The Effect Of Butterfly Pea Flower Tea On Brain Weight In Wistar Rats

After D-Galactose Induction

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**ABSTRACT**

Introduction: Butterfly pea flowers (Clitoria ternatea L.) currently popular in society as high-antioxidant supplement. One of the organs affected by aging is the brain. D-galactose is a compound that is widely used for induction of aging in animal studies. The aim of this study was to assess the effect of butterfly pea flower tea on changes in brain weight in mice induced by D-galactose.

Method: 24 rats were divided into four groups consisting of control, groups with 1%, 5%, and 10% butterfly pea flower tea intervention after being induced by aging for 6 weeks. The brain organs were then weighed to assess changes in each group.

Result: There was an increase in body weight but no significant differences between each group either before or after the intervention. No significant differences were found in the average brain weight of each group, with the brain weight of the P3 group being lower than that of the other intervention groups. There is a positive correlation between body weight and brain weight.

Conclusion: Although the butterfly pea flower has no statistically significant effect, at concentrations of 1% and 10% it can have an effect on brain weight after induction of aging by D-galactose.

**Keywords:** aging, antioxidant, brain, butterfly pea, natural product.

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**INTRODUCTION**

Recently, butterfly pea flowers (Clitoria ternatea L.) have become increasingly popular in Indonesia as flowers that provide many health benefits (Marpaung, 2020). Serving in the form of drinks or other food is increasingly easy to find in restaurants. Fresh or dried butterfly pea flowers are increasingly use for commercial (Marpaung, 2020). Many people also plant butterfly pea flowers in their yards for their family's needs or just as ornamental plants (Marpaung, 2020). Apart from that, butterfly pea flowers can be a natural dye for textiles, but its implementation is still challenging because production costs are higher than synthetic dyes (Deepak et al., 2022). The use of butterfly pea flowers has increased along with the many studies regarding the benefits of consuming butterfly pea flowers that have been researched since the 1950s (Oguis et al., 2019).

Butterfly pea flowers are perennial vines belonging to the Fabaceae or legume family. Butterfly pea is a special herb in traditional medicine (Oguis et al., 2019). All parts - from roots to flowers - are known to have healing effects and strengthen organ performance (Oguis et al., 2019). Based from the phytochemical review, butterfly pea flowers have several active ingredients that have pharmacological potential due to the presence of various antioxidants such as tannins, saponins, , flavanol, and one of the most commons is anthocyanins (Jeyaraj, Lim and Choo, 2021). The pharmacological potential of butterfly pea flowers is as an antioxidant, antibacterial, anti-inflammatory, analgesic, antiparasitic and antiviral, anti-diabetic, anti-cancer, antihistamine, and immunomodulator (Vidana Gamage, Lim and Choo, 2021). It has also the potential to play a role in improving the function of the central nervous system (CNS) (Weerasinghe et al., 2022). Other parts of this plant, namely the leaves, and roots, also have their potential in traditional medicine (Oguis et al., 2019).

The aging process can cause a decline in body function both anatomical and physiological (Abrams and Thompson, 2014). Problems that arise in the elderly can be caused by physiological changes that occur in the body related to movement ability and function (Abrams and Thompson, 2014). One of the organs affected is the brain, which functions as a 10% decrease in brain weight (Grimm and Eckert, 2017). In addition, aging causes a decrease in sensory perception and motor responses in the CNS (Kuehn et al., 2018). The reduced brain weight in the elderly is related to the reduced protein and fat content in the brain so the brain becomes lighter.(Cole et al., 2019). Dendrites, which function for communication between cells, become thinner and lose contact between cells (Grimm and Eckert, 2017). Nerve conductivity decreases and axons in the spinal cord decrease so that impulses become slower (Grimm and Eckert, 2017). These changes result in decreased cognitive, coordination, balance, muscle strength, reflexes, changes in posture, and reaction time (Kuehn et al., 2018). Currently, several studies on the effect of aging on the nervous system use the compound D-galactose to induce physiological aging.

D-galactose is a natural aldohexose that is included in the reducing sugar group (Shwe et al., 2018). Excessive administration of galactose can react with the amine group (NH2) and also through metabolism by the enzymes dldose reductase and galactose oxidase which will produce metabolites that contribute to the formation of reactive oxygen species (ROS) compounds (Shwe et al., 2018). In experimental animals of mice and rats, chronic administration of D-galactose has a decreasing effect on cognitive function, heart function, and mobility which is similar to physiological aging conditions (Li et al., 2016). Thus, induction with D-galactose is widely used as a model in research related to the aging process (Shwe et al., 2018). Some natural products are known to have high antioxidant content and also have anti-aging effects, but there are lack of studies that explore practical products and their benefits on certain organs. Based on the above, this research will examine the effect of steeping butterfly pea flower tea (Clitoria ternatea L) on brain weight in aging rats model after D-galactose induction.

