The Effect of Combination Ovariectomy and D-galactose Administration on Alzheimer's Animal Model

Faradila F. 1*, Yuliarni S. 2, Rika S. 3, Nur I. Liputo 2

1Department of Biomedical Science, Faculty of Medicine Andalas University, Padang, Indonesia
2Department of Neurology, Faculty of Medicine Andalas University, Padang, Indonesia
3Department of Forensic Medicine and Medicolegal, Faculty of Medicine Andalas University, Padang, Indonesia

*Corresponding author: faradila1991@hotmail.com
E-mails address: syafrita.yuliarni@gmail.com, rikasusanti1976@gmail.com, indra.liputo@gmail.com

Received 19/8/2020, Accepted 11/7/2021, Published Online First 20/3/2022, Published 1/10/2022

Abstract:
Background and purpose: Animal model helps researchers to evaluate new treatment plan for human and understand pathological mechanism involved in a development of disease. The use of rats as an animal model for Alzheimer's research has become a favorite among researchers. Rats are capable in mimicking Alzheimer disease due to their intelligence and quick adaptation to nature. At present there are several methods that can be used to induce Alzheimer's animals, but each method has advantages and disadvantages. We need to learn other methods that can provide many advantages and few disadvantages. The Amyloid-beta 42 (Aβ-42) and Reactive Oxygen Species (ROS) are thought to play an important role in the pathology of Alzheimer’s disease. This study aims to investigate whether ovariectomy and D-galactose can be an effective method for inducing Alzheimer's animal models. This was an experimental study with control group design. Twelve female Sprague Dawley rats were involved and grouped into two groups. Control group who did not undergo ovariectomy and the experiment group underwent ovariectomy and was given intraperitoneal D-galactose 500 mg/kg.bw. Amyloid beta-42 plasma and Y-Maze test were conducted after 6 weeks. The results showed that the experiment group has lower mean Y-Maze score (42.79 ± 6.97) compared with control group (74.27 ± 4.01) and the Amyloid-beta 42 plasma was higher in the control group. In conclusion ovariectomy and D-galactose are proven to induce cognitive decline and higher plasma Amyloid-beta 42 in the Alzheimer’s animal model.

Keywords: Alzheimer’s, D-galactose, Ovariectomy, Sprague Dawley, Y-Maze

Introduction:
Higher life expectancy worldwide leads to higher elderly population. High population of elderly brings various health issues such as neurodegenerative disease, Alzheimer’s 1. There is no medical therapy available to cure Alzheimer. The current therapy works on slowing down Alzheimer’s progressivity and its risk factors. The pathology of Alzheimer’s is not yet fully understood so thus more study needs to be done to understand this disease more thoroughly 2. Animal model helps researchers to evaluate new treatment plan for human and understand pathological mechanism involved in a development of disease. Due to limited knowledge regarding the cause of Alzheimer, every available model shows limitation so thus its application must be considered carefully. At present, no animal model is able to mimic Alzheimer's disease in a natural way, therefore the majority of studies are carried out using phenotype simulations in animal models, or using transgenic animals as the most advanced techniques 3.

Although transgenic animals show many advantages in research, they still show some weaknesses. Besides there may be a high mortality rate and might show deleterious effects, transgenic animals are also very expensive and will further burden research funding. In some studies that do not provide transgenic animals, rats are the animal of choice in Alzheimer study. Due to their intelligence and quick adaptation, rat are capable of mimicking Alzheimer disease in human. Rats have memory and cognitive abilities that can be measured using

| DOI: [http://dx.doi.org/10.21123/bsj.2022.5486](http://dx.doi.org/10.21123/bsj.2022.5486) |  |  |

---

*Corresponding author: faradila1991@hotmail.com*

E-mails address: syafrita.yuliarni@gmail.com, rikasusanti1976@gmail.com, indra.liputo@gmail.com

Received 19/8/2020, Accepted 11/7/2021, Published Online First 20/3/2022, Published 1/10/2022

