นิพนธ์ต้นฉบับ . Original Article

ผลของสารเฮสเพอริดินต่อภาวะความจำบกพร่องในหนูที่ถูกเหนี่ยวนำโดยกรดวอลโพรอิก

โสรยา แก้วงาม[1], ธนพร อะโนศรี[1], ราม ประจิตต์[1], กรรวี สุวรรณโคตร[1], นัตยา ศรีตะวัน[1], อนุสรา อารณะโรจน์[1], อภิวัฒน์ ศิริโชติ[1], วนัสนันท์ แป้นนางรอง[1], จริยา อำคา เวลบาท[1,2*]

[1]*ภาควิชากายวิภาคศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น*

[2]*กลุ่มวิจัยและพัฒนาด้านประสาท มหาวิทยาลัยขอนแก่น*

Effect of Hesperidin on Memory Impairments Induced by Valproic acid in Rats

Soraya Kaewngam[1], Tanaporn Anosri[1], Ram Prajit[1], Kornrawee Suwannakot[1], Nataya Sritawan[1], Anusara Aranarochana[1], Apiwat Sirichoat[1], Wanassanan Pannangrong[1], Jariya Umka Welbat[1,2*]

[1]*Department of Anatomy, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand.*

[2]*Neuroscience Research and Development Group, Khon Kaen University, Khon Kaen 40002, Thailand.*

Received: 21 October 2020

Accepted: 15 December 2020

หลักการและวัตถุประสงค์: การลดลงของการสร้างเซลล์ประสาท (neurogenesis) ในชั้น subgranular zone (SGZ) ของ dentate gyrus (DG) ในสมองส่วนฮิปโปแคมปัส สัมพันธ์กับภาวะความจำบกพร่อง มีการศึกษาในสัตว์ทดลองที่ได้รับกรดวอลโพรอิก (valproic acid; VPA) พบว่ามีการสร้างเซลล์ประสาทในชั้น SGZ ลดลง ซึ่งส่งผลให้เกิดความจำบกพร่อง เฮสเพอริดิน (hesperidin; Hsd) เป็นสารสกัดจากธรรมชาติจากพืชจำพวกฟลาโวน ซึ่งมีฤทธิ์ในการเพิ่มและกระตุ้นการเรียนรู้และความจำ ดังนั้นการศึกษาครั้งนี้จึงมีวัตถุประสงค์เพื่อศึกษาผลของ Hsd ต่อภาวะความจำบกพร่องในหนูที่ถูกกระตุ้นด้วย VPA

วิธีการศึกษา: หนูแรทเพศผู้สายพันธุ์ Sprague Dawley แบ่งออกเป็น 4 กลุ่ม กลุ่มละ 6 ตัว ได้แก่ กลุ่ม vehicle, VPA, Hsd และ VPA+Hsd โดย VPA (300 มก./กก.) ให้โดยการฉีดเข้าช่องท้อง วันละสองครั้ง เป็นเวลา 14 วัน Hsd (100 มก./กก./วัน) ให้โดยการป้อนทางปาก วันละครั้ง เป็นเวลา 21 วัน ในระหว่างการให้สารหนูจะถูกชั่งน้ำหนักและนำมาวิเคราะห์ และหลังจากสิ้นสุดการให้สาร 3 วัน หนูถูกทดสอบความจำโดยทำการทดสอบ novel object location (NOL) และ novel object recognition (NOR)

ผลการศึกษา: ผลของน้ำหนักและผลการทดสอบ NOL และ NOR ค่าระยะเวลาการสำรวจวัตถุทั้งหมดในแต่ละกลุ่มไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($p>0.05$) การทดสอบ NOL พบว่าหนูกลุ่ม vehicle, Hsd และ VPA+Hsd สามารถแยกความ

Background and objective: Decreasing of neurogenesis in the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus is linked to memory deficits. In animal studies, treatment with valproic acid (VPA) impairs neurogenesis in the SGZ resulting in memory impairment. Hesperidin (Hsd), a plant flavanone, is a natural extract, which enhances learning and memory. Therefore, the aim of this study was to investigate the effect of Hsd on memory impairment in rats induced by VPA.

Methods: Male Sprague Dawley rats were divided into 4 groups (6 animals/group) including vehicle, VPA, Hsd and VPA+Hsd groups. VPA (300 mg/kg) was administrated by intraperitoneal (i.p.) injection twice daily for 14 days. Hsd (100 mg/kg/day) was administrated by oral gavage once a day for 21 days. Body weight was weighed and recorded every day. Three days after the treatment, rats were tested for memory using the novel object location (NOL) and novel object recognition (NOR) tests.

