นิพนธ์ต้นฉบับ . Original Article

สารสกัดผลลูกไหนมีผลต่อการป้องกันการลดลงของความจำในหนูแรทตัวเต็มวัยที่ถูกเหนี่ยวนำให้แก่ชราด้วยดีกาแลคโทส

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Prunus domestica L. Crude Extract Protects against Reductions of Memory in D-Galactose-Induced Aging in Adult Rats

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Received: 2 June 2022 / Revised: 14 August 2022 / Accepted: 16 August 2022

บทคัดย่อ

หลักการและวัตถุประสงค์: การลดลงของความจำเป็นพยาธิสภาพหนึ่งที่พบได้มากในผู้สูงอายุ การเสื่อมสภาพของสมองขณะที่อายุมากขึ้นสัมพันธ์กับภาวะเครียดออกซิเดชันซึ่งเหนี่ยวนำอนุมูลอิสระที่เพิ่มขึ้น การที่เซลล์ถูกทำลายและการตายของเซลล์ประสาท การศึกษาในสัตว์ทดลองพบว่าดีกาแลคโทส (D-galactose; D-gal) มีผลทำให้เกิดการลดลงของวงจรประสาทและการสร้างเซลล์ประสาทในชั้น SGZ ของ DG ในสมองส่วนฮิปโปแคมปัส ส่งผลให้สูญเสียความจำ *Prunus domestica* L. (PD) หรือลูกไหนเป็นผลไม้ที่มีสารประกอบประเภทฟีโนลิกและฟลาโวนอยด์ การศึกษาก่อนหน้านี้ได้รายงานผลของสารสกัดลูกไหนมีประโยชน์ต่อการเรียนรู้และความจำ ดังนั้นการศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของสารสกัดลูกไหนต่อภาวะความจำบกพร่องในหนูที่ถูกเหนี่ยวนำให้เกิดภาวะความชราด้วย D-gal
วิธีการศึกษา: หนูแรทเพศผู้สายพันธุ์ Sprague Dawley ถูกแบ่งออกเป็น 8 กลุ่ม ได้แก่ vehicle, D-gal, PD 75, PD 100, PD 150, D-gal + PD 75, D-gal + PD 100 และ D-gal + PD 150 โดย D-gal (50 มก/กก.) ให้โดยการฉีดเข้าช่องท้อง และ PD (75, 100 และ 150 มก/กก.) ให้โดยการป้อนทางปาก วันละครั้ง เป็นเวลา 8 สัปดาห์ ในระหว่างการให้สารหนูถูกชั่งน้ำหนัก หลังจากนั้นหนูถูกทดสอบความจำด้วยการทดสอบ novel object location (NOL) และ novel object recognition (NOR)
ผลการศึกษา: ไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($p>0.05$) ของน้ำหนักและการเคลื่อนไหวของหนูทุกกลุ่ม ส่วนการทดสอบความจำ NOL และ NOR กลุ่ม vehicle, PD 75, PD 100, PD 150, D-gal + PD 75, D-gal + PD 100 และ D-gal + PD 150 สามารถแยกความแตกต่างอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) ระหว่างวัตถุวางในตำแหน่งเก่าและใหม่หรือวัตถุเก่าและวัตถุใหม่ ในทางตรงข้ามกลุ่ม D-gal ไม่สามารถแยกได้ในทั้งสองการทดสอบ
สรุป: การศึกษาในครั้งนี้แสดงว่า สารสกัดลูกไหน (75, 100 และ 150 มก/กก.) มีผลที่เป็นประโยชน์ดีต่อการลดภาวะความจำบกพร่องที่เกิดจากการเหนี่ยวนำโดย D-gal ได้

