นิพนธ์ต้นฉบับ ▪ Original Article

ผลของไครซินต่อภาวะความจำบกพร่องในหนูชราภาพที่ถูกเหนี่ยวนำโดยดีกาแลคโทส

ราม ประจิตต์[1], นัตยา ศรีตะวัน[1], กรรวี สุวรรณโคตร[1], สาลินี แนวหล้า[1], อนุสรา อารณะโรจน์[1], อภิวัฒน์ ศิริโชติ[1], วนัสนันท์ แป้นนางรอง[1], จริยา อำคา เวลบาท[1, 2*]

[1]*ภาควิชากายวิภาคศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น*

[2]*กลุ่มวิจัยและพัฒนาด้านประสาทวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น*

The Effect of Chrysin on Memory Impairments in Aging Rats Induced by D-galactose

Ram Prajit[1], Nataya Sritawan[1], Kornrawee Suwannakot[1], Salinee Naewla[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*

หลักการและวัตถุประสงค์: ภาวะสมองเสื่อมเป็นหนึ่งในปัญหาของความชราภาพที่ส่งผลกระทบต่อเซลล์ประสาทต้นกำเนิดในชั้น subgranular zone (SGZ) ของ dentate gyrus (DG) ในสมองส่วน hippocampus และแสดงออกมาในรูปของความจำบกพร่อง ดีกาแลคโทส (D-galactose; D-gal) เป็นสารที่สามารถเหนี่ยวนำให้เซลล์ประสาทตายจากภาวะเครียดออกซิเดชันและกระบวนการการอักเสบ จึงถูกนำมาใช้ในการศึกษาภาวะสมองเสื่อม ไครซิน (chrysin) เป็นสารสกัดจาก flavonoid ที่มีคุณสมบัติในการฟื้นฟูความจำ การศึกษานี้จึงมีวัตถุประสงค์ในการศึกษาผลของ chrysin ต่อภาวะความจำบกพร่องในหนูชราภาพที่ถูกเหนี่ยวนำโดย D-gal

วิธีการศึกษา: หนูแรทเพศผู้สายพันธุ์ Sprague Dawley ถูกแบ่งออกเป็น 6 กลุ่ม ได้แก่ control, D-gal, chrysin 10, chrysin 30, D-gal + chrysin 10 และ D-gal + chrysin 30 โดย D-gal (50 มก/กก.) ให้โดยการฉีดเข้าช่องท้อง chrysin (10 และ 30 มก/กก.) ให้โดยการป้อนเป็นเวลา 8 สัปดาห์ จากนั้นน้ำหนักและการเคลื่อนไหวของหนูถูกนำมาวิเคราะห์ และหนูถูกทดสอบความจำด้วยการทดสอบ novel object location (NOL) และ novel object recognition (NOR)

ผลการศึกษา: ผลของน้ำหนักและการเคลื่อนไหวของหนูทุกกลุ่มไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ในส่วนการทดสอบความจำ กลุ่ม control, chrysin 10, chrysin 30, D-gal + chrysin 10 และ D-gal + chrysin 30 มีความแตกต่างอย่างมีนัยสำคัญทางสถิติในการแยกตำแหน่งและวัตถุใหม่ในการทดสอบ NOL และ NOR ในขณะที่กลุ่ม D-gal ไม่สามารถแยกตำแหน่งและวัตถุใหม่ได้ในทั้งสองการทดสอบ

Background and Objective: Brain aging is one of aging problems that affects to the neural stem cells in the subgranular zone (SGZ) of the dentate gyrus (DG) in hippocampus, which exhibits cognitive impairments. D-galactose (D-gal) induces neuronal apoptosis caused by oxidative stress and inflammation. It is used in several brain aging studies. Chrysin, one of flavonoid, has many neuroprotective effects that can improve memory. This study investigated the effects of chrysin on memory impairments in aging rats induced by D-gal.

Methods: Male Sprague Dawley rats were divided into 6 groups; control, D-gal, chrysin 10, chrysin 30, D-gal + chrysin 10 and D-gal + chrysin 30 groups. D-gal (50 mg/kg) was administrated by intraperitoneal (i.p.) injection. Chrysin (10 and 30 mg/kg) was administrated by oral gavage. Both of D-gal and chrysin were administrated for 8 weeks. After treatments, the body weight and locomotor activity were determined. The memories were evaluated using novel object location (NOL) and novel object recognition (NOR) tests.

Results: The results showed that the body weight and locomotor activity did not significantly differ among all groups. In the memory tests, control, chrysin 10, chrysin 30, D-gal + chrysin 10 and D-gal + chrysin 30 groups showed significant differences to discriminate

*Corresponding author : Jariya Umka Welbat, Department of Anatomy, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand. E-mail: jariya_umka@yahoo.com

สรุป: การศึกษาในครั้งนี้พบว่า D-gal สามารถเหนี่ยวนำให้เกิดความจำบกพร่องได้ อย่างไรก็ตาม chrysin สามารถบรรเทาภาวะความจำบกพร่องที่เกิดจากการเหนี่ยวนำโดย D-gal ได้

คำสำคัญ: ดีกาแลคโทส, ไครซิน, ความจำ, ชราภาพ

the novel location and object in the NOL and NOR tests. By contrast, D-gal group showed no significant difference of discrimination in the both tests.

Conclusion: This study demonstrates that D-gal induced memory impairments. However, chrysin could attenuate the memory impairments caused by D-gal.

Keywords: D-galactose, chrysin, memory, aging

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Introduction

The subgranular zone (SGZ), a part of dentate gyrus (DG) in hippocampus, is an area of neurogenesis that neural stem cells proliferate and differentiate into mature neurons or glial cells. Neural circuits in the hippocampal area play an effective role with memory [1, 2].

Aging, a process of life that depends on time lapse, is a cause of many diseases. Biological mechanism of aging is an imbalance between cell regeneration and cell death[2, 3]. Brain aging is one of aging problems, which exhibits cognitive impairments[4]. According to previous studies, brain aging has been reported to have a defect of neurogenesis due to oxidative stress, inflammation and neuronal apoptosis [5, 6].

D-galactose (D-gal) is one of monosaccharides that is found in natural fruits and vegetables[7]. High concentration of D-gal can generate galactitols, reactive oxygen species (ROS), advanced glycation end-products (AGEs), which can induce oxidative stress or inflammation[8, 9]. D-gal is used widely in several studies that are associated with aging or cellular senescence[5, 6]. Moreover, D-gal also induces recognition and spatial memory impairments in rodents[4].

Chrysin or 5, 7-dihydroxyflavone is one of flavonoids that is found in honey and propolis[10]. Chrysin has many neuroprotective effects such as antioxidant activities, anti-inflammation and anti-depressants[11, 12]. Several evidences have shown that chrysin can improve spatial memory in aging mice as well as in cerebral hypoperfused models[13, 14].

The SGZ is the area of neurogenesis where is related with memory. Therefore, this study hypothesized that chrysin would improve spatial and recognition memory impairments in aging rats induced by D-gal.

Materials and Methods

Animals

Sixty-six adult male Sprague-Dawley rats, age 8 weeks old, body weight 280-300 grams (Nomura Siam International Co., Ltd. Pathumwan, Bangkok), were used in this study. This experimental protocol was approved by the Khon Kean University Ethics Committee in Animal Research (IACUC-KKU-22/61). The animals were controlled in the environment with 12:12 hour, light/dark cycle and stable room temperature at 23-25 °C with food and water ad libitum. The animals were divided into 6 groups (11 animals per group) and lived without any drug administration for 4 weeks.