Results: The results showed that the body weight and total exploration time were not significantly different among groups in both NOL and NOR tests ($p>0.05$). In the NOL test, rats in the vehicle, Hsd and

*Corresponding author : Jariya Umka Welbat, Department of Anatomy, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand. E-mail: jariya@kku.ac.th

แตกต่างระหว่างวัตถุในตำแหน่งใหม่และตำแหน่งเก่าได้อย่างมีนัยสำคัญทางสถิติ ($p<0.05$) และเมื่อทดสอบ NOR พบว่าหนูกลุ่ม vehicle, Hsd และ VPA+Hsd สามารถแยกความแตกต่างระหว่างวัตถุใหม่และวัตถุเก่าได้อย่างมีนัยสำคัญทางสถิติ ($p<0.05$) ในขณะที่หนูในกลุ่ม VPA ไม่สามารถแยกแยะได้ทั้งสองการทดสอบ

สรุป: การศึกษาในครั้งนี้พบว่าเฮสเพอริดิน สามารถฟื้นฟูความจำบกพร่องที่เกิดจากการเหนี่ยวนำโดยกรดวอลโพรอิกได้

คำสำคัญ: เฮสเพอริดิน; กรดวอลโพรอิก; ความจำบกพร่อง

VPA+Hsd groups could significantly discriminate between the novel and familiar locations ($p<0.05$). In the NOR test, similarly, rats in the vehicle, Hsd and VPA+Hsd groups could significantly discriminate between the novel and familiar objects ($p<0.05$). In contrast, rats in the VPA group could not significantly performed in both tests.

Conclusion: This study demonstrates that hesperidin could improve the memory impairments induced by valproic acid.

Key word: Hesperidin; Valproic acid; Memory impairment

ศรีนครินทร์เวชสาร 2564; 36(2): 142-148. ● Srinagarind Med J 2021; 36(2): 142-148.

Introduction

Dementia is one of chronic disorders caused by the deterioration of the brain[1]. Decreasing of neurogenesis in the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus[2–5] induces memory deficits. In animal researches, treatment with valproic acid (VPA) causes neurogenesis reduction in the SGZ resulting in memory impairment[4,6–8].

Memory functions are related to neurogenesis[9], which is a process that new neurons are continuously generated from neural stem cells throughout life[10]. Previous studies have shown that VPA can cause cognitive impairment and reduce neurogenesis in the hippocampal dentate gyrus[4].

Hesperidin (Hsd), a flavonoid compound, is a natural extract of flavanone group and richly found in the citrus fruits such as lemons and oranges[11,12]. It has the anti-inflammatory, antioxidant, anti-apoptotic and neuroprotective properties[13–15]. A recent study has shown that Hsd (100 mg/kg/day) decreases memory impairment in methotrexate-treated rats by recovering levels of neurogenesis in the hippocampal dentate gyrus[16].

Therefore, this study investigated the effect of Hsd on memory impairment induced by VPA in rats. The spatial memory was tested using the novel object location (NOL) and recognition memory was tested using novel object recognition (NOR) tests.

Materials and Methods

Animals

Twenty-four male Sprague Dawley rats (age: 4-5 weeks, 120-170 g) were provided from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornprathom. The experimental method was accepted by the Khon Kaen University Ethics Committee in Animal Research (ACUC-KKU-57/62). The rats were adjusted to the experimental condition for one week before the experiment. Four rats were housed in a cage and maintained under standard laboratory conditions (12 h light-dark cycle, 23±2 °C temperature). They were fed with food and water ad libitum.

Drug administration

VPA (Sigma-Aldrich, Inc., St. Louis, USA) and Hsd (ChemFaces Biochemical, Wuhan, China) were freshly prepared every day before the drug administration. VPA at a dose of 300 mg/kg was dissolved in 0.9% saline solution by intraperitoneal (i.p.) injection two times every day at 10 a.m. and 3 p.m. for 14 days. Hsd at a dose of 100 mg/kg/day was dissolved in propylene glycol (Ajax Finechem Pty Ltd., Auckland, New Zealand) and given by oral gavage for 21 days. Rats were randomly divided into 4 groups. The vehicle group received 0.9% saline solution (i.p.) and propylene glycol by oral gavage. The VPA group received 300 mg/kg of VPA (i.p.) at 10 a.m. and 3 p.m.[4,17]. The Hsd group received 100 mg/kg of Hsd by oral gavage. The VPA+Hsd group administered with VPA and Hsd at the same dose and time as in the VPA and Hsd groups.

