Role of Ovarectomy and D-Galactose Load to Cognitive Function and Behavior of Sprague Dawley

Linda Rosalina[1], Nelly Mariati[2], Loso Judijanto[3], Siti Hamidatul Aliyah[4], Tanti Tri Lestary[5], Aulia Asman[6]

1. *linda.rosalina@fpp.unp.ac.id Universitas Negeri Padang, Indonesia*
2. *nellyrachel09@gmail.com Universitas Muhammadiyah Banjarmasin, Indonesia*
3. *losojudijantobumn@gmail.com IPOSS Jakarta, Indonesia*
4. *sitihamidatula@gmail.com Center For Biomedical Research, Research Organizational For Health, National Research And Innovation Agency (BRIN), Indonesia*
5. *tanti@borneo.ac.id Universitas Borneo Tarakan, Indonesia*
6. *aulia.asman@gmail.com Universitas Negeri Padang, Indonesia*

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ABSTRACT
The interplay between hormonal imbalance and oxidative stress plays a critical role in age-related cognitive decline. This study investigates the effects of ovariectomy (OVX) and D-galactose (D-Gal) administration on cognitive function and behavior in Sprague Dawley rats. Ovariectomy is commonly used to model post-menopausal conditions in animals, while chronic D-Gal exposure induces oxidative stress, mimicking aging.In this experiment, female Sprague Dawley rats were divided into four groups: a control group, an OVX group, a D-Gal group, and an OVX+D-Gal group. Cognitive performance was assessed using maze tests, while behavioral changes were evaluated through activity and anxiety-related behavior assays. Biochemical analyses were performed to measure oxidative stress markers in the brain tissue.The results indicated significant cognitive impairment and behavioral changes in the OVX and D-Gal groups, with the OVX+D-Gal group showing the most pronounced deficits. Oxidative stress levels were elevated in the OVX+D-Gal group, suggesting that the combination of hormonal deficiency and oxidative stress accelerates cognitive decline. These findings highlight the importance of addressing both endocrine and oxidative stress pathways in the prevention of age-related cognitive dysfunction.This study provides valuable insights into the mechanisms underlying neurodegenerative processes and suggests potential therapeutic targets for cognitive decline associated with aging and menopause.

INTRODUCTION

Progressive loss of neuronal cell structure or function, including neuronal cell death, is referred to as a neurodegenerative condition. The development of this illness will alter the physiological processes that the body uses to process sensory and motor information as well as higher functions (Pakaya& Susilowati, 2020). Elderly people frequently experience a variety of neurodegenerative conditions, ranging in severity from mild to severe, including Parkinson's disease (PP), Alzheimer's disease (PA), and Huntington's disease PH. After Alzheimer's disease, Parkinson's disease (PP) is one of the most prevalent neurological conditions. (Alia et Al., 2022). The most critical health issue is Alzheimer's disease. is a serious neurological condition that worsens over time and accounts for the majority of causes of age-related dementia (Bertram et al., 2005). It is characterized by cognitive loss and the buildup of Amyloid Beta (A) deposits and tangles in the brain. Age is the primary determinant of Alzheimer's disease risk, and diagnosis often occurs after age 65. However, Alzheimer's disease can also affect individuals younger than 65 (Wildah et al., 2020).

The geriatric population will dramatically increase in Indonesia, a growing nation. In Indonesia, the percentage of the elderly was initially rather low—it was only about 4.5 percent in 1971. But the percentage of the old population will noticeably rise between 1990 (6.6%) and 2020 (11%) (Affandi, 2009). The two syndromes that best describe neurodegenerative diseases are Parkinson's disease and Alzheimer's disease (Suharti, 2020). A neurodegenerative illness associated with aging and increased oxidative stress is

Alzheimer's disease. For purposes of both preventive and therapeutic intervention, it is crucial to identify the causes and factors that affect the development and course of this disease. In Indonesia, a developing country, there will be a significant rise in the elderly population. The proportion of elderly people in Indonesia was initially rather low; in 1971, it was only around 4.5 percent. But between 1990 (6.6%) and 2020 (11%) the proportion of the elderly population will significantly increase (Affandi, 2009). Parkinson's disease and Alzheimer's disease are the two syndromes that best represent neurodegenerative illnesses (Suharti, 2020). Alzheimer's disease is a neurodegenerative condition linked to oxidative stress and aging. Identification of the causes and variables influencing the onset and progression of this disease is essential for therapeutic and preventive action.