Drug administration

Control group was administrated with 0.9% normal saline solution 1 milliliter/kilogram and propylene glycol (Ajax Finechem Pty Ltd., Australia) 1 milliliter/kilogram. D-gal group received D-gal (Sigma Aldrich, Inc., St. Louis, USA) 50 milligram/kilogram dissolved in 0.9% normal saline solution. Chrysin 10 group was given chrysin (Sigma Aldrich, Inc., St. Louis, USA) 10 milligram/kilogram dissolved in propylene glycol. Chrysin 30 group received chrysin 30 milligram/kilogram dissolved in propylene glycol. D-gal + chrysin 10 group was given D-gal and chrysin similar dose to the D-gal and chrysin 10 groups. D-gal + chrysin 30 was administered with D-gal and chrysin at an equivocal dose to the D-gal and chrysin 30 groups (Figure 1).

The 0.9% normal saline solution and D-gal were administered by intraperitoneal (i.p.) injection whereas chrysin and propylene glycol were administered by oral gavage. All of the agents were given for 8 weeks. Three days after the drug administration, memories were investigated using novel object location (NOL) and novel object recognition (NOR) tests.

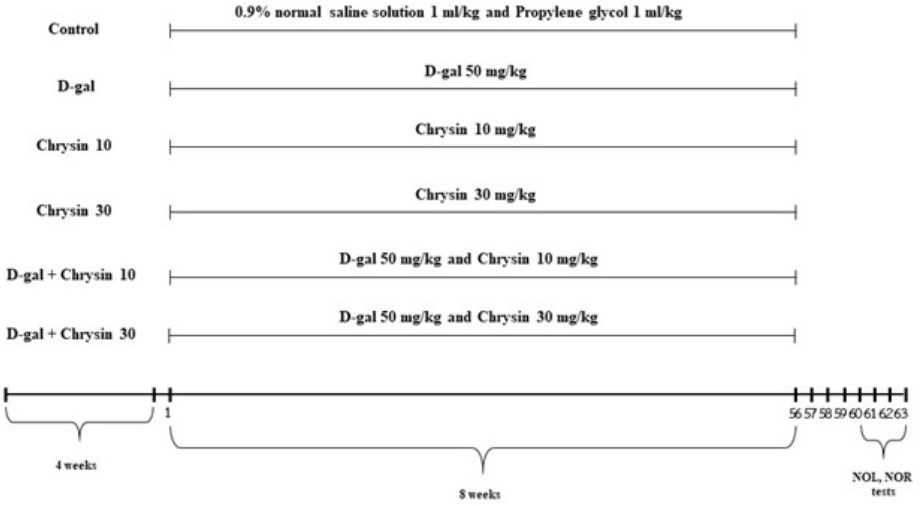

Figure 1 Timeline of drug administration and behavioral tests.

Behavioral tests

Behavioral apparatus composed of an arena (width x length x high = 50 x 50 x 50 cm.) and plastic bottles. Video tracking software of EthoVision® XT (EthoVision®, XT version[12], Noldus, Wageningen, Netherlands) was used in both of the NOL and NOR tests to record an exploration time of the animals.

Novel object location (NOL) test

The NOL test is separated into three parts, including habituation, familiarization and choice trails. In the habituation trial, the animals were habituated freely in the empty arena for 30 minutes. The next day, they were habituated in the empty arena for 3 minutes prior the familiarization trial. Then, two identical objects were placed randomly at two different corners of the arena. In the familiarization trial, they were allowed to explore the objects for 3 minutes and then returned to their cage for 15 minutes. In the choice trail, the objects were placed in the arena at the familiar and novel locations. Then, the animals were allowed to explore the objects for 3 minutes. The arena and objects were cleaned with 20% ethanol every time after finishing each trial. Exploration time was used to calculate discrimination index (DI) that is defined as the ability to discriminate between novel and familiar locations[15, 16].

Novel object recognition (NOR) test

The NOR test is divided into three parts, which is similar to the NOL test. In the habituation trial, the animals were allowed to habituate freely in the empty arena for 30 minutes. One day later, they were habituated in the empty arena for 3 minutes. Then, two identical objects were placed randomly at two different corners of the arena. In familiarization trial, they were placed in the arena to explore the objects for 3 minutes and then returned to the cage for 15 minutes. Before performing in the choice trail, one of the objects was changed to a novel object and then placed into the same location of the familiarization trail. After that, they were allowed to explore the objects for 3 minutes. The arena and objects were cleaned with 20% ethanol every time when the animal finished in each trial. DI was calculated using differences between the exploration time of the novel and familiar objects[4, 15, 16].

Statistical analysis

GraphPad Prism 5.0 software was used to analyze the data and significance was determined as $p< 0.05$. Two-way ANOVA was used to analyze body weight. One-way ANOVA was used to determine total exploration time. Discrimination index was compared using one sample t-test.

Results

Body weight

In this study, body weight of the animals in all groups were not significantly different throughout the experiment ($p>0.05$, Fig. 2). This result indicates that D-gal and chrysin did not have a negative effect on the body weight of the animals.

Behavioral tests

During the behavioral tests, total exploration times were recorded to evaluate locomotor activity of the animals. The data showed no significant differences in both of NOL and NOR tests ($p>0.05$, Fig. 3A and 3B), indicating that the animals in all groups had a similar locomotor activity and the drug administration did not decrease their movement.

The DI was calculated in the choice trail in both of the behavioral tests. In the NOL test, a spatial memory test, the control, chrysin 10, chrysin 30, D-gal + chrysin 10 and D-gal + chrysin 30 groups showed significant differences in the DI ($p<0.05$, Fig. 4A), but did not find in the D-gal group ($p>0.05$, Fig. 4A). The NOR test, a recognition memory task, the control, D-gal + chrysin 10 and D-gal + chrysin 30 groups showed significant differences in the DI ($p<0.05$, Fig. 4B). Moreover, chrysin 10 and 30 groups also showed significant difference in DI ($p<0.001$, $p<0.01$, respectively, Fig. 4B). In contrast, the D-gal group had no significant difference in DI of NOR test ($p>0.05$, Fig. 4B). These results indicate that chrysin dose 10 and 30 milligram/kilogram could improve spatial and recognition memory impairments.

Discussion

The present study demonstrates that D-gal induced brain aging due to memory impairment. On the other hand, this study has found that chrysin dose 10 and 30 milligrams/kilogram could improve memory in aged rats induced by D-gal. However, D-gal and chrysin did not affect to body weight and locomotor activity in this study.

From the results, the body weight of the animals administrated with D-gal and chrysin did not different. In some similar studies, the body weight of animals received D-gal is not altered when compared to the other groups[6, 8]. In a spinal cord injury model, interestingly, rats received chrysin can attenuate weight loss[13]. Whereas, several studies of chrysin in brain aging do not mention about changes of the body weight[14, 15].

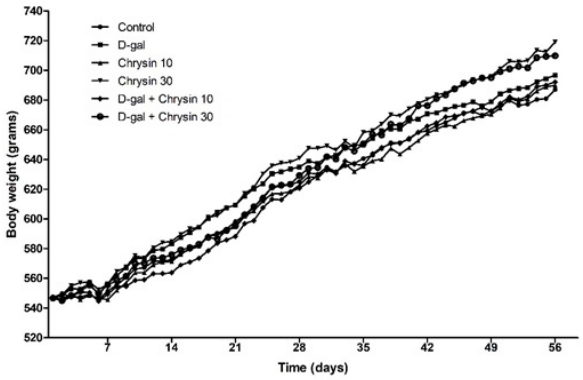

Figure 2 Body weight of animals throughout the drug administration.

In the present study, the animals received both D-gal and chrysin agents had no effect of locomotor activity, evaluated using total exploration time. According to previous studies, distance move, speed and number of crossing were used to determine the locomotor activity in aging model. The data of those studies also found that D-gal and chrysin did not affect to the locomotor activity[4, 15].

The DI is an ability to discriminate between novel and familiar locations (or objects). A positive DI value indicates that animals spend more time to explore novel locations or objects. In contrast, a negative DI value indicates that animals spend more time to explore the familiar locations or objects. If DI value is equal to zero, this reflects that animals cannot discriminate locations or objects[16, 17].

Spatial memory is a memory that involves in hippocampus-dependent spatial memory, including locations, configurations and routes[4, 18, 19]. The present study has found that the animals received D-gal spent more time exploring the object in the familiar location, indicating that the animals had spatial memory impairment. Several studies have found that D-gal induces neuronal apoptosis in the DG caused by oxidative stress and inflammation[4-6, 8]. Therefore, spatial memory deficit probably occurs from neuronal apoptosis in the DG. The animals in co-administration groups were able to discriminate the objects in different location and spent more time exploring the novel location, according to the positive DI value, suggesting that chrysin attenuated spatial memory deficit in these animals. Various evidences have supported that chrysin can improve memory by attenuating oxidative stress and inflammation and increasing sodium-potassium ATPase activity, brain-derived neurotrophic factor and nerve growth factor levels in the hippocampus[11, 12, 14, 15]. Moreover, several studies have found that improving of spatial memory is related to generating of new neurons in the DG[17, 19, 20]. For these reasons, the neuroprotective effects of chrysin may increase cell survival of neural stem cells or immature neurons and then attenuate the negative effects of D-gal.

Recognition memory is a memory, which is

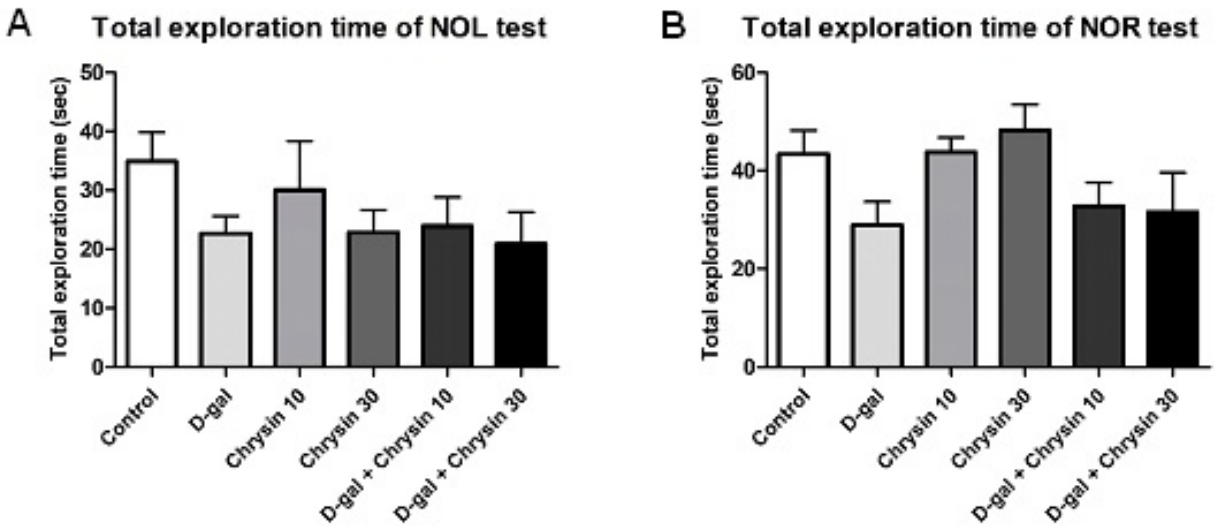

Figure 3 Total exploration time of the animal exploratory activity all objects in NOL (A) and NOR (B) tests.

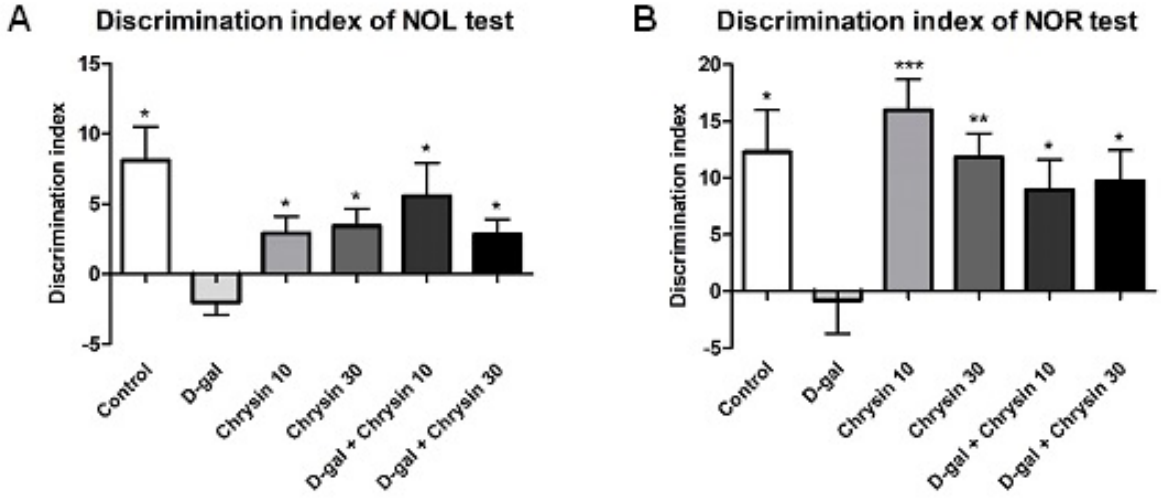

Figure 4 Discrimination index of the NOL (A) and NOR (B) tests (*p<0.05, ** p<0.01 and *** p<0.001 significant differences compared to zero).

associated with the hippocampal and cortical functional integrity [4]. Furthermore, this memory also involves in perirhinal cortex, the area that receives visual, olfactory and somatosensory stimuli before entering into the hippocampus. The perirhinal cortex damage reduces preference of novel objects in animals[21, 22]. In this study, the animals which received D-gal could not discriminate between the familiar and novel objects, indicating that they had impairment of recognition memory. Previous studies have found that D-gal decreases antioxidant enzyme activities and increases reactive astrocyte levels in the hippocampus, cortex and corpus callosum[4, 6, 23]. Therefore, recognition memory impairment likely occurred from neuronal damage caused by D-gal. By contrast, the animals in the co-administrated groups could discriminate the objects between the familiar and novel objects, referred to the positive DI value. There are many evidences that have supported improvement of chrysin on spatial memory in aged and chronic cerebral hypoperfused rats[14, 15]. Although, the effect of chrysin on recognition memory still unclear, chrysin can increase antioxidant enzyme activities and decrease pro-inflammatory cytokines in cortex[11, 14, 15]. So, chrysin may be attenuated the negative effects on recognition memory that induced by D-gal.

The SGZ, an area of the DG in hippocampus, is the location of neural stem cells that can give rise to mature neurons[1, 2]. Therefore, this area is involved in spatial and recognition memory[4, 18, 19]. The present study has found the memories impairments in aged rats induced by D-gal. Whereas, chrysin can improve the memory impairment. It is likely that D-gal proba-

bly induces oxidative stress or inflammation to damage neural stem cells or immature neurons in the SGZ. However, the negative effect of D-gal attenuated chrysin may be due to improvment of antioxidant enzyme activities or inhibited pro-inflammatory cytokines.

Conclusion

This study demonstrates that chrysin improved memory impairments in aged rat induced by D-gal. This study suggests that chrysin has a benefit effect to attenuate age-related memory impairment.

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