Behavioral testing

The NOL and NOR tests were adapted from Dix and Aggleton[18], which are spatial and recognition memory tasks. Both tests were divided into three parts

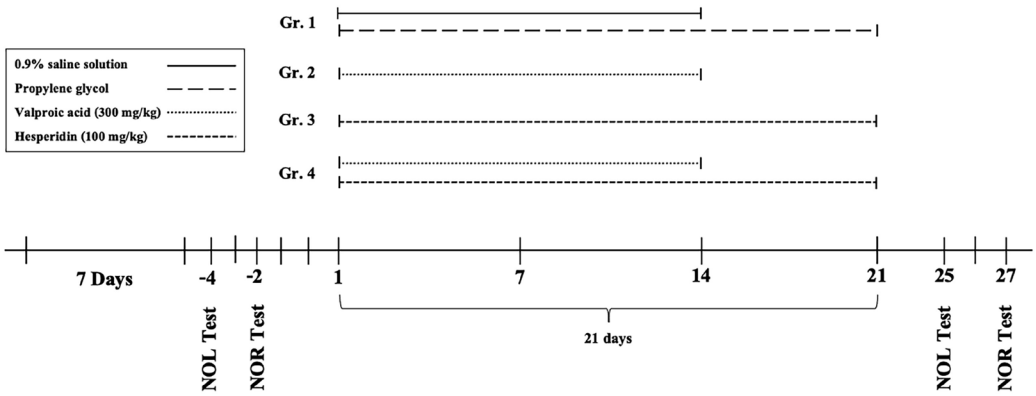

Figure 1 Timeline of drug administration and behavioral tests.

including habituation, familiarization and choice trials. One day before the NOL and NOR testing, all rats were habituated to the environment of an arena (open field black acrylic 50 x 50 x 50 cm.) without objects for 30 minutes. The objects used in these tests composed of the identical objects (plastic bottles and triangles). The behavioral tests were recorded using EthoVision® XT (EthoVision®, XT version 12, Noldus, Wageningen, Netherlands)

Novel object location (NOL) test

On the testing day, all rats were habituated to the arena without objects for 3 minutes. In the familiarization trial, they were allowed to survey two similar objects put in different positions for 3 minutes and then returned to their cage for 15 minutes. In the choice trial, they were enabled to explore two similar objects, one of which was placed in the same location and the other one was placed in a new location for 3 minutes.

Novel object recognition (NOR) test

On the testing day, all rats were habituated to the arena without objects for 3 minutes. In the familiarization trial, rats were placed at the center of the arena to explore two similar objects placed in different locations for 3 minutes and then moved to home cage for 15 minutes. During the choice trial, rats were put back to the arena and enabled to explore one familiar object and a novel object in the same locations for 3 minutes.

The exploratory activity of rats was measured when their noses directed to the object from a distance within 2 cm.[5,18]. The exploration time was used to calculate discrimination index (DI) that is defined as the ability to discriminate between novel and familiar locations or objects. A positive DI value means the rats can discriminate the objects in the novel location or novel object from the objects in the familiar location or familiar object. By contrast, a negative DI values reflect the rats cannot discriminate the objects in the novel location or novel object from the objects in the familiar location or familiar object. If the DI value is not significantly higher than 0, indicating that rats cannot discriminate between two objects by showing equal exploration[4,8,16].

Statistical analysis

The data were expressed as mean ± standard error of mean (SEM) using GraphPad Prism 8.0 software (GraphPad software Inc., San Diego, CA, USA). Two-way repeated measure analysis of variance (ANOVA) was used to analyze body weight. One-way ANOVA was used to determine total exploration time. The DIs were compared using one sample t-test. A significance was considered as $p<0.05$.

Results

1. Body weight

In this study, body weight of rats in all groups were not significantly different throughout the experiment ($p>0.05$, Fig 2). The result indicates that Hsd and VPA did not have a negative effect on the body weight of all rats.

2. Behavioral tests

During the behavioral tests, total exploration times were recorded before and after three days of drugs administration to evaluate locomotor activity of rats. The DIs were calculated in the choice trial in both of the behavioral tests.