---

**Abstract:**

Background and purpose: Animal model helps researchers to evaluate new treatment plan for human and understand pathological mechanism involved in a development of disease. The use of rats as an animal model for Alzheimer's research has become a favorite among researchers. Rats are capable in mimicking Alzheimer disease due to their intelligence and quick adaptation to nature. At present there are several methods that can be used to induce Alzheimer's animals, but each method has advantages and disadvantages. We need to learn other methods that can provide many advantages and few disadvantages. The Amyloid-beta 42 (Aβ-42) and Reactive Oxygen Species (ROS) are thought to play an important role in the pathology of Alzheimer’s disease. This study aims to investigate whether ovariectomy and D-galactose can be an effective method for inducing Alzheimer's animal models. This was an experimental study with control group design. Twelve female Sprague Dawley rats were involved and grouped into two groups. Control group who did not undergo ovariectomy and the experiment group underwent ovariectomy and was given intraperitoneal D-galactose 500 mg/kg.bw. Amyloid beta-42 plasma and Y-Maze test were conducted after 6 weeks. The results showed that the experiment group has lower mean Y-Maze score (42.79 ± 6.97) compared with control group (74.27 ± 4.01) and the Amyloid-beta 42 plasma was higher in the control group. In conclusion ovariectomy and D-galactose are proven to induce cognitive decline and higher plasma Amyloid-beta 42 in the Alzheimer’s animal model.

**Keywords:** Alzheimer’s, D-galactose, Ovariectomy, Sprague Dawley, Y-Maze

**Introduction:**

Higher life expectancy worldwide leads to higher elderly population. High population of elderly brings various health issues such as neurodegenerative disease, Alzheimer’s 1. There is no medical therapy available to cure Alzheimer. The current therapy works on slowing down Alzheimer’s progressivity and its risk factors. The pathology of Alzheimer’s is not yet fully understood so thus more study needs to be done to understand this disease more thoroughly 2. Animal model helps researchers to evaluate new treatment plan for human and understand pathological mechanism involved in a development of disease. Due to limited knowledge regarding the cause of Alzheimer, every available model shows limitation so thus its application must be considered carefully. At present, no animal model is able to mimic Alzheimer's disease in a natural way, therefore the majority of studies are carried out using phenotype simulations in animal models, or using transgenic animals as the most advanced techniques 3.

Although transgenic animals show many advantages in research, they still show some weaknesses. Besides there may be a high mortality rate and might show deleterious effects, transgenic animals are also very expensive and will further burden research funding. In some studies that do not provide transgenic animals, rats are the animal of choice in Alzheimer study. Due to their intelligence and quick adaptation, rat are capable of mimicking Alzheimer disease in human. Rats have memory and cognitive abilities that can be measured using...
behavioral tests which are very important features in choosing an Alzheimer's animal model. Previous study showed rat capable in future decision making based on their current knowledge. This ability is called metacognitive which previously considered one of primate unique feature.

At present there are several methods that can be used to induce Alzheimer's animals, but each method has advantages and disadvantages, so we need to learn other methods that can provide many advantages and few disadvantages. Increased Amyloid-beta 42 (Aβ42) production and Reactive Oxygen Species (ROS) are widely believed to be the main causes in Alzheimer's pathology. D-galactose is a reducing sugar that is easy to react with amino acids in peptides, to form Advanced Glycation End Products (AGEs). Chronic administration of D-galactose at low doses has shown to induce changes that mimic natural aging processes in animals, includes cognitive decline, oxidative stress, and metabolic disorders. Premature aging in animals which induced by chronic exposure to D-galactose is commonly used as a model for studying the neurodegenerative disease.

This study aims to investigate whether ovariectomy and D-galactose can be an effective method for inducing Alzheimer's animal models. The combination of ovariectomy and D-galactose as a ROS stimulator is thought to support each other to induce Alzheimer's by disrupting the amyloid-beta cascade pathway and damaging the mitochondria by increasing ROS. This method is based on principle that estrogen is endogen Aβ regulator. Ovariectomy diminishes the negative feedback to Luteal Hormone (LH), this process leads to increased LH level that supports Aβ production through amyloidogenic pathway.

In normal condition, estrogen in the brain plays role in maintaining synapses plasticity and hippocampal neuronal growth that are involved in periodical memory formation. Estrogen also preserves the nerve from beta amyloid toxicity, lowers brain inflammation, and prevents hyperphosphorylation of tau protein. Estrogen regulation on Aβ has not been fully understood, but the mechanism might involve indirect action of estradiol aromatization pathway, and gonadotropin and gonad hypothalamus-hypophyses pathway. In cell culture experiment, estradiol directly reduce the Aβ oligomer level and indirectly increases Aβ clearance.

There are two estrogen receptors in the brain, alpha and beta. Each of these receptors has a different role. Alpha receptor acts as a stimulator meanwhile beta receptor acts as an inhibitor. A study shows that estrogen level in the plasma is decreased significantly after ovariectomy and correlated with risk factor of age-related Alzheimer disease. Decreased estrogen level after ovariectomy causes lower neural cell viability and leads to cell death (apoptosis). Furthermore, ovariectomy also decreases alpha receptor in hippocampus that leads to excessive tau protein expression. Based on a previous animal study, estrogen has a protective effect in the rats neuronal hippocampal that underwent Aβ toxicity and this protective effect is related with increased mitochondria respiration function. This phenomenon shows estrogen-induced neuronal induction mechanism in degenerative process is estrogen activation function of cellular mechanism and the end result is improvement of mitochondria viability. Therefore mitochondria is an ideal targeted therapy in neurodegenerative disease.

Materials and Methods:

Chemicals and reagent kits
D-galactose were purchased from PUDAK Scientific and Rats Aβ42 ELISA Kit was purchased from Elabscience.

Animal grouping and treatment
The study design was approved by the Ethics Committee of Faculty of Medicine Andals University, Indonesia. This was an experimental study with 12 female Sprague Dawley rats (12 weeks old, 150-200 gram) and kept in SysLab Laboratory, Bogor. The rats were fed with standard pelleted and distilled water ad libitum. The room temperature was maintained at 24±25°C with constant dark-light cycle. They were divided into two groups consisted of 6 rats. The control group, which did not undergo ovariectomy and the experimental group which undergo ovariectomy and given intraperitoneal D-galactose 500 mg/kg.bwt.

Ovactomized and D-galactose Group
Intra-muscular anesthesia was performed on rats using ketamine (50 mg / KgBW) with a 1 cc syringe. The anesthesia will take about 45 minutes. Shave the lateral sides of the rats then disinfect with 70% alcohol and iodine solution. Make a 2 cm incision following the spine at a distance of 1.5 cm. Find the ovaries and tie them with the catgut chromic. Then, sew the muscles back with catgut chromic followed by the skin with silk chromic using a simple interrupted technique. Observed wound recovery every day. 7 days post-ovariectomy, start administration of D-galactose 500mg/kg.bwt intraperitoneally daily for 6 weeks.

Behavioural experiments and blood collection
After 6 weeks cognitive function was assessed using Y-Maze and blood sample was
collected for Aβ-42 test. Y-Maze test is defined as spontaneous switch percentage if the score is greater than 50%, which indicated normal cognitive function. Spontaneous switch percentage score was tested with T test to investigate mean difference between groups. Y-Maze Spontaneous alternation test is a behavior test to assess rats instinct in exploring new environment. Rats tend to explore the new labyrinth arm compared with going back to the previous arm. Various brain structures including hippocampus, septum, base of forebrain, and prefrontal cortex are involved in this process. Up to this day, Y-Maze is often used as a method to investigate learning ability and behavior evaluation among animal model so this is a good method in reflecting animal memory ability.

Y-Maze consists of three arms, rats tend to enter the different arm as their natural instinct for searching food. Counting the mistakes in entering the arm may reflect spatial memory and learning ability in animal model. In other word, Y-maze test is practical to investigate memory function and capable to assess cognitive impairment in rats. The method of inducing cognitive impairment by ovariectomy has a principle that estrogen is an endogenous regulator of Aβ. This study involved female rats that underwent ovariectomy when they reached 3 months old because at this age rats are prone to hormonal change. The previous study, using 3 months old rats showed that serum estrogen level was higher compared with 6 months old female rats. High dose D-galactose administration aims to trigger metabolic disturbance that leads to excessive ROS production. ROS and mitochondria function are two contributing variables. High levels of ROS in the mitochondria cause cell damage and thus contribute to the pathological process in the brains of rats undergoing ovariectomy which can be assessed from Aβ-42 levels.

At the end, the rats were sacrificed for blood collections. Blood is collected by cardiac puncture to investigate plasm Aβ-42.

Statistical analysis

Data obtained were presented as mean ± SEM. The statistical significance was determined using T-Test Comparisons producing a p value < 0.05 were considered significant. The analysis was performed using SPSS software (version 16).

Results:

Subjects including 12 female rats aged 12 weeks with body weights ranging from 150-200 grams. The weighing of rats aimed to ensure that the rats body weight meets the inclusion criteria. The analysis of Y-maze was conducted at the Syslab Bogor and ELISA quantitative measurement for plasma Aβ-42 was performed at the Biomedical Laboratory of Andalas University and all research was conducted between October 2018 – June 2019. To find out the differences in rat weight, parametric analysis was performed using the T-test. The analysis showed that the p value was 0.27 (p > 0.05), which means that there was no significant difference in sample weight between groups and all the rats were included into the inclusion criteria.

The percentage of spontaneous alternation was expressed as mean ± standard deviation. It was calculated by counting the number of arms sequentially visited by the rats. The results showed that the experimental group has a lower percentage of spontaneous alternation compared to the control group Fig.1. The control group had an average percentage of spontaneous alternation 74.27±6.97 which represents a normal cognitive function while the experimental group showed the percentage of spontaneous alternation 42.79±6.97 which represents impaired cognitive function, with p value 0.01. According to the Stanford Behavioral and Functional Neuroscience Laboratory, the percentage of spontaneous alternation of Y-Maze Test values of more than 50% is considered normal.

Discussion:

Cognitive function assessment based on the principle that rats have a natural instinct to explore new objects. Y-Maze is used to study the ability of rodents in environmental recognition and memory functions so that this method is effective in reflecting the ability of animals to function their memory. Y-Maze consists of three arms, rats will
Spatial memory and learning - neuronal function consists in reduction of Aβ through mitochondria can influence mitochondrial function stimulating energy requirements. Decreased brain cell energy requirements because it is sensitive and double chain so that hydrogen ions are moved. This process is called lipid peroxidation. Brain is the easiest organ to experience oxidative processes because it has a high level of ROS in mitochondria (ROS) and mitochondrial function stimulate abilities and memory function and an increase of secretage and cytoskeletal changes, including the appearance of epitopes that trigger NFT. This causes an increase in ROS production which induces interference with oxidative phosphorylation which results in a decrease of ATP level needed for normal energy homeostasis. As a result, more and more neurons will die. Clinically, this will be seen in a decline in cognitive function. This study showed a significant difference between the control group and the experimental group. This can occur due to increased Aβ and mitochondrial disorders caused by increased ROS.

Conclusions and Recommendations:
Animal induction in Alzheimer's models with ovariectomy and D-galactose has been shown to cause cognitive impairment in experimental model. Further research is needed to understand the mechanism in influencing cognitive function. However, for studies with limited time and funding, induction of Alzheimer's model rats with ovariectomy and D-Galactosa is worth considering.

Acknowledgment:
We would like to thank the Indonesian Ministry of Research Technology and Higher Education, for sponsoring full research through the PMDSU Grant (059/SP2HL/DRPM/IV/017).

Authors' declaration:
- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for republication attached with the manuscript.
- The author has signed an animal welfare statement.
- Authors sign on ethical consideration’s approval.
- The study was approved by the Ethical Review Committee of the Medical Faculty, Andalas University (No.530/KEP/FK/2018).

Authors' contributions statement:
F. F., Y. S., R. S., and N. I. L. contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

Reference:
1. Zhang T, Han Y, Wang J, Hou D, Deng H, Deng YL, Song Z. Comparative Epidemiological Investigation of Alzheimer's Disease and Colorectal Cancer: The Possible Role of Gastrointestinal Conditions in the...
Pathogenesis of AD. Front. Aging Neurosci. 2018; 10:176.

2. Mayeux R, Stern Y. Epidemiology of Alzheimer's disease. Cold spring habf perspect med. 2012; 2(8):269–73.

3. Benedikz E, Ewa K, Winblad B. The Rat as an animal model for Alzheimer Disease. J Cell mol med. 2009; 13(6):1032–42.

4. Götz J, Bodea L, Goedert M. Rodent models for Alzheimer disease. Nat Rev Neurosci. 2018; 19:583–98.

5. Yuki S, Okanoya K. Rats Show Adaptive Choice in a Metacognitive Task With High Uncertainty. Journal of Experimental Psychology: Animal Learning and Cognition. 2017; 43(1):109–18.

6. Gao J, He H, Jiang W, Chang X, Zhu L, Luo F, et al. Salidroside ameliorates cognitive impairment in a D-galactose-induced rat model of Alzheimer’s disease. Behav Brain Res. 2015; 93:27–33.

7. Ali AA, Ahmed HI, Abu-Elfotuh K. Modeling stages mimic Alzheimer’s disease induced by different doses of aluminum in rats: focus on progression of the disease in response to time. J Alzheimers Disease. 2018; 51(3):618–26.

8. Chiroma SM, Mohd MMA, Mat Taib CN, Baharuldin MTH, Amon Z. d-galactose and aluminium chloride induced rat model with cognitive impairments. Biomed Pharmacother. 2018;103:1602–8.

9. Brinton RD. Estrogen regulation of glucose metabolism and mitochondrial function: therapeutic implications for prevention of Alzheimer’s disease. Adv Drug Deliv Rev. 2008; 60:1504–11.

10. Henderson VW. Cognitive Changes after menopause: Influence of estrogen. Clin Obstet Gynecol. 2008; 51(3):618–26.

11. Henderson VW, St John JA, Hodos LN, McCleary CA, Shoupe D, Stanczyk FZ, et al. Cognitive effects of estradiol after menopause: A randomized trial of the timing hypothesis. Neurology. 2016; 87(7):699–708.

12. Bowen RL, Atwood CS. Living and Dying for Sex. Gerontol. 2004; 50(5):265–90.

13. Lei Y, Renyuan Z. Effects of Androgens on the Amyloid-β Protein in Alzheimer's Disease. Endocrinology. 2018; 159(12):3885–94.

14. Gray NE, Zweig JA, Kawamoto C, Quinn JF, Copenhagen PF. STX, a novel memran estrogen Receptor Ligand, Protects Against Amyloid-β Toxicity. J Alzheimer Dis. 2016; 51(2):391-403.

15. Agca C, Klakotsaia D, Stopa EG, Schachtmann T, Agca Y. Ovariectomy Influences Cognition and Markers of Alzheimer’s Disease. J Alzheimer Dis. 2020:529–41.

16. Miedel CF. Assessment of Spontaneous Alternation, Novel Object Recognition and Limb Clasping in Transgenik Mouse Models of Amyloid-β and Tau Neuropathology. J Vis Exp. 2017; 12:1-12.

17. Yu-bin J, Li Z, Xin G, Wei C, Hong-jian Z. Study on cognitive impairment in diabetic rats by different behavioral experiment. 2017. IOP Conf. Ser : Earth Environ. Sci.

18. Chen CF, Lang SY, Zuo PP, Yang N, Wang XQ, Xia C. Effect of D-Galaktosa on the expression of hippocampal peripheral-type benzodiazepine receptor and spatial memory performance in rats. Psychoneuroendocrinol. 2006; 7:805-1.

19. Aydin F, Kalaz EB, Kucukgergin C, Coban J, Dogru-Abbasoglu S, Uysal M. Carnosine Treatment Diminished Oxidative Stress and Glycation Products in Serum and Tissues of D-Galactose-Treated Rats. Curr Aging Sci. 2018;11(1):10–15.

