ANTIDIABETIC EFFECT TEST OF *CENTELLA ASIATICA* EXTRACT IN HYPERCHOLESTEROLEMIA RAT MODELS

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ABSTRACT

Introduction: High blood sugar levels can lead to hypercholesterolemia, a condition where cholesterol levels in the blood exceed normal values. The active compounds in *Centella asiatica* (Pegagan) have the potential to act as antidiabetic agents by stimulating insulin production by pancreatic beta cells.

Objective: This study aims to determine the effect of *Centella asiatica* extract on lowering fasting blood glucose (FBG) levels in hypercholesterolemia rat models.

Methods: Thirty-two test animals were divided into 4 groups: normal control, negative control, positive control (Simvastatin 1.8 mg/kgBW/day), and treatment (*Centella asiatica* extract 500 mg/kgBW/day). Hypercholesterolemia was induced using 2% cholesterol and 0.2% cholic acid. FBG measurement was performed after 5 weeks of administration of *Centella asiatica* extract and Simvastatin. Data analysis used one-way ANOVA and post hoc Bonferroni tests.

Results: The mean FBG (mg/dL) in P1=70.85±1.89; P2=151.82±2.91; P3=92.34±1.86; and P4=83.89±2.17. The one-way ANOVA test results showed statistically significant differences ($p<0.01$). The post hoc Bonferroni test results showed significant differences between all experimental groups.

Conclusion: Administration of *Centella asiatica* extract at a dose of 500 mg/kgBW can significantly lower FBG levels in hypercholesterolemia rat models compared to the administration of Simvastatin 1.8 mg/kgBW/day.

Keywords: *Centella asiatica*, Simvastatin, Antidiabetic, Fasting blood glucose, Hypercholesterolemia.

ABSTRAK

Pendahuluan: Tingginya kadar gula darah dapat menyebabkan hiperkolesterolemia, suatu kondisi di mana kadar kolesterol dalam darah melebihi nilai normal. Senyawa aktif dalam *Centella asiatica* (Pegagan) berpotensi bertindak sebagai agen antidiabetes dengan merangsang produksi insulin oleh sel beta pankreas.

Tujuan: Studi ini bertujuan untuk menentukan pengaruh ekstrak *Centella asiatic*a terhadap penurunan kadar glukosa darah puasa (GDP) pada model tikus hiperkolesterolemia.

Metode: Tiga puluh dua hewan uji dibagi menjadi 4 kelompok: kontrol normal, kontrol negatif, kontrol positif (Simvastatin 1.8 mg/kgBB/hari), dan perlakuan (ekstrak *Centella asiatica* 500 mg/kgBB/hari). Hiperkolesterolemia diinduksi menggunakan kolesterol 2% dan asam kholat 0.2%. Pengukuran GDP dilakukan setelah 5 minggu pemberian ekstrak *Centella asiatica* dan *Simvastatin*. Analisis data menggunakan ANOVA satu arah dan uji *post hoc Bonferroni*.

Hasil: Rata-rata GDP (mg/dL) pada P1=70,85±1,89; P2=151,82±2,91; P3=92,34±1,86; dan P4=83,89±2,17. Hasil uji ANOVA satu arah menunjukkan perbedaan yang signifikan secara statistik ($p<0,01$). Hasil uji *post hoc Bonferroni* menunjukkan perbedaan yang signifikan antara semua kelompok eksperimental.

Kesimpulan: Pemberian ekstrak *Centella asiatica* dengan dosis 500 mg/kgBB dapat menurunkan kadar GDP secara signifikