



# Differences in Spatial Memory Impairment in Mice after Oral D-Galactose Administration and Intraperitoneal Injection

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## Abstract

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**BACKGROUND:** The aging process can increase the incidence of dementia, such as spatial memory impairment to remember space, recognize shapes and distances. Research on dementia in mice was carried out by administering d-galactose through intraperitoneal injection, while oral d-galactose administration had not received enough attention.  
**AIM:** This study aims to prove the differences in the occurrence of spatial memory impairment in rats induced by d-galactose through two different routes, oral and intraperitoneal injection.  
**METHODS:** This study is an experimental study using a post-test control group design. The sample criteria were 20 male Wistar rats aged 12–14 weeks, weighing 200–300 g divided into two groups which are the oral and intraperitoneal injection. Spatial memory assessment based on spontaneous alternation using the Y-maze test was carried out at the end of week 8.  
**RESULTS:** In this study, the average spatial memory score after d-galactose administration in the injection group (51.572±4.388) was lower compared to the oral group (66.058±1.551). The Shapiro–Wilk normality test shows that the data are normally distributed with  $p > 0.05$ . Independent t-test showed a significant difference in the incidence of spatial memory disorders between the injection and oral groups with  $p = 0.010$  ( $p < 0.05$ ).  
**CONCLUSION:** The conclusion of this study is that d-galactose administration by oral route or intraperitoneal injection causes a decrease in spatial memory in mice. Spatial memory in the injection group was lower than in the oral group. This might be related to the decrease in synaptophysin in the hippocampus of mice due to d-galactose administration by intraperitoneal injection.

## Introduction

The aging process causes progressive deterioration in tissues and organs, accompanied by an increased incidence of neurodegenerative diseases such as dementia. Dementia is characterized by a gradual loss of cognitive performance, memory, and spatial ability [1]. The main characteristic of Alzheimer's dementia (AD) is cognitive impairment related to disorders of the hippocampus for the storage of spatial memory and emotional behavior. Spatial memory in animals is related to the ability to remember space, recognize shapes and distances that play a role in finding locations, where food is available and safety in sustaining life [2]. The Y-maze test was developed to assess working memory based on spatial learning by assessing the activity of mice in exploring new environments and looking for food quickly and efficient [3]. It is useful for assessing memory skills, which depend on the function of various areas of the brain, especially the hippocampus, septum, basal forebrain, and prefrontal cortex [4].

D-galactose induces advanced glycation end products (AGEs) and reactive oxygen species that cause mitochondrial dysfunction, oxidative stress, inflammation, and apoptosis in nerve cells [5], so d-galactose can be used as a model for aging in AD and other memory impaired. Brain aging induced by d-galactose is highly dose

dependent, starting from 100 mg/kg/day to 500 mg/kg/day with the administration for 6–8 weeks [6]. Most studies in the accessible literature state that d-galactose can be used for mice with dementia models when administered by subcutaneous or intraperitoneal injection. However, several studies have shown that oral administration of d-galactose also causes age-related changes [7].

There are many studies of dementia model mice with a focus on d-galactose administration through intraperitoneal and subcutaneous injection, whereas oral d-galactose administration has not received enough attention [8]. Most authors mention that chronic administration of d-galactose can cause learning and memory impairment in mice, while the acute effect of d-galactose administration is unclear [7]. Hence, this study is planned to evaluate the impacts of d-galactose induction in Wistar rats through two different routes that are intraperitoneal injection and oral administration of spatial memory using the Y-maze test.

## Methods and Procedures

This research is an experimental study with a post-test control group design research. Male Wistar rats

aged 12–14 weeks weighing 200–300 g were kept in the Animal Laboratory Unit of the Pharmacology Unit of the Faculty of Medicine, Udayana University. The study was conducted for 8 weeks from June to August 2019. The subjects were 20 rats divided into two groups of 10 rats each as an oral group who received the oral d-galactose and 10 rats as an injection group who received the intraperitoneal d-galactose injection. Induction of d-galactose at a dose of 100 mg/kg/day orally through a nasogastric tube once every day at 08.00 am and intraperitoneal injection induction is done every day by changing the side of the injection between the right and left. The Wistar rat was conducted according to the policy of research ethics with ethical clearance (No.1799/UN14.2.2.VII.14/LP/2019) from the ethical commission at the Faculty of Medicine, Udayana University. Spatial memory assessment based on spontaneous alternation using the Y-maze test measured for 5 min and recorded using video. Y-maze test was carried out in a closed and quiet room in both groups at the end of week 8. Spatial memory is expressed by spontaneous alternation (%) when mice can enter three different arms sequentially according to the overlapping set of triplets.