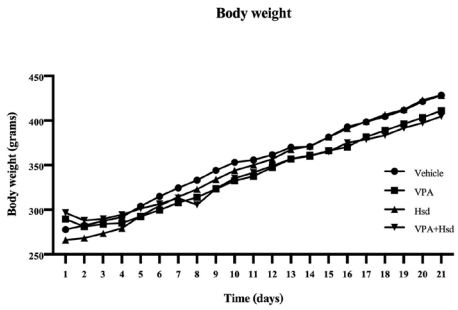

Figure 2 Body weight of rats throughout the drugs administration.

2.1 NOL test

The total exploration times in the NOL test were not significant differences among groups before ($p>0.05$, one-way ANOVA, Fig 3A) and after treatment ($p>0.05$, one-way ANOVA, Fig 3B). This result indicates that the rats in all groups had similar locomotor activity. Additionally, the DIs of all groups before treatment were higher than 0 ($p<0.05$, one sample t-test, Fig 4A). The results indicate that rats in all groups could discriminate between two identical objects in different locations. After treatment, the DIs of the vehicle, Hsd and VPA+Hsd groups were significantly different from zero ($p<0.05$, one sample t-test, Fig 4B). Whereas, the DI of the VPA group was not significantly different from zero ($p>0.05$, one sample t-test, Fig 4B). These results indicate that rats in the vehicle, Hsd and VPA+Hsd groups could discriminate between two identical objects in different locations but rats in the VPA group could not discriminate. These indicate that Hsd could counteract VPA-induced spatial memory deficits.

2.2 NOR test

The total exploration times in the NOR test were not significant differences among groups before and after treatment ($p>0.05$, one-way ANOVA, Fig 5A-B). This result indicates that rats in all groups had a similar locomotor activity. In addition, the results of the DIs before treatment were significant differences among groups ($p<0.05$, one sample t-test, Fig 6A). These indicate that all rats were able to discriminate between the novel and familiar objects. For the DI analysis after treatment, there were significant differences in the vehicle, Hsd and VPA+Hsd groups ($p<0.05$, one sample t-test, Fig 6B) but it was not found in the VPA group ($p>0.05$, one sample t-test, Fig 6B). The results indicate that rats in the vehicle, Hsd and VPA+Hsd groups had the ability to discriminate between the novel and familiar objects. However, rats

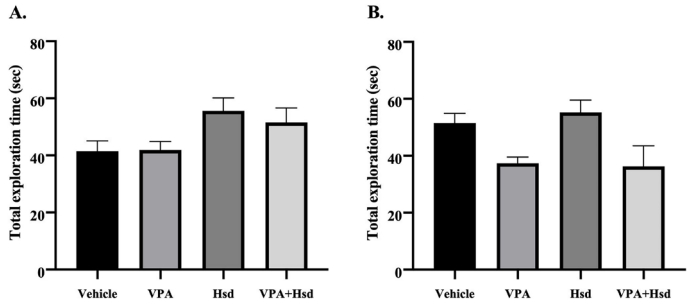

Figure 3 Total exploration time of the NOL test before (A) and after treatment (B).

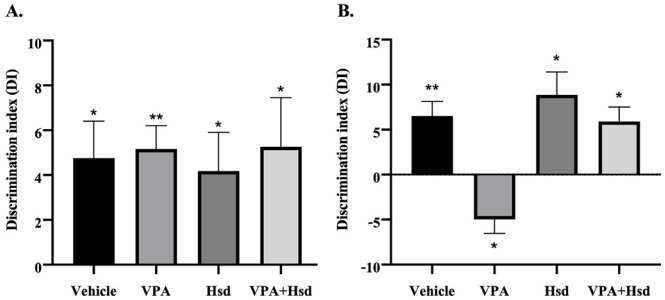

Figure 4 The discrimination index of the NOL test before (A) and after treatment (B). (*$p<0.05$, **$p<0.01$ significant difference compared to zero)