20. Casadeus G, Webber KM, Atwood CS, Pappolla MA, Perry G, Bowen RL, et al. Lutenizing hormones modulates cognition and amyloid-β deposition in Alzheimer APP transgenic mice. Biochim Biophys Acta. 2006; 4:447-52.

21. Peng N, Clark JT, Prasain J, Kim H, White CR, Wyss JM. Antihypertensive and cognitive effects of grape polyphenols in estrogen-depleted, female spontaneously hypertensive rats. Am J Physiol Regul Integr Physiol. 2005; 289(3):771-5.

22. Khajuria DK, Razdan R, Mahapatra DR. Description of a new method of ovariectomy in female rats. Rev Bras Reumatol. 2012; 52(3):462-70.

23. Parameshwaran K, Michael HL, Kosta S, Carl AP. D-galactose Effectiveness in Modeling Aging and Therapeutic Antioxidant Treatment in Mice. Rejuven Research.2010; 13:1-9.

24. Koffe RM, Hyman BT, Spires-Jones TL. Alzheimer's disease ; synapse gone cold. Mol Neurodegener. 2011; 6:63-71.

25. Peña-Bautista C, Baquero M, Vento M, Cháfer-Pericás C. Free radicals in Alzheimer's disease: Lipid peroxidation biomarkers. Clinica Chimica Acta. 2019; 491:85-90.
تأثير الجمع بين استئصال المبيض وتناول D-جالاكتوز على النموذج الحيواني لمرض الزهايمر

فردالا. ف. بولاري . س. ريکا. س. نور.ا. لبوت1

1 قسم العلوم الطبية الحيوية ، كلية الطب ، جامعة الأندلس ، بادانج ، إندونيسيا
2 قسم المح والاغصاب ، كلية الطب ، جامعة الأندلس ، بادانج ، إندونيسيا
3 قسم الطب الشرعي ، كلية الطب ، جامعة الأندلس ، بادانج ، إندونيسيا

الخلاصة:

استحداث الأمراض وعلاجها مختبرياً (باستخدام الحيوانات المختبرية) يعطي معلومات قيمة حول ميكانيكية حدوث الأمراض وتطورها. ومن أهم هذه الأمراض مرض الزهايمر حيث توجد طرق عديدة لإحداث المرض في الجرذان وكل طريقة محسنة وعيوب تحدي الأكبر هو الوصول إلى طريقة لإحداث المرض ذات محسنة عالية وعيوب قليلة. يُعتقد أن أميلويد بيتا 42 (Aβ-42) وأنواع المركبات الغير مستقرة التي تحتوي على الأكسجين في تركيبها ROS تلعب دور مهم في امراضية الزهايمر. تهدف هذه الدراسة إلى إحداث مرض الزهايمر لدى إناث الجرذان من خلال استئصال البيض وحقن أميلويد بيتا 42 (Aβ-42) وهل تعتبر هذه الطريقة هي الطريقة الأفضل. تعتبر هذه الدراسة دراسة مختبرية مع تصميم المجموعة الضابطة. تم تضمين 12 من إناث الجرذان وتقسيمهم إلى مجموعتين المجموعة الضابطة التي لم تخضع إلى استئصال المبايض والمجموعة الثانية التي خضعت إلى عملية استئصال المبايض وحقن D-galactose 500 ملغم لكل كجم. تم إجراء فحصي Amyloid beta 42 검사 (Y-Maze) وY-Maze検査 بعد ستة أشهر من استئصال المبايض. أظهرت هذه الدراسة أن المجموعة الثانية لديها مستوى دخول في المazes أを作る (74 ± 6.97) أقل من المجموعة الضابطة (79 ± 4.01). ونذكر أنه تم استخدام D-galactose 42 في البلازما في النموذج الحيوي للمرض الزهايمر.

الكلمات المفتاحية: مرض الزهايمر، D-جالاكتوز، استئصال المبيض، جرذ المختبر نوع (Sprague Dawley)