## Results and Discussion

Results of mean spatial memory scores in this study after d-galactose administration in the injection group ( $51.572 \pm 4.388$ ) were lower than those in the oral group ( $66.058 \pm 1.551$ ). The Shapiro–Wilk normality test shows that the data are normally distributed with  $p > 0.05$ . An independent t-test was used to assess the differences in spatial memory scores between the oral and injection groups after the administration of d-galactose. Independent t-test obtained a significant difference in the incidence of spatial memory impairment between the injection and oral groups with  $p = 0.010$  ( $p < 0.05$ ). The analysis results are presented in Table 1.

**Table 1: Mean spatial memory scores after d-galactose administration**

Group	Mean spatial memory scores	p
Oral	$66.058 \pm 1.551$	0.010
Injection	$51.572 \pm 4.388$	

Chronic administration of d-galactose, regardless of the route, is used as a model of cognitive impairment and aging. The development of aging is characterized by a gradual loss of cognitive performance, memory, and spatial ability [1]. In this study, it was found that d-galactose administration caused a decrease in spatial memory in both oral and injection groups. The results obtained in this study are similar to studies conducted by Cardoso *et al.* that systemic exposure to d-galactose results in biochemical changes and morphological aging in several organs, including the central nervous system. Exposure to d-galactose can

shorten lifespan and cause behavioral changes such as learning and memory disorders and other cognitive declines [9]. Giving d-galactose can be used as a model of aging that is fast, economical, and widely used for anti-aging pharmacological research. Other advantages are its easy application, low tumor incidence, and high animal survival rate during the study period [9].

The mean spatial memory score in this study after administration of d-galactose in the injection group ( $51.572 \pm 4.388$ ) was lower than the oral group ( $66.058 \pm 1.551$ ). Lin *et al.* suggested that the administration of d-galactose by intraperitoneal injection significantly increased the A $\beta$  level in rat hippocampus. The previous study also stated that the administration of d-galactose by intraperitoneal injection could raise the aggregation and deposition of A $\beta$  in the brain, and this process subsequently leads to a cellular cascade of neuronal loss and dementia [8], while other evidence suggests that oral administration of d-galactose can have a protective effect on AD animal models induced by streptozotocin. A study comparing chronic systemic administration of d-galactose with oral results shows that the route of oral administration can improve cognitive deficits in streptozotocin-induced AD models in mice, where the protective effect of d-galactose depends on the concentration or route of administration. Hence, there is still controversy surrounding the use of d-galactose through this route orally [7].

The difference in the occurrence of spatial memory impairment between the injection and oral groups in this study was assessed using an independent t-test obtained  $p = 0.010$  ( $p < 0.05$ ). The results of this study can be concluded that there are significant differences in the incidence of spatial memory impairment in rats induced by oral d-galactose and intraperitoneal injection. Spatial memory in the injection group was lower than in the oral group. One study also observed that synaptophysin (one of the main protein components in synaptic vesicles responsible for neuron transmission) is reduced in elderly individuals with cognitive impairment and dementia, suggesting that synaptic loss is a significant contributor to dementia in the elderly. Another study showed that d-galactose 120 mg/kg/day for 60 days, administered intraperitoneally, could induce a decrease of synaptophysin in rat hippocampus, in contrast, d-galactose given orally did not change synaptophysin after 4 or 6 weeks of treatment [8].

Galactose has protecting effect to cholinergic neurons during hypoglycemia conditions at low pH levels (pH 6.7–6.9) to maintain brain function under hypometabolism [10]. Prolonged d-galactose administration could interfere with the body's natural ability to convert galactose to glucose, causing an increase in galactitol and aldose reductase activation, resulting in oxidative damage to cells and forms AGE. The previous studies have shown that chronic administration of d-galactose injected through the intraperitoneal route at a dose of 100 mg/kg induces cognitive impairment after 8 or 12 weeks of administration. In our study, d-galactose

administered through the oral route also produced memory deficits in mice, suggesting that continuous administration by the oral route can cause interference with behavior. The oral route can minimize stress levels and damage due to carbohydrate administration compared to using the intraperitoneal route for an extended period [8].

## Conclusion

Based on the results of this study, it was concluded that there were differences in the incidence of spatial memory disorders in rats induced by oral d-galactose and intraperitoneal injection. Spatial memory impairment appears to be lower in d-galactose by intraperitoneal injection than oral administration. This might be related to the decrease in synaptophysin in the hippocampus of mice due to d-galactose administration by intraperitoneal injection.

Further research needs to be done to understand the effects of oral d-galactose administration in rats with a longer time to ascertain spatial memory disorders that have occurred and clarify their therapeutic potential as well as subsequent studies using antioxidants in d-galactose-induced mice to observe improvements in spatial memory.

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