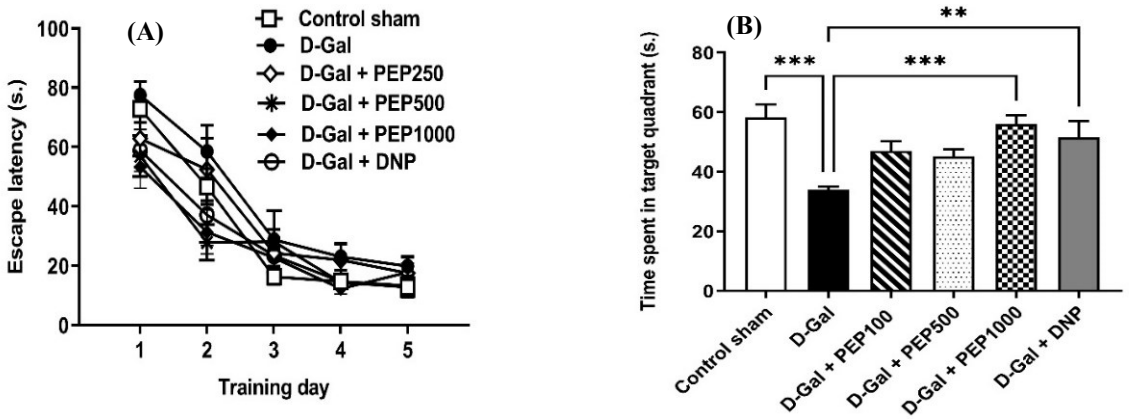

Figure 2 Effect of PEP extract on the spatial memory of rats: (A) escape latency from training days 1 - 5 and (B) time spent in the target quadrant in the probe trial. Data are expressed as the mean ± SEM; N = 7 rats per group. $^{**}p < 0.01$ and $^{***}p < 0.001$ responses are significantly different compared with the D-Gal group. D-Gal = d-galactose, PEP = purple eggplant, DNP = donepezil, s = second.

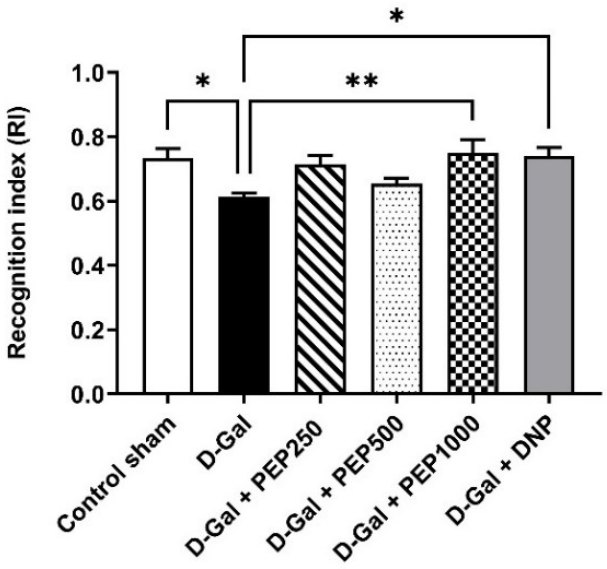

Figure 3 Effect of PEP extract on the recognition memory of rats: Graphs demonstrate the recognition index (RI) using the NOR test. Data are expressed as the mean ± SEM; N = 7 rats per group. $^{*}p < 0.05$ and $^{**}p < 0.01$ responses are significantly different compared with the D-Gal group. D-Gal = d-galactose, PEP = purple eggplant, DNP = donepezil.

Effects of eggplant extract on against oxidative stress in the hippocampus

Oxidative stress refers to a state of physiological imbalance that arises from the production of ROS exceeding the capacity of antioxidant systems to remove them, resulting in damage to cellular components. ROS have the potential to induce lipid peroxidation, a process commonly assessed by measuring the amount of MDA. SOD, an antioxidant enzyme, can transform superoxide radical, a primary ROS generated by the electron transport chain in the mitochondria, into hydrogen peroxide. The latter is subsequently decomposed into water by either catalase (CAT) or glutathione peroxidase (GPx) [39]. Animals treated with D-Gal exhibit age-related alterations such as impaired learning and memory, elevated generation of MDA, and decreased levels of antioxidant enzyme activities [25,40]. To determine the antioxidation property of EP extract, MDA levels and SOD activity were measured.