**METHOD**

This research is an experimental study with a posttest group design only. The subjects of this research were 2-month-old male Wistar rats. A total of 24 mice were divided into four groups consisting of:

1. Group 1 (P1) is the control group (aquades).

2. Group 2 (P2) is the treatment group with 1% steeping of butterfly pea flower tea.

3. Group 3 (P3) is the treatment group with 5% steeping of butterfly pea flower tea.

4. Group 4 (P4) is the treatment group with 10% steeping of butterfly pea flower tea.

The D-galactose induction process was carried out at a dose of 150 mg/KgBW orally for 6 weeks in each group. After being induced with D-Galactose, the mice were then given 1, 5, and 10% of butterfly pea flower tea for 2 weeks, respectively. To harvest organs, the rat's brain was anesthetized using ketamine, then the tissue was reperfused using NaCl for 15 minutes until the fluid that came out was clear. Once finished, take the brain tissue and then weigh it using a digital scale.

Brain weight data is then presented in the form of mean ± standard deviation. The average results were then analyzed between the averages of each group using the One-Way ANOVA test to assess the differences between each group. Statistical analysis was carried out with SPSS 23 software.

**RESULT AND DISCUSSION**

The average change in body weight between all groups before intervention (mean 158.37 ± 17.27 grams) increased to 219.83 ± 25.13 grams. There was a significant difference between body weight before and after intervention (P < 0.001), but no significant difference was found in body weight between groups either before or after intervention. The P4 group had greater body weight at the end of the intervention than the control and other treatment groups.

**Table 1.** Body and brain weight results in rats.

|  |  |  |
| --- | --- | --- |
| Group | Body weight (gram) | Brain weight (gram) |
| Pre-intervention | Post-intervention |
| P1 | 162.83 ± 17.87 | 221.83 ± 28.42 | 1.84 ± 0.12 |
| P2 | 151.50 ± 12.37  | 216.66 ± 32.90 | 1.85 ± 0.12 |
| P3 | 161 ± 18.37  | 217.83 ± 26.47 | 1.77 ± 0.06 |
| P4 | 158.16 ± 21.75  | 223 ± 16.74  | 1.88 ± 0.06 |

After termination, the brain organ was taken to measure the weight of the organ. The average mouse brain weight was 1.83 ± 0.1 grams. The brain weight of mice in the P3 group was lower than the control group or the treatment group. There were no statistically significant differences in either brain weight between groups or the proportion between body weight and brain weight (Table 1), but there is a positive correlation was found between body weight and brain weight (Pearson = 0.599, p = 0.002).

In this study, we found that butterfly pea flower extract did not significantly affect body weight and brain organs. However, at concentrations of 1% and 10%, the average brain weight was greater than in the model group with D-galactose. Changes in brain weight are directly proportional to the increase in mouse body weight as physical growth occurs. The brain is an organ that is susceptible to the influence of changes in various conditions, both physiological (aging) and pathological (the influence of diet induction, changes in organ components, etc.) (Grimm and Eckert, 2017; Kesler et al., 2021; Adnan et al., 2022). These changes can affect body functions such as motor skills, cognition, and psychology as a result of changes in brain structure and the affected nervous system (Grimm and Eckert, 2017). In addition, structural changes are also frequently found in pathological conditions such as Alzheimer's and Parkinson's diseases and worsen the patient's prognosis (De Pablo-Fernández et al., 2019).

The aging process is mediated by the formation of advanced glycation end products (AGEs) which are formed from the accumulation of D-galactose with free amino acids that accumulate as the aging process occurs (Bo-Htay et al., 2018). In several studies, D-galactose induction is known to induce brain mitochondrial dysfunction through increased hydrogen peroxide (H2O2) which produces a decrease in superoxide dismutase, a natural antioxidant in cells (Shwe et al., 2018). This process then increases the lipid peroxidation process in the cell membrane and damages redox homeostasis as a process of eliminating oxidative damage (Shwe et al., 2018). As a result, further accumulation will damage neurons. Apart from the peroxidation process, the accumulation of D-galactose will increase osmotic stress due to the metabolic process of D-galactose into galactitol which leads to increased accumulation of ROS (Shwe et al., 2018). The mitochondrial dysfunction process induced by the accumulation of D-galactose will trigger apoptosis due to the release of cytochrome complex (cyt-c) and increase the proapoptotic gene Bax (Shwe et al., 2018).