The specific causation of Alzheimer's disease is still a mystery. The amyloid cascade theory is regarded as the primary hypothesis in the pathogenic process of Alzheimer's despite the fact that many other hypotheses have surfaced. Beta amyloid (Aβ) accumulates excessively as a result of disruptions in the metabolism of amyloid precursor protein (APP), an integral membrane glycoprotein in the brain, which involves the β-site amyloid precursor protein cleaving enzyme (BACE-1) and is neurotoxic and causes oxidative stress (Samaey et al., 2019). It has been demonstrated that oxidative stress induces a positive feedback loop that leads to increased BACE-1 expression, greater A buildup, the development of neurofibrillary tangles (NFTs), and neuronal death.

It has been demonstrated that giving catechins, such as those found in green tea, to older rats speeds up their aging process and protects them against oxidative stress and alcohol-induced damage to serum lipids. Mice make a good choice for aging research since they have a lot of Aβ-deposition and can serve as an animal model for AD (Pepeu, 2004).

The phospholipids of poly unsaturated fatty acids (PUFA) will be attacked by free radicals, resulting in the production of numerous aldehydes with varied carbon chain lengths, including malondialdehyde (MDA), acrolen, and 4-hydroxyxinonenal. Adaptive metabolic responses do not take place in the brains of ovariectomized rats. The direct switch from fatty acid use to ᴺᴸ-amyloid production in the hippocampus of ovariectomized mice suggests accelerated brain metabolic aging (59–61) (Brinton, 2012). In the brains of Alzheimer's transgenic mice, the administration of low and high doses of antioxidant therapy (curcumin) dramatically reduced protein oxidation, and at low doses of insoluble Aβ and soluble Aβ, plaque formation decreased significantly by 43-50% (Lim et al., 2001).

Rats' inflammatory response and oxidative stress can be reduced by administering a natural anti-oxidant and anti-inflammatory therapy (Shakti et al., 2019). The load of Aβ deposits also dramatically diminished, and this result was followed by considerable drops in Aβ 1-40 and Aβ 1-42 in the neocortex and hippocampus. In senile plaques, amyloid beta builds up as insoluble extracellular deposits, creating a typical neuropathological disease in PA. In addition to their dual roles as antioxidants and anti-inflammatories, curcumin and the polyphenol group will be highly helpful in the treatment of PA because of their structural ability to interact with beta aggregation and metal dyshomeostasis (Ruan & Yao, 2020).

West Sumatra is regarded as a barometer of gambier producing centers in Indonesia, and Indonesia supplies the majority (80%) of the world's gambier (Rosalina & Sari, 2019). Fifty Cities and Pesisir Selatan districts have the greatest gambir land among the 19 regencies and cities in West Sumatra (S. Friskila, 2017). Gambir from the SigunturPesisir Selatan district is regarded as the top grade gambier and has the greatest catechin content (96.17% 0.18), thanks to pre-purification processing (Gitawati, 2012). Gambir's pure catechin content

satisfies the Indonesian Herbal Pharmacopoeia (FHI) standards for a pharmacologically active chemical for therapeutic use. Gambier's promise as a source of catechins hasn't, however, been fully realized.

A female Sprague Dawley rat served as the study's animal model. Mice offer various advantages to employing rabbits since they are more practical, simple to procure, and have an omnivorous diet. In a preliminary investigation using 5 rats for 4 weeks, ovariectomies plus D galactose 500 mg/kgbb with 95% pure catechins at a dose of 60 mg/200 gbb, the findings of the histological analysis revealed evidence of destruction of neuronal cells and glia in the hippocampus in samples that had ovariectomies plus D galactose 500 mg/kg, 2 weeks. Two weeks after catechins 60 mg/200 grbb treatment, neurons and glial cells in the hippocampus showed signs of cell recovery.

Based on the aforementioned context, researchers are curious to learn the neuroprotective effects of catechins in rats with neurodegenerative disorders by evaluating levels of beta amyloid, Bace-1, 4-HNE, and measuring the process of forming pathogenic plaques in the brain to support the question: Is there a relationship between ovarectomy and d-galactose and cognitive/behavioral rats?

METHODS

This study is an experimental one that was conducted in two stages with a post test only control group design. By executing ovarectomy procedures and intraperitoneal D-galactose administration, the status of female rat animal models related with cognitive impairment was ascertained in the initial stage of the investigation. The second stage of the research examined the possibility of administering catechin therapy from Gambir to rodent models of cognitive impairment, plasma levels of β-amyloid and 4-hydroxynonenal, levels of beta-amyloid and BACE CSF, and the presence of amyloid plaques in the rodent models' brains for Alzheimer's disease. The Sprague Dawley female white rats used in this study were obtained from the laboratory of the faculty of veterinary medicine, Bogor Agricultural University's pet development section. The population represented by the sample satisfies the inclusion and exclusion requirements.

The inclusion criteria were as follows: 1) 12 weeks of age, 2) 100-150 mg body weight, 3) No physical impairment, 4) Female white rats with neurodegenerative diseases caused by ovarectomy and injection of D-Galactose as measured by the Open Field test and Y-maze test. While the exclusion criteria were: 1) Rats that lost physical condition, 2) Rats who passed away during the trial, and 3) Sick rats who lost 20–30% or more of their starting body weight. The accuracy and precision of laboratory examination results are ensured by quality assurance in research by consistently improving quality at all stages. Pre-, analytical, and post-analytical activities are all included in the range of quality assurance items. To verify that everything is done correctly and that the legitimacy of the data obtained can be properly accounted for academically, quality assurance is employed. The Republic of Indonesia Health Laboratory Center's standard operating procedures are used in all quality assurance procedures in this investigation. Editing, coding, processing, and cleaning techniques were used for data processing and analysis. Using SPSS 21, data were computerized processed. The impact of administering gambier as a source of catechins on different markers of cognitive impairment and clinical symptoms of cognitive impairment was examined statistically through analysis. The t-test and an ANOVA will be used in

statistical analysis to compare the two means and see whether there are any differences between more than two groups.

RESULT AND DISCUSSION

A post-test only control group design was used in an experimental study to determine the impact of gambir catechins on the levels of beta amyloid, 4 hydroxynonenal, and beta amyloid expression in the brains of female Sprague Dawley rats that were 12 weeks old, weighed between 100 and 140 grams, and had up to 25 tails. The rats were obtained from the pet development unit of the Laboratory of the Faculty of Veterinary Medicine, Bogor Agricultural University.

The first step in the research process involved conducting an ovarianectomy and administering D-galactose intraperitoneally to a female rat animal model to establish the condition associated with cognitive impairment. The second phase of the study examined the possibility of administering catechin treatment from Gambir to rat model animals with cognitive and behavioral abnormalities, looking at the relationship between plasma levels of β amyloid, BACE-1, and 4-hydroxynonenal and pathological brain alterations.

The study's findings provide a description of the data and the findings of hypothesis testing in accordance with how the problem was formulated. All of the rats were weighed before the study, according to an analysis of the research data. The appendix contains the weighting findings for the rats. Using the One Sample Kolmogorof-Smirnov test, a normality test was carried out to determine the pattern of distribution of rat body weight prior to treatment in each group. All of the data groupings have an Asymp score, according to the test results. Sig. > 0.050 indicates that all data on the mice's pre-treatment body weight were normally distributed. Levene's test was used to determine whether the sample was homogeneous. As a result of the test's significance value of 0.087 ($p > 0.05$), which indicates that the sample is homogeneous because 0.087 > 0.050, the sample is significant.

One way ANOVA was used in a parametric analysis to assess whether there was a significant difference in the body weight of the rats prior to treatment. Given that the probability value derived from the analysis was 0.995 ($p > 0.05$), it was concluded that there was no substantially different body weight sample between the groups.

Summary results of one way ANOVA can be seen in Table 5.1 below.

Tabel 5.1 Summary of Results of Test Analysis of Differences in Weight of Rats between Groups before Treatment

Variable	Group	N	Mean	SD	Sig
Rat Body Weight Before Treatment	Group I	5	1.582	4.816	0.995
	Group II	5	1.558	9.121	
	Group III	5	1.580	1.212	
	Group IV	5	1.574	1.064	
	Group V	5	1.574	9.044	

Group I: Negative control, Group II: Positive control, Group III: Catechins at a dose of 20 mg/kg BW, Group IV: Catechins at a dose of 40 mg/kg BW, Group V: Catechins at a dose of 60 mg/kg BW.

Four weeks after ovarectomy and d-galactose administration, cognitive testing was done in the treatment and good control groups. Cognitive skills, memory, and anxiety levels are all evaluated. The Appendix contains information on the rats' % alternation Y-Maze test results in each group prior to catechin treatment. The following diagram shows the average% alternation value on the Y-Maze test for rats prior to catechin administration.

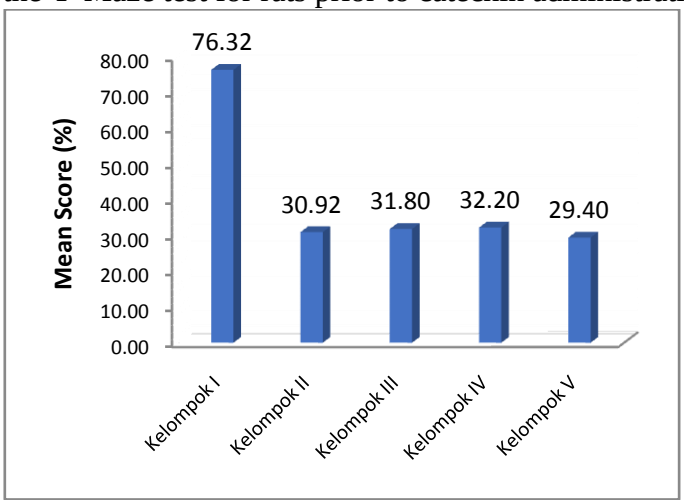

Figure 1.The alternation average % value on the Y-Maze Test Mice

Group I: Negative control, Group II: Positive control, Group III: Catechins at a dose of 20 mg/kg BW, Group IV: Catechins at a dose of 40 mg/kg BW, Group V: Catechins at a dose of 60 mg/kg BW

These results demonstrate that the rats in the K (-) group had an alternation value of 76.32% (> 50%) prior to catechin treatment. Mice in the treatment groups (I-III) and K (+) exhibited alternation values below 50%, in contrast. This suggests that group II and the therapy group of rats have a neurodegenerative disease.

The One Sample Kolmogorof-Smirnov test was used to conduct a normality test in order to determine the distribution pattern of the% alternation y-maze test conducted prior to treatment in each group. All of the data groupings have an Asymp score, according to the test results. Sig. > 0.050 denotes a normally distributed distribution for all % alternation y-maze test data prior to treatment. Levene's test was used to determine whether the sample was homogeneous. Since the score is 0.499 > 0.050, the test's significance value of 0.499 (p > 0.05) indicates that the sample is homogeneous.

One way ANOVA was used in a parametric analysis to see whether there was a significant difference between the rats' pre-treatment scores on the alternation y-maze test and those of other rats. Because the score was 0.011 0.05 and the analysis yielded a probability value of 0.011 (p > 0.05), it was concluded that there was a significant difference in the percentage of samples used in the alternation y-maze test between groups.

Summary results of one way ANOVA can be seen in Table 5.2 below.

Table. 5.2 Summary of Results of Analysis of Differences in % alternation y-maze test of mice between groups before treatment

Variable	Group	N	Mean	SD	Sig
% alternation y-maze test	Group I	5	7.632	1.234	0.011
	Group II	5	3.092	1.330	
	Group III	5	3.180	1.482	
	Group IV	5	3.220	8.467	
	Group V	5	2.940	1.101	

Group I: Negative control, Group II: Positive control, Group III: Catechins at a dose of 20 mg/kg BW, Group IV: Catechins at a dose of 40 mg/kg BW, Group V: Catechins at a dose of 60 mg/kg BW.

According to the data that has been evaluated, there was a difference in the rats' % alternation y-maze test results prior to catechin administration. This indicates that, prior to catechin treatment, there was a statistically significant difference in the results of the % alternation y-maze test between the five groups of mice. Duncan's Test was used in a Post Hoc test to compare the scores of the various groups. The Duncan test results for the % alternation y-maze test are listed below.

Table 5.3. Results of Duncan's Post Hoc Analysis

Sample Group	Mean Score	Interpretation
Group V	2.94	Not Significantly Different
Group III	3.18	
Group IV	3.22	
Group II	3.09	
Group I	7.63	Significantly different

Group I: Negative control, Group II: Positive control, Group III: Catechins at a dose of 20 mg/kg BW, Group IV: Catechins at a dose of 40 mg/kg BW, Group V: Catechins at a dose of 60 mg/kg BW.

According to the results of the Post Hoc test, rats in the negative control group significantly differed from the other four groups in terms of the results of the % alternation Y-maze test. The three treatments and the positive control group did not, however, vary from one another significantly ($p > 0.05$).

Conclusion

The ovarectomy and intraperitoneal D-galactose administration for a period of six weeks served as the first stage of the research's construction of the condition of the female rat animal model related with cognitive impairment. Five groups of rats were created at random: a negative control group, a positive control group, and three treatment groups. In this investigation, pre-pubertal female rats were employed, which are thought to be the most capable of displaying the signs of hormonal deprivation since they are highly sensitive to ovarian hormone depletion. Cognitive testing was done in the treatment and positive control groups 6 weeks following induction via ovarectomy and d-galactose administration. Cognitive skills, memory, and anxiety levels are all evaluated.

The Y-maze test was one of the criteria used in the initial stage of this investigation to demonstrate the presence of neurodegenerative diseases in mice (Faradila et al., 2022). The simplest labyrinth exam is called Y labyrinth, and it is shaped like the letter Y. According to Gok et al. (2015), the metrics measured are the frequency of repeated errors entering the arm and the frequency of errors entering the empty arm. The Stanford Behavioral and Functional Neuroscience Laboratory defines normal as a% alternation Y-maze test value > 50%. Rats in the negative control group (K-), which did not have any ovariectomies, had an alternation value of 76.32% (more than 50% is regarded as typical). Rats that received ovariectomy and those in the positive control group and treatments III, IV, and V, on the other hand, had an alternation value of 50%, indicating the presence of cognitive impairment.

Spatial working memory errors are those that enter the repeating arm, whereas spatial reference memory errors are those that enter the empty arm (Tao et al., 2020). Being able to remember and identify shapes, distances, and areas in plane spaces as well as one's own direction or location are all aspects of spatial memory. Without spatial memory, people will struggle to comprehend their own position, see forms and planes, recall the location or direction of an object, and gauge the distance to a location (Kelly et al., 2003). The hippocampus is primarily responsible for working memory (Pepeu, 2004).

In order to identify the phenotype of a brain condition manifesting as anxiety, which is a sign of panic disorder, specific and social phobias, obsessive-compulsive disorder, depression, and post-traumatic stress disorder, another parameter is the Open field test (Ruan & Yao, 2020). The Open field test, which evaluates the average number and duration of quadrant I, is the initial part of this research.