The hippocampal MDA level of the Control sham rats (7.99 ± 0.23 nmol/mg proteins) was significantly lower than ($p < 0.01$) that observed in the aging model group (11.03 ± 0.42 nmol/mg proteins). In this work, we observed that prolonged exposure to D-Gal led to significant oxidative stress, as evidenced by elevated levels of lipid peroxidation, indicating the occurrence of oxidative damage in the hippocampus. Interestingly, in D-Gal-induced aging rats treated with PEP extract (1,000 mg/kg) and DNP, the MDA level in hippocampal tissue (8.58 ± 0.52 and 7.55 ± 0.56 nmol/mg proteins, respectively) was significantly decreased ($p < 0.05$ and $p < 0.01$, respectively) when compared to the aging model group (**Figure 4A**).

However, the results of the present study demonstrated a nonsignificant difference between the SOD activity in the hippocampal tissues of the Control sham group (97.48 ± 0.39 % inhibition) and the aging model group (95.49 ± 0.88 % inhibition) ($p > 0.05$) (**Figure 4B**). A previous study reported that the activity of SOD in the brain was not significantly influenced by age [41]. According to Yoo and Kim [40], a recent study found that there was no statistically significant difference in the protein expression of SOD in the brain tissues of rats in the control group and those in the D-Gal group. In contrast, CAT activity has been demonstrated to decrease in the brain of the group treated with D-Gal. It is possible that the reduction of one antioxidant may be compensated for by the enhancement of another antioxidant [40,41].

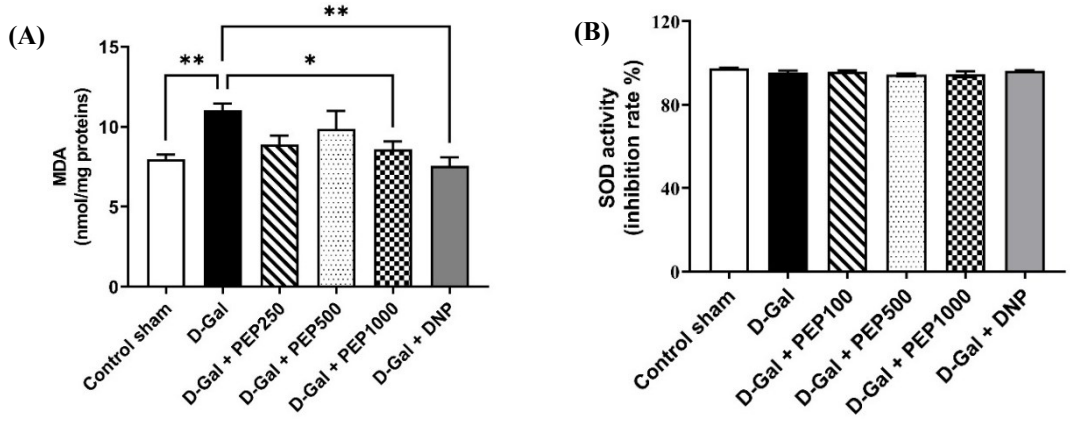

Figure 4 Effect of PEP extract on oxidative stress markers in the hippocampus of rats: (A) MDA level and (B) SOD activity. Data are expressed as the mean ± SEM; N = 7 rats per group. $^{*}p < 0.05$ and $^{**}p < 0.01$ responses are significantly different compared with the D-Gal group. D-Gal = d-galactose, PEP = purple eggplant, DNP = donepezil, MDA = malondialdehyde, SOD = superoxide dismutase.