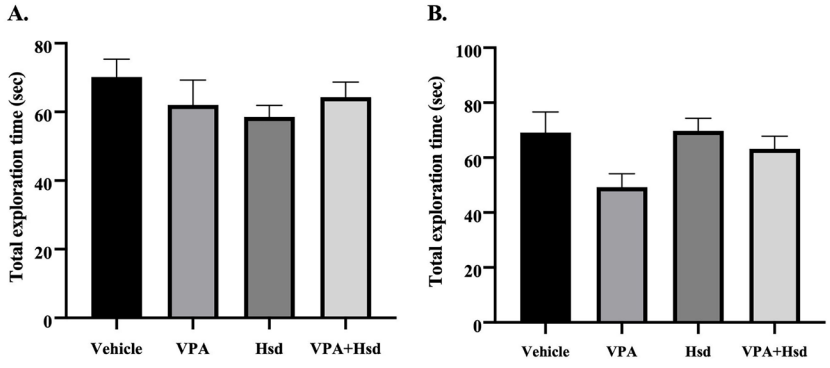

Figure 5 Total exploration time of the NOR test before (A) and after treatment (B).

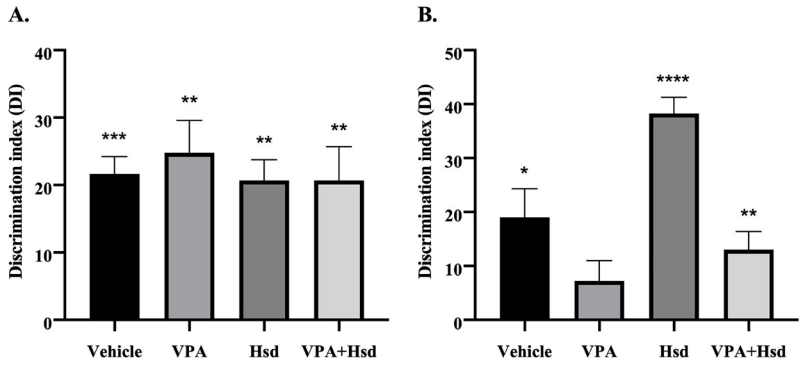

Figure 6 The discrimination index of the NOR test before (A) and after treatment (B). (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 significant difference compared to zero)

in the VPA group could not discriminate between the novel and familiar objects. These findings reveal that Hsd could improve VPA-impaired the recognition memory in rats.

Discussion

The present study demonstrates the effect of Hsd on memory impairment after receiving VPA treatment. During this study, we found that Hsd could improve VPA-induced memory deficits in rats. However, both Hsd and VPA did not affect body weight and locomotor activity.

The results after administration show that the body weight of rats in all groups were not significantly different, indicating that Hsd and VPA did not affect body weight. This finding suggests that Hsd did not affect body weight which is consistent with previous studies[19,20]. Besides the effect of Hsd on body weight, VPA treatment for 14 days was not significantly different in other groups. Similarly, the body weight of rodent pups exposed to VPA in utero was not significantly different when compared to the controls[21].

In this study, the rats administrated with both Hsd and VPA had no effect on locomotor activity that was evaluated using total exploration time. This indicating that all rats had normal movement and could perform the behavioral tests. Accordingly, previous studies have found that Hsd and VPA did not have negative effect on locomotor activity using distance moved, speed and number of crossing analysis[6,8,16].

Both the NOL and NOR tests were used to evaluate memory impairments, which are dependent on hippocampus. The NOL test is a test for spatial memory that involved in hippocampus-dependent memory related to spatial locations, configurations or routes[22]. The NOR test is a test for recognition memory that is associated with the hippocampus and perirhinal cortex. The perirhinal cortex is an area that receives the information including visual, olfactory and somatosensory stimuli before entering into the hippocampus[23]. The potential of spatial and recognition memory is associated with neurogenesis in the SGZ of the hippocampal DG[24–26]. The hippocampus plays a role in learning and memory[27]. The results in this study demonstrates that VPA induced memory impairments, which are consistent with previous studies[4,8,28]. VPA treatment can induce memory impairments, which are associated with decreasing in hippocampal neurogenesis including cell proliferation, survival and differentiation[4,6,8,29]. In the NOL test, the DIs show that rats treated with VPA could

not discriminate between two identical objects in the familiar and novel locations, indicating that VPA impaired spatial memory. In the NOR test, similarly, rats in the VPA group showed an inability to discriminate between the familiar object and novel object, indicating an impairment in recognition memory. However, impairments of both spatial and recognition memory were improved by Hsd co-administration. This was confirmed by the DIs of NOL and NOR tests. Likewise, the effect of Hsd (100 mg/kg) improved learning and memory impairment in a mouse model of Alzheimer's disease[30]. Hsd promotes cell survival by increasing the number of new neurons derived from hippocampal neurogenesis[16]. The ability of learning and memory is linked to the number of generated neurons in the SGZ of the DG in the hippocampus[31]. Therefore, further study of neuroprotective effects of Hsd should focus on the relationship of hippocampal neurogenesis and cognition.

This study confirmed that VPA treatment could induce both spatial and recognition memory impairments however Hsd could improve these memory impairments. Hsd, a flavanone glycoside, is one of the most important antioxidants, and also functions as a neuroprotective agent. A previous study has reported that Hsd is a mediated of improvement of neural growth factors and endogenous antioxidants defense, alleviating neuro-inflammatory and apoptotic pathways[15]. Similarly, a recent report has shown that effects of Hsd reduces the negative effects of methotrexate (MTX) on neurogenesis, oxidative stress, and antioxidant enzymes[24]. Moreover, Tamilselvam et al.[32] reported that Hsd protects against rotenone-induced oxidative stress and apoptosis in a cellular model for Parkinson's disease. These studies support our findings that Hsd can improve memory impairments in rats induced by VPA.

Conclusion

This study reveals that Hsd significantly improved memory impairments induced by VPA in adult rats. This finding postulates the ability of Hsd to improve memory impairments caused by the deterioration of the brain functions.

Acknowledgement

The author would like to thank invitation research from Faculty of Medicine (IN63242), Khon Kaen University for financial support.

References

1. Cahill S. WHO's global action plan on the public health response to dementia: some challenges and opportunities. Aging Ment Health 2020; 24: 197–199.
2. ELBeltagy M, Mustafa S, Umka J, Lyons L, Salman A, Gloria Tu C-Y, et al. Fluoxetine improves the memory deficits caused by the chemotherapy agent 5-fluorouracil. Behav Brain Res 2010; 208: 112–117.
3. ELBeltagy M, Mustafa S, Umka J, Lyons L, Salman A, Dormon K, et al. The effect of 5-fluorouracil on the long term survival and proliferation of cells in the rat hippocampus. Brain Res Bull 2012; 88: 514–518.
4. Umka J, Mustafa S, ElBeltagy M, Thorpe A, Latif L, Bennett G, et al. Valproic acid reduces spatial working memory and cell proliferation in the hippocampus. Neuroscience 2010; 166: 15–22.
5. Lyons L, ELBeltagy M, Bennett G, Wigmore P. The effects of cyclophosphamide on hippocampal cell proliferation and spatial working memory in rat. Homayouni R, editor. PLoS ONE 2011; 6: e21445.
6. Umka Welbat J, Sirichoat A, Chaijaroonkhanarak W, Prachaney P, Pannangrong W, Pakdeechote P, et al. Asiatic acid prevents the deleterious effects of valproic acid on cognition and hippocampal cell proliferation and survival. Nutrients 2016; 8: 303.
7. Welbat JU, Chaisawang P, Chaijaroonkhanarak W, Prachaney P, Pannangrong W, Sripanidkulchai B, et al. Kaempferia parviflora extract ameliorates the cognitive impairments and the reduction in cell proliferation induced by valproic acid treatment in rats. Ann Anat - Anat Anz 2016; 206: 7–13.
8. Aranarochana A, Chaisawang P, Sirichoat A, Pannangrong W, Wigmore P, Welbat JU. Protective effects of melatonin against valproic acid-induced memory impairments and reductions in adult rat hippocampal neurogenesis. Neuroscience 2019; 406: 580–593.
9. Lledo P-M, Alonso M, Grubb MS. Adult neurogenesis and functional plasticity in neuronal circuits. Nat Rev Neurosci 2006; 7: 179–193.
10. Ming G, Song H. Adult neurogenesis in the mammalian brain: significant answers and significant questions. Neuron 2011; 70: 687–702.
11. Garg A, Garg S, Zaneveld LJD, Singla AK. Chemistry and pharmacology of the citrus bioflavonoid hesperidin. Phytother Res 2001; 15: 655–669.
12. Crozier A, Jaganath IB, Clifford MN. Dietary phenolics: chemistry, bioavailability and effects on health. Nat Prod Rep 2009; 26: 1001.

13. Cho J. Antioxidant and neuroprotective effects of hesperidin and its aglycone hesperetin. Arch Pharm Res 2006; 29: 699–706.

14. Thenmozhi AJ, Raja TRW, Janakiraman U, Manivasagam T. Neuroprotective Effect of hesperidin on aluminum chloride induced Alzheimer's disease in Wistar rats. Neurochem Res 2015; 40: 767–776.

15. Hajialyani M, Hosein Farzaei M, Echeverría J, Nabavi S, Uriarte E, Sobarzo-Sánchez E. Hesperidin as a neuroprotective agent: A review of animal and clinical evidence. Molecules 2019; 24: 648.

16. Naewla S, Sirichoat A, Pannangrong W, Chaisawang P, Wigmore P, Welbat JU. Hesperidin alleviates methotrexate-induced memory deficits via hippocampal neurogenesis in adult rats. Nutrients 2019; 11: 936.

17. Nissinen J, Pitkänen A. Effect of antiepileptic drugs on spontaneous seizures in epileptic rats. Epilepsy Res 2007; 73: 181–191.

18. Dix SL, Aggleton JP. Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. Behav Brain Res 1999; 99: 191–200.

19. de Oliveira D, Dourado GKZS, Cesar T. Hesperidin associated with continuous and interval swimming improved biochemical and oxidative biomarkers in rats. J Int Soc Sports Nutr 2013; 10: 27.

20. Tomazini Gonçalves T, Lazaro C, De Mateo F, Campos M, Mezencio J, Claudino M, et al. Effects of glucosyl-hesperidin and physical training on body weight, plasma lipids, oxidative status and vascular reactivity of rats fed with high-fat diet. Diabetes Metab Syndr Obes Targets Ther 2018; 11: 321–332.

21. Kotajima-Murakami H, Kobayashi T, Kashii H, Sato A, Hagino Y, Tanaka M, et al. Effects of rapamycin on social interaction deficits and gene expression in mice exposed to valproic acid in utero. Mol Brain 2019;12: 3.

22. Kessels RPC, de Haan EHF, Kappelle LJ, Postma A. Varieties of human spatial memory: a meta-analysis on the effects of hippocampal lesions. Brain Res Rev 2001; 35: 295–303.

23. Pourmemar E, Majdi A, Haramshahi M, Talebi M, Karimi P, Sadigh-Eteghad S. Intranasal Cerebrolysin attenuates learning and memory impairments in D-galactose-induced senescence in mice. Exp Gerontol 2017; 87: 16–22.

24. Welbat JU, Naewla S, Pannangrong W, Sirichoat A, Aranarochana A, Wigmore P. Neuroprotective effects of hesperidin against methotrexate-induced changes in neurogenesis and oxidative stress in the adult rat. Biochem Pharmacol 2020; 178: 114083.

25. 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: 1100.

26. Sritawan N, Prajit R, Chaisawang P, Sirichoat A, Pannangrong W, Wigmore P, et al. Metformin alleviates memory and hippocampal neurogenesis decline induced by methotrexate chemotherapy in a rat model. Biomed Pharmacother 2020; 131: 110651.

27. Patestas MA, Gartner LP. A textbook of neuroanatomy. Malden, MA: Blackwell Pub; 2006: 454.

28. Takuma K, Hara Y, Kataoka S, Kawanai T, Maeda Y, Watanabe R, et al. Chronic treatment with valproic acid or sodium butyrate attenuates novel object recognition deficits and hippocampal dendritic spine loss in a mouse model of autism. Pharmacol Biochem Behav 2014; 126: 43–49.

29. Welbat JU, Sangrich P, Sirichoat A, Chaisawang P, Chaijaroonkhanarak W, Prachaney P, et al. Fluoxetine prevents the memory deficits and reduction in hippocampal cell proliferation caused by valproic acid. J Chem Neuroanat 2016; 78: 112–118.

30. Wang D, Liu L, Zhu X, Wu W, Wang Y. Hesperidin alleviates cognitive impairment, mitochondrial dysfunction and oxidative stress in a mouse model of Alzheimer's disease. Cell Mol Neurobiol 2014; 34: 1209–1221.

31. Kitabatake Y, Sailor KA, Ming G, Song H. Adult neurogenesis and hippocampal memory function: new cells, more plasticity, new memories? Neurosurg Clin N Am 2007; 18: 105–113.

32. Tamilselvam K, Braidy N, Manivasagam T, Essa MM, Prasad NR, Karthikeyan S, et al. Neuroprotective effects of hesperidin, a plant flavanone, on rotenone-induced oxidative stress and apoptosis in a cellular model for Parkinson's disease. Oxid Med Cell Longev 2013; 2013: 1–11.

SMJ