In this study, brain weight did not differ significantly between groups. However, in the group with 5% D-galactose induction there was a decrease in brain weight compared to other intervention groups. A decrease in brain weight was also found in study by Nam et al that D-galactose induced a decrease in hippocampal weight in mice, although brain weight was not significantly different compared to controls (Nam et al., 2019). Apart from that, the study also found a reduction in the number of neuroblasts and immature neuron cells in the brains of mice after D-galactose induction (Nam et al., 2019). The decrease in the number of neuron cells due to D-galactose induction will then have an impact on the restoration of neuron function through the plasticity of brain neurogenesis (Nam et al., 2019).

Our results showed heavier brain weight in the group given the butterfly pea flowers tea with a concentration of 1% and 10% although there was no statistically significant difference. This difference can be influenced by the dose concentration that crosses the blood-brain barrier and has an impact on improving oxidative stress and changes in brain structure (Pinto et al., 2020). Supplementation with butterfly pea flower tea affects increasing levels of superoxide dismutase (SOD), a natural antioxidant component in the body to reduce oxidative stress and neuroinflammatory responses which damage nerve cells (Terracina et al., 2022). In addition, antioxidant components in butterfly pea flowers, namely anthocyanins, also have anti-apoptotic effects which are mediated by the aging process (Vauzour et al., 2021). However, further studies are needed regarding the mechanism of the effective ingredients in butterfly pea flower tea, especially regarding the concentration that is effective in the aging process.

The findings from our study showed a significant increase in body weight compared to before the intervention, however, the study by Chen et al (2017) showed the opposite results after being induced by D-galactose for eight weeks (Chen, Chen and Zhou, 2018). This change is also mediated by ROS-induced pro-inflammatory cytokine activity which is one of the results of the D-galactose peroxidation reaction (Chen, Chen and Zhou, 2018). D-galactose-induced weight loss in the aging process can occur through mitochondrial dysfunction in skeletal muscle which increases oxidative stress and abnormal contractility by muscles (Sumbalová et al., 2022). Our study did not analyze body components that contribute to increased body weight such as muscle or fat, so further studies are needed to analyze the outcomes of body components after induction with D-galactose.

Although it is known that D-galactose is a model of induction of aging in animals, it is known to have therapeutic effects when given in acute doses. Study findings by Chogtu et al (2017) show that administration of D-galactose for 2 weeks either orally or subcutaneously has the effect of improving memory function and learning, but this beneficial effect is reduced in chronic administration (Chen, Chen and Zhou, 2018). These results may be influenced by brain cell resistance from D-galactose exposure and induced oxidative stress reactions, which may be influenced by differences in dose and stress threshold of individual animals (Chogtu et al., 2018; Shwe et al., 2020). Further studies regarding potential D-galactose resistance are needed to assess effective dose thresholds for stimulation of aging and therapeutic effects in the future.

In this study, we did not identify antioxidant components in butterfly pea flowers, but several other studies found that one of the most common antioxidant components found in butterfly pea flowers is anthocyanins (Vidana Gamage, Lim and Choo, 2021). The anthocyanin content of butterfly pea flowers can remain up to 80% at low temperatures but can decrease at high temperatures (Vidana Gamage, Lim and Choo, 2021). The antioxidant components of butterfly pea flower extraction have also been studied regarding the effect of increasing natural antioxidant levels in the body and reducing oxidative stress (Thilavech et al., 2021). In further conditions, antioxidants from butterfly pea flowers can reverse the effects of organ damage due to oxidative stress by suppressing the expression of angiotensin-converting enzyme activity, plasma angiotensin II, and cardiac angiotensin II type 1 receptor, nuclear factor-kappa B, and tumor necrosis factor-alpha expression which plays an important role in damage to blood vessels and the heart (Maneesai et al., 2021).

**CONCLUSION**

Brewing butterfly pea flower tea with a concentration of 1% and 10% has the effect of increasing the brain weight of mice induced by D-Galactose. Butterfly flower tea with a concentration of 10% can also increase the body weight of mice induced by the aging process, but the components that influence body weight are not yet known. The high antioxidant content in butterfly pea flowers can improve changes in brain structure due to the aging process induced by D-galactose. However, our study did not analyze the antioxidant content specifically at each tea concentration. Also, we also do not analyze structural changes histologically, so a specific analysis of organ structure is needed to assess the optimal effect of antioxidants on the brain. Further research is needed with higher concentration doses of butterfly pea flowers and their antioxidant components for pharmacological mechanisms explanation in practical products to provide health benefits in the future.

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