คำสำคัญ: ความจำ, ลูกไหน, ดีกาแลคโทส, ชราภาพ

Abstract

Background and Objective: A decline in memory is the most common pathology of aging. Brain degeneration during aging is related to upregulating the reactive oxygen species (ROS) levels, which induce oxidative stress, neuronal damage, and neuronal apoptosis. In animal studies, D-galactose caused down-regulation of neuronal circuitry and neurogenesis in the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) resulting in memory impairment. *Prunus domestica* L. (PD) or look-nai is a fruit that contains phenolic compounds and flavonoids. Previous studies have reported the beneficial effects of PD extract on learning and memory. Therefore, this study aimed to study the effect of PD extract on memory impairment in aging rats induced by D-gal.
Methods: Male Sprague Dawley rats were divided into 8 groups: Vehicle, D-gal, PD 75, PD 100, PD 150, D-gal + PD 75, D-gal + PD 100, and D-gal + PD 150. D-gal (50 mg/kg) and PD (75, 100 and 150 mg/kg) were given by intraperitoneal injection and digestive gavage once a day for 8 weeks, respectively. Rats was weighed during the experiment. Then, memory was tested using the novel object location (NOL) and novel object recognition (NOR) tests.
Result: There were no statistically significant differences ($p>0.05$) in body weight and locomotor activity among the groups. For the NOL and NOR memory tests, the vehicle, PD 75, PD 100, PD 150, D-gal + PD 75, D-gal + PD 100, and D-gal + PD 150 groups were able to discriminate between the objects placed at the familiar and novel locations or the familiar and novel objects ($p<0.05$). On the other hand, the D-gal group was unable to distinguish in both tests.
Conclusion: This study demonstrates that PD (75, 100 and 150 mg/kg) had a beneficial effect to ameliorate D-gal-induced memory impairment.

Keywords: memory, *Prunus domestica* L., D-galactose, aging

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Introduction

Brain degeneration during aging is related to upregulating reactive oxygen species (ROS) levels, which induce oxidative stress, stimulating neuronal damages, and apoptosis of neurons. These alterations eventually lead to neuronal loss, dysfunction of neuronal plasticity, and decreasing neurogenesis in the brain[1,2]. Neurogenesis in the brain is found in two important neurogenic regions: the subgranular zone (SGZ) of the dentate gyrus in the hippocampus and the subventricular zone (SVZ) in the lateral ventricle. The hippocampal function is associated with working memory such as spatial memory. Navigational performance declines have been found in elderly people[3].

D-galactose (D-gal) is an uncomplicated monosaccharide sugar and found in several fruits and vegetables[4]. It is well-known about D-galactose that can induce aging in adult rats by increasing the production of ROS, oxidative stress, dysfunctional mitochondria, and neural degeneration in the hippocampus[5,6]. Moreover, D-gal-induced aging in rats revealed impairments of recognition and spatial memory associated with a decline of cell proliferation, neuronal cell survival and immature neurons. Thus, aging rats exhibited a reduction of hippocampal neurogenesis related to memory impairment[7].

Prunus domestica L. (PD) is one of plum species. It is also a member of Rosacea family and prunus genus. Plums have been demonstrated to have several pharmacological capacities and utilized as anti-inflammatory and anxiolytic effects. Plums is also used as sedatives for neurasthenia and prevention of aging in folk Iranian medicine to prevent Alzheimer's disease[8,9]. According to many researchers, plums contain phenol and flavonoid compounds higher than other fruits[10].

Therefore, this study aims to investigate neuroprotective effects of PD against memory deficits in aging rats induced by D-gal. Memory was assessed using the novel object location (NOL) and novel object recognition (NOR) tests, which are spatial and recognition memories, respectively[11-13].

Materials and Methods

Animals

Ninety-six adult male Sprague-Dawley rats (age: 8 weeks old, body weight: 280-300 g) were obtained from Nomura Siam International Co., Ltd. Pathumwan, Bangkok. This experimental protocol was performed in concordance with ethical guidelines and approved by Khon Kaen University Ethics Committee in Animal Research (IACUC-KKU-49/64). Animals were assigned 4 animals per a plastic cage with wood shaving bedding in a well-ventilated room preserved at 23-25^{0}C. All animals were given a 12-hour light/dark cycle, a standard balanced diet, and free access to food and tap water ad libitum. Animals will be raised without any drug administration for a 4-week period for acclimatization.