The rat's mobility during the chamber Open Field test is shown by the center number. There is a tendency to raise the middle number in groups II, II, IV, and V. Mice who spent more time exploring the middle region exhibited anxious traits. Anxiety is a symptom of an unidentified threat (Samaey et al., 2019). The frequency of this characteristic represents the level of concern as it rises. According to Rosalina et al. (2017), increased freezing and grooming frequency is a sign of growing anxiety. A low mean number is expected for healthy mice. Middle hour, which also encapsulates worry. Additionally, the quadrant I time was evaluated. A low level of panic indicated by a high quadrant I time showed amygdala injury. A low quadrant I time is indicative of healthy or normal mice. There is amygdala injury because all therapies exhibit an upward trend (blue hue) indicating a decrease in the amount of panic.

The best way to create Alzheimer's model mice that can imitate changes in neurochemistry, histopathology, and cognitive impairment is to induce Alzheimer's disease through ovarian removal and administer 500 mg/kg of D-galactose intraperitoneally for six

weeks. It has been demonstrated that estrogen deficiency causes anatomical, metabolic, and functional abnormalities that eventually lead to a decline in memory, behavior, and cognitive abilities (Suharti, 2020).

High doses of D-galactose administration will cause metabolic abnormalities that lead to increased ROS generation. ROS will cause modifications to APP metabolism, which will result in further A buildup. Because of the loss of cholinergic neurons in the forebrain and the degradation of connections in the hippocampus and cerebral cortex, mice will suffer significant memory and cognitive impairment (Hua et al., 2007).

A negative control group (one without any neurodegenerative disorders) and four groups with neurodegenerative disorders were obtained after stage I was finished. The rats in the negative control group (group I) received simply a conventional food (30-50 gram pellet + 200-250 mL distilled water), whereas the rats in the positive control group (group II) received a standard diet. In addition, the five groups underwent therapy for around four weeks. with a standard diet dancatechin dose 1 (20 mg/200 gram BW), a placebo (NaCl 0.9% 1 mL), treatment group IV with a standard diet dancatechin dose 2 (40 mg/200 gram BW), and treatment group V with a standard diet dancatechin dose 3 (60 mg/200 gram BW) in order to assess the potential effects of catechin therapy on the cognitive function of rat animal models.

Rats typically spend more time exploring the outer regions, typically those that are in close proximity to the wall (thigmotaxis), than the interior regions (center). Rats who spend more time in the middle exhibit anxious behaviors.

The rat's mobility during the chamber Open Field test is shown by the center number. Due to amygdala injury, a high middle number denotes a low level of anxiety. A low mean number is expected for healthy mice. Middle of the night, which also sums the anxiety level.

A high quadrant I time indicates a low level of panic which indicates damage to the amygdala. Normal or healthy mice will have a low quadrant I time. All treatments showed low duration (except K- which also had the highest Q1 time) and did not differ significantly. In groups III, IV, V there is a decreasing time trend in the middle number. The time trend decreased (although not significantly) in Group V indicating an improvement in stress or panic levels. Anxiety is a sign of a threat that is still unknown (Pan et al., 2007) (Hobfoll, 1989). The frequency of this characteristic represents the level of concern as it rises. The more often a person freezes or grooms, the more anxious they are feeling (Nuraeni&Ratnaya, 2023). A low mean number is expected for healthy mice. Additionally, the quadrant I time was evaluated. A low level of panic indicated by a high quadrant I time showed amygdala injury. A low quadrant I time is indicative of healthy or normal mice. The fact that all therapies exhibit a declining trend (green color) indicating an increase in terror shows that cognitive function has improved.

According to Rosalina et al. (2017), catechin treatment has the effect of enhancing cognitive function and reducing brain vatropy in experimental mice. Additionally, it has been demonstrated that catechins lower levels of 8-oxodeoxyguanosine (8-oxodG), a marker of DNA oxidative damage in the kidney and liver (Gok et al., 2015). According to Rosalina et al. (2017), catechins are thought to be more effective than vitamins E and C at scavenging free radicals.

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