Effects of eggplant extract on AChE activity in the hippocampus

ACh is a prominent modulatory neurotransmitter within the brain that plays a crucial role in the processes of learning and memory. AChE is an enzyme that breaks down the ACh in the synaptic cleft. It plays a vital role in regulating cholinergic signalling by rapidly terminating the action of ACh. The increase in AChE activity could potentially be attributed to a direct neurotoxic impact on the plasma membrane, which is caused by an elevation in lipid peroxidation. This alteration in the plasma membrane has the potential to impact the cholinergic system's integrity and functionality, which can contribute to cognitive impairment [42]. This study examined the effect of EP extract on cognitive impairment in aging rats induced by D-Gal with DNP, an AChE inhibitor, as the standard positive control. Evidence shows that DNP can reduce the AChE activity and MDA level in the hippocampus of D-Gal-induced aging mice [38].

AChE is a marker of cholinergic neuron degeneration in the brain. The Control sham group was significantly lower ($p < 0.001$) in the AChE activity (0.22 ± 0.01 nmol of ATCI hydrolyzed/min/mg protein) when compared to the aging model group (0.30 ± 0.01 nmol of ATCI hydrolyzed/min/mg protein). Administrations of PEP extract (1,000 mg/kg) and DNP significantly induced increase in the AChE activity (0.23 ± 0.01 and 0.23 ± 0.01 nmol of ATCI hydrolyzed/min/mg protein, respectively) compared with the aging model group ($p < 0.001$) (**Figure 5**).

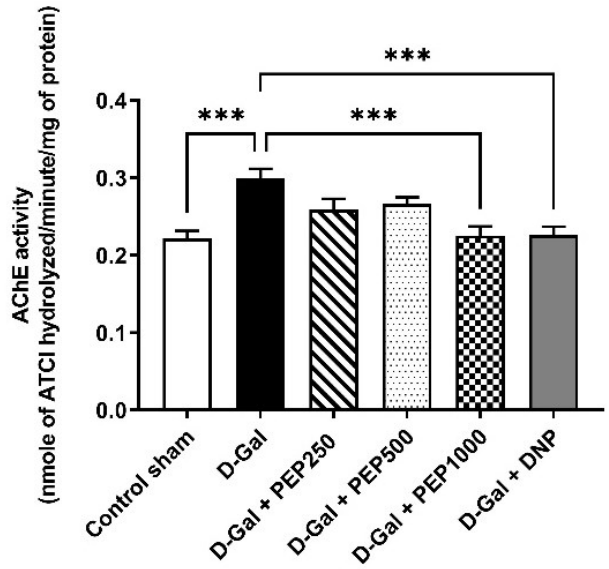

Figure 5 Effect of EP extract on AChE activity in the hippocampus of rats: Data are expressed as the mean ± SEM; N = 7 rats per group. $^{***}p < 0.001$ responses are significantly different compared with the D-Gal group. D-Gal = d-galactose, EP = eggplant, DNP = donepezil, AChE = acetylcholinesterase.

In the current study, we observed an increase in MDA level and AChE activity in the hippocampus of D-Gal induced aging model rats; however, PEP extract (1,000 mg/kg) and DNP significantly prevented this increase. Our findings indicate that PEP extract ameliorated D-Gal-induced cognitive impairment by decreasing MDA and AChE levels in the hippocampus. Based on these findings, PEP extract might be a useful dietary supplement for slowing down the aging process and delaying neurological conditions. However, more research into the molecular mechanisms underlying PEP extract's effects on oxidative stress and cholinergic pathways is necessary.

Conclusions

Our findings show that the administration of PEP extract has the potential to improve cognitive impairment induced by D-Gal. This improvement is attributed, in part, to the restoration of cholinergic functions through the regulation of AChE activity and the reduction of oxidative damage by decreasing the level of MDA in the hippocampus of D-Gal-induced aging rats.

Acknowledgements

The authors you are thankful to the School of Medical Sciences, University of Phayao, Thailand and the Institutional Animal Care of University of Phayao, Thailand, for providing the necessary facilities. This research study was funded by the University of Phayao capital contract no. FF65-RIM115.

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