Plant collection and extraction

PD fresh fruits were collected at the end of February 2021 at latitude/ longitude: 20° 11' 57" N / 99° 53' 5" E from Mae Chan District, Chiang Rai Province, Thailand. The voucher specimen was authenticated and deposited at Medicinal Plants Innovation Center, Mae Fah Luang University, Thailand. The mature undamaged fruits were selected and washed thoroughly with distilled water. After that, it was macerated with 95% (v/v) ethanol for a week. The combined extract was filtered through filter paper. Then, we used the rotary to do solvent extraction. The yield of the crude extracts was 4.84 %. Finally, it was dissolved in a proper amount of distilled water to prepare the dose of extract as needed.

Drugs and administration schedules

D-gal were purchased from Sigma Aldrich, Inc., St. Louis, USA, while PD crude extract was obtained from Medicinal Plant Innovation Center of Mae Fah Luang University, Muang, Chiang Rai, Thailand. The other chemicals employed were scientific standard. D-gal was dissolved in 0.9% normal saline for intraperitoneal (i.p.) administration, whereas PD extract was dissolved in distilled water for oral administration. All animals were randomly divided into 8 groups, with each group comprising of 12 animals as follows:

Group I: Vehicle group received 0.9% normal saline by i.p. injection and distilled water by intra-gastric gavage.

Group II: D-galactose group received D-gal 50 (mg/kg/day, i.p.).

Group III, IV, V: PD 75, 100, 150 groups received orally PD 75, 100, 150 mg/kg/day, respectively.

Group VI, VII, VIII: D-gal + PD 75, D-gal + PD 100, D-gal + PD 150 groups received co-administration of D-gal 50 (mg/kg/day, i.p.) and PD orally 75, 100, 150 mg/kg/day, respectively.

The D-gal and PD doses were chosen based on published literatures research[7,14].

All animals were treated for 8 weeks. Three days after the drug administration, all animals were evaluated behavioral tests using the NOL and NOR tests (Fig 1).

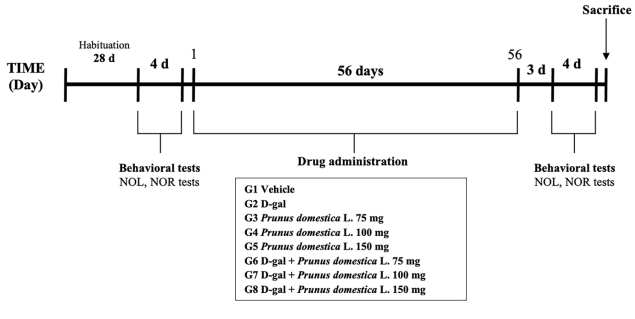

Figure 1 Timeline of drug administration and behavioral testing

Behavioral tests

The behavioral tests were performed before and after drug administration using the NOL and NOR tests. The equipment includes a 50-cm-square arena and water-filled plastic bottles. For data collection, the arena was placed under a digital camera attached to a computer recording method (EthoVision®, XT Version 12, Noldus, Wageningen, Netherlands).

NOL and NOR tests

The NOL test includes three parts of trials: habituation, familiarization, and choice. For the habituation, the animals were able to explore an open-field arena for 30 minutes without stimulations a day foregoing testing. One day later, the animals were given 3 minutes to explore the arena without stimulations. Two similar objects were deposited in different locations in the arena for the animals to explore for 3 minutes in the familiarization trial. After exploring, animals were caged for 15 minutes (inter-trial interval; ITI). To remove odors, the arena and two objects were wiped with 20% alcohol. The choice trial was performed by placing one object at a familiar location (FL) and the other at a new location (novel location; NL) and then the animals explored the objects for 3 minutes.

The NOR test employed the same procedure as the NOL test both the habituation and familiarization trials. During the choice trial, one of the duplicates or familiar objects (FO) and a novel object (NO) were set at the same location in the arena and the animals were allowed to explore the objects for 3 minutes. When the animals actively explored the objects by smelling, licking, or pointing the nose towards the object at a range less than 2 cm, the exploration time of the NOL and NOR tests was recorded. Results were expressed the locomotor activity of the animals and discrimination index (DI) (the capacity to distinguish between novel and familiar locations or objects). Animals typically spend more time exploring a new location or object than a familiar one in both tests. Consequently, the DIs must be significantly greater than zero.

Statistical analysis

The data of the total exploration time in the NOL and NOR tests were analyzed using One-way analysis of variance (ANOVA), while the DI was analyzed using One-sample t-test followed by Bonferroni's post hoc test. Two-way repeated measures ANOVA was used to determine the body weight (in gram) of animals among groups. Comparative significance was determined using $p<0.05$ as the cut-off point for statistical tests. Results were then demonstrated as mean standard error of the mean (SEM). The analysis was performed using GraphPad Prism (Version 9.0; GraphPad Software Inc., San Diego, CA, USA).

Results

1. Effects of D-gal and PD on weight gain

Weighing the animals every day was a routine part of the experiment as shown in Figure 2. No significant differences of the body weight were detected among groups ($p>0.05$). Based on these findings, it appears that D-gal and PD did not have a negative effect on body weight.

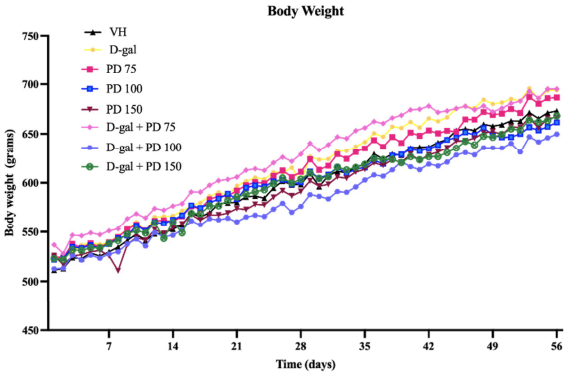

Figure 2 Body weight of animals throughout the drug administration.

2. Behavioral tests

2.1 Effects of D-gal and PD crude extract on locomotor activities

The animal locomotor activity was evaluated using the total exploration time recorded during the tests. One-way ANOVA revealed that statistically significant differences were not revealed in both the NOL and NOR tests in all animal groups ($p>0.05$ Fig. 3 and 4). As a result of evidence suggests that the drug administration does not impair motor integration.

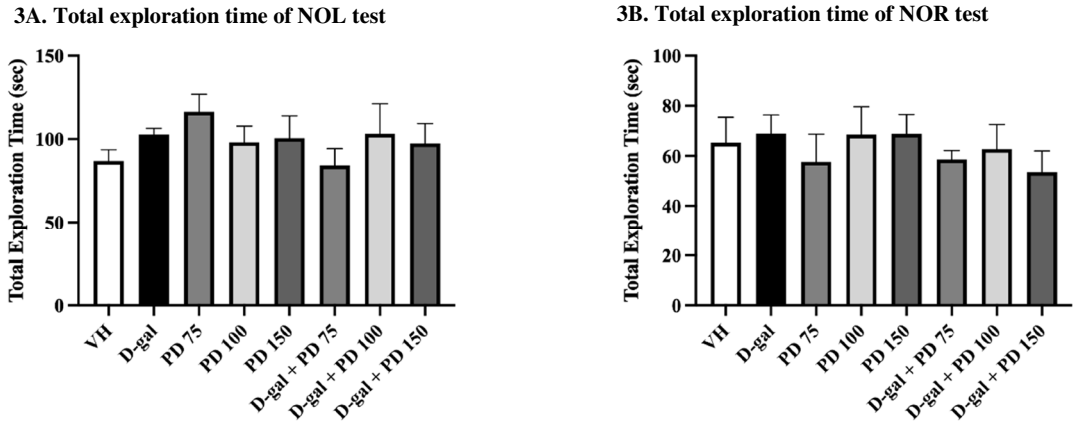

Figure 3 Total exploration time of all objects explored by the animals in the NOL (A) and NOR (B) tests before drug administration. All groups revealed no significant difference.

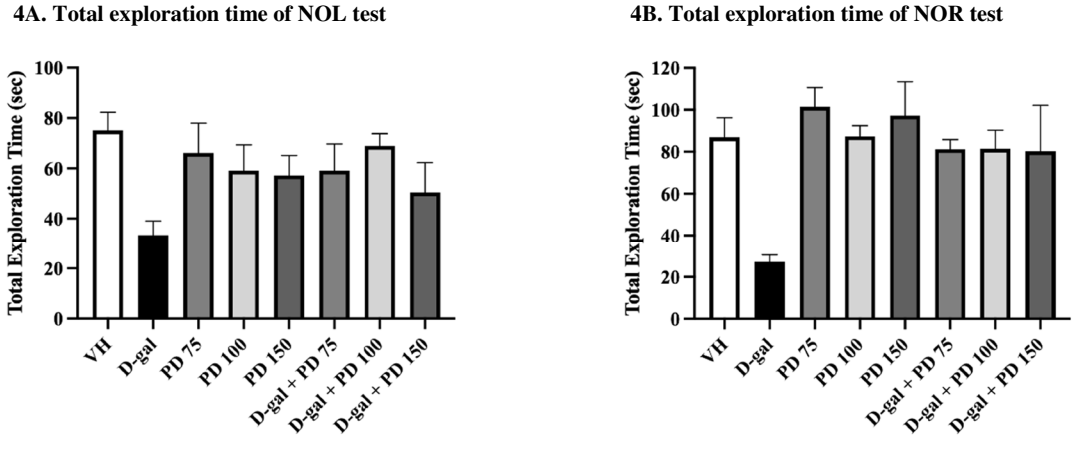

Figure 4 Total exploration time of all objects explored by the animal in the NOL (A) and NOR (B) tests after drug administration. All groups revealed no significant difference.

2.2 Effects of D-gal and PD crude extract on discrimination index (DI)

As part of the choice trail, the DI was calculated for the NOL and NOR tests. Before treatment, there were significant differences in DI among groups ($p<0.05$, Fig. 5A and 5B). A p-value less than 0.05 indicates the ability of all animals to distinguish between an object that had been relocated to a novel location and a novel object. The DI analysis performed after treatment revealed that there were significant differences in the DI in comparison with 0 in the vehicle, PD, and co-treatment with the PD groups. On the other hand, the D-gal group did not show any significant differences in the DI when compared to 0 ($p<0.05$, Fig. 6A and 6B). According to these findings, PD may help to improve memory impairments related to spatial navigation and recognition.

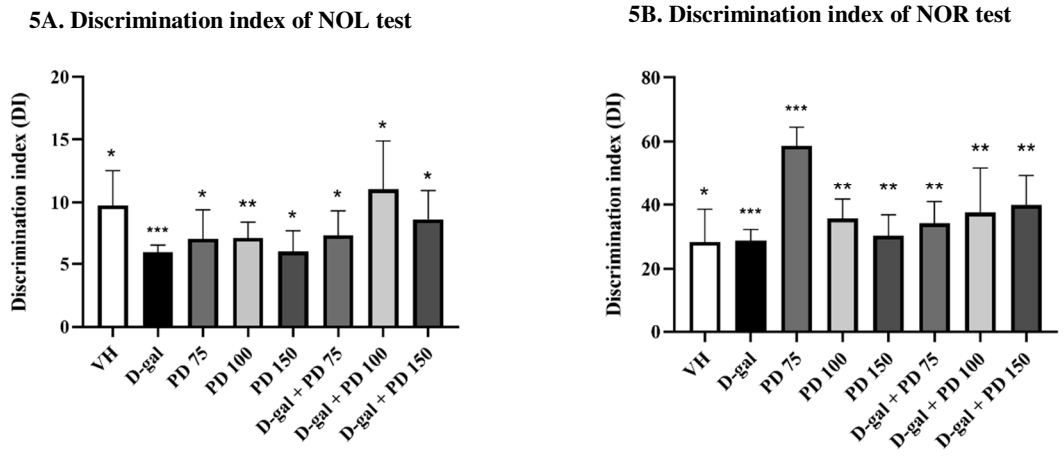

Figure 5 Before drug administration, the DI of the animals in the NOL (A) and NOR (B) tests. (*p<0.05, ** p<0.01, and *** p<0.001 significant differences compared to zero)

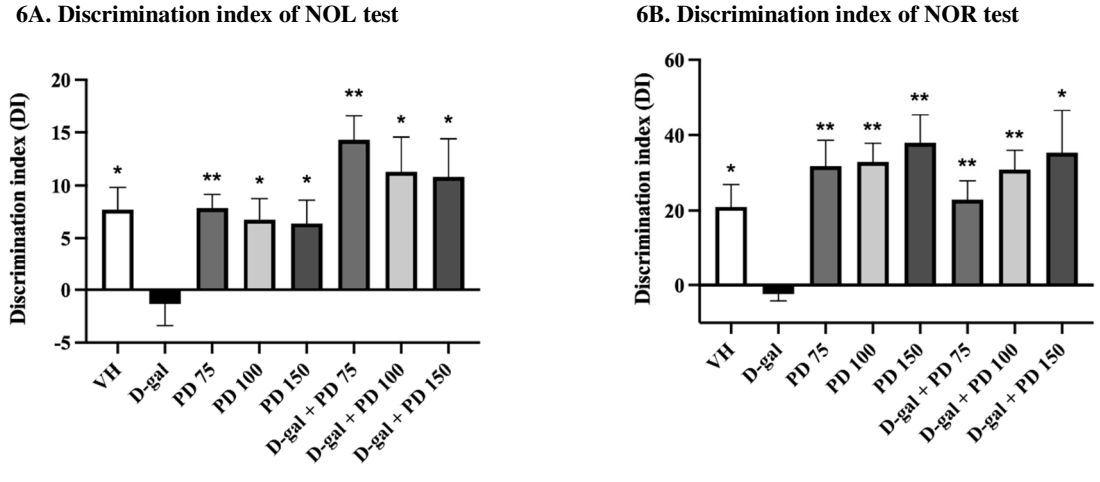

Figure 6 After drug administration, the DI of the animals in the NOL (A) and NOR (B) tests. (*$p<0.05$ and ** $p<0.01$ significant differences compared to zero)

Discussion

Numerous research have shown that accumulation of D-gal stimulates brain aging by initiating oxidative stress and inflammation, leading to loss of neuronal cells and memory. In this study, we used a D-gal-induced aging model to evaluate the protective effect of PD crude extract on the memory impairment caused by aging. In addition, co-treatment with PD in this study has a beneficial role in decreasing impairment induced by D-gal in rats[15]. Previous studies revealed no toxicity of PD in an *in vivo* study at 75,100,150 mg/kg doses and an *in vitro* study using MTT assay[14,16].

After administration, the results showed that a significant difference in the body weight of the animals was not detected in all groups. This suggests that D-gal and PD did not affect to the body weight. Previous studies also revealed the body weight of animals received D-gal which is not altered when compared to the other groups. Even the treatment with hydro-alcoholic extract of plum does not show significant differences in body weight of the animals[14,17,18].

Under the experimental circumstances of this study, receiving D-gal or PD, as well as D-gal with PD, has no effect on the animal locomotor activity, as measured by the total exploration time in all the animal groups. Therefore, the behavioral tests carried out in this investigation were not likely to be affected by motor impairments. D-gal has offered up comparable results, as reported[7].

This study is based on an animal's inherent preference for novelty and does not require any additional external reinforcement. The NOL and NOR are two effective behavioral tasks that are commonly used to determine the function and health of specific memory-related brain regions. The NOL focuses on spatial learning, which is heavily influenced by activity in the hippocampal brain region. A non-spatial learning of object identity, on the other hand, is evaluated by the NOR, which relies on a multiple of brain regions[19,20]. After drug administration, the D-gal group in this study was unable to distinguish between objects placed in the familiar and novel locations during the NOL test. In the same way as in the NOR test, the animals in the D-gal group were unable to recognize the difference between the familiar and novel objects. Memory and cognitive impairments are shown to be caused by D-gal, which is supported by findings that are consistent with previous studies[7,18]. Since D-gal can accelerate aging process in many organs by upregulating oxidative stress, ROS, apoptosis that can induce lipid peroxidation in the cell membranes and disrupt redox equilibrium, resulting in neuronal injury[17,21]. However, Positive DI values significantly greater than zero indicates that animals administered with both D-gal and PD were able to distinguish between an object placed in a novel location and a novel object. PD has the ability to reduce the effect of D-gal on memory impairments. Conversely, PD prevented the D-gal-mediated ROS accumulation, by regulating the antioxidant mechanisms through their interactions with activating the extracellular signal-regulated kinase (ERK) and the Akt signaling pathways, enhanced production of a transcription factor. This response induces the exhibition of neurotrophins that is essential for long-term memory and long-term potentiation (LTP) process[22,23]. However, further investigation in molecular testing may help to understand about hippocampal neurogenesis that relates to memory and develop PD as a memory supplement in aging.

Conclusion

According to the findings of the current research, PD is able to protect against the memory impairment that is caused by D-gal in aged rats. This study suggests that PD has a beneficial effect on an animal model of age-related memory deficits.

Acknowledgement

The authors would like to thank the Invitation Research from Faculty of Medicine (IN65224), Khon Kaen University for financial support.

References

1. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Kawas CH, et al. The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's Dement 2011;7(3):263–9.
2. Yassa MA, Lacy JW, Stark SM, Albert MS, Gallagher M, Stark CEL. Pattern separation deficits associated with increased hippocampal CA3 and dentate gyrus activity in nondemented older adults. Hippocampus 2011;21(9):968–79.
3. Ming GL, Song H. Adult Neurogenesis in the Mammalian Brain: Significant Answers and Significant Questions. Neuron 2011;70(4):687–702.
4. Acosta PB, Gross KC. Hidden sources of galactose in the environment. Eur J Pediatr 1995;154(7 Suppl 2):87-92.

5. Hong XP, Chen T, Yin NN, Han YM, Yuan F, Duan YJ, et al. Puerarin Ameliorates D-Galactose Induced Enhanced Hippocampal Neurogenesis and Tau Hyperphosphorylation in Rat Brain. J Alzheimer's Dis 2016;51(2):605–17.
6. Cannizzo ES, Clement CC, Sahu R, Follo C, Santambrogio L. Oxidative stress, inflamm-aging and immunosenescence. J Proteomics 2011; 74(11):2313-23.
7. Prajit R, Sritawan N, Suwannakot K, Naewla S, Aranarochana A, Sirichoat A, et al. Chrysin protects against memory and hippocampal neurogenesis depletion in D-galactose-induced aging in rats. Nutrients 2020;12(4):1100.
8. Egbekun MK, Akowe JI, Ede RJ. Physico-chemical and sensory properties of formulated syrup from black plum (Vitex doniana) fruit. Plant Foods Hum Nutr 1996;49(4):301-6.
9. Bouayed J, Rammal H, Dicko A, Younos C, Soulimani R. Chlorogenic acid, a polyphenol from *Prunus domestica* (Mirabelle), with coupled anxiolytic and antioxidant effects. J Neurol Sci 2007;262(1-2):77-84.
10. Kayano SI, Kikuzaki H, Fukutsuka N, Mitani T, Nakatani N. Antioxidant activity of prune (*Prunus domestica* L.) constituents and a new synergist. J Agric Food Chem 2002;50(13):3708–12.
11. Fardell JE, Vardy J, Shah JD, Johnston IN. Cognitive impairments caused by oxaliplatin and 5-fluorouracil chemotherapy are ameliorated by physical activity. Psychopharmacology (Berl) 2012;220(1): 183–93.
12. Sirichoat A, Chaijaroonkhanarak W, Prachaney P, Pannangrong W, Leksomboon R, Chaichun A, et al. Effects of Asiatic acid on spatial working memory and cell proliferation in the adult rat hippocampus. Nutrients 2015;7(10):8413–23.
13. Chaisawang P, Sirichoat A, Chaijaroonkhanarak W, Pannangrong W, Sripanidkulchai B, Wigmore P, et al. Asiatic acid protects against cognitive deficits and reductions in cell proliferation and survival in the rat hippocampus caused by 5-fluorouracil chemotherapy. PLoS One 2017;12(7):1–14.
14. Shahidi S, Setareye S, Mahmoodi M. Effect of *Prunus domestica* L. (mirabelle) on learning and memory in mice. Anc Sci Life 2013;32(3):139-43.
15. Kumar A, Prakash A, Dogra S. *Centella asiatica* Attenuates D-Galactose-Induced Cognitive Impairment, Oxidative and Mitochondrial Dysfunction in Mice. Int J Alzheimers Dis 2011;2011:1-9.
16. Ullah H, Sommella E, Santarcangelo C, D'Avino D, Rossi A, Dacrema M, et al. Hydroethanolic Extract of *Prunus domestica* L.: Metabolite Profiling and In Vitro Modulation of Molecular Mechanisms Associated to Cardiometabolic Diseases. Nutrients 2022;14(2):340.
17. Hsieh HM, Wu WM, Hu ML. Soy isoflavones attenuate oxidative stress and improve parameters related to aging and Alzheimer's disease in C57BL/6J mice treated with d-galactose. Food Chem Toxicol 2009;47(3):625–32.
18. Nam S, Seo M, Seo J, Rhim H, Nahm S, Cho I et al. Ascorbic Acid Mitigates D-galactose-Induced Brain Aging by Increasing Hippocampal Neurogenesis and Improving Memory Function. Nutrients 2019; 11(1):176.
19. Akirav I, Maroun M. Ventromedial prefrontal cortex is obligatory for consolidation and reconsolidation of object recognition memory. Cereb Cortex 2006;16(12):1759-65.
20. Murai T, Okuda S, Tanaka T, Ohta H. Characteristics of object location memory in mice: Behavioral and pharmacological studies. Physiol Behav 2007; 90(1):116-24.
21. Roser M, Josic D, Kontou M, Mosetter K, Maurer P, Reutter W. Metabolism of galactose in the brain and liver of rats and its conversion into glutamate and other amino acids. J Neural Transm 2009; 116(2):131–9.
22. Tomás-Barberán FA, Gil MI, Cremin P, Waterhouse AL, Hess-Pierce B, Kader AA. HPLC-DAD-ESIMS analysis of phenolic compounds in nectarines, peaches, and plums. J Agric Food Chem 2001; 49(10):4748-60.
23. Spencer JP. Food for thought: the role of dietary flavonoids in enhancing human memory, learning and neuro-cognitive performance. Proc Nutr Soc 2008;67(2):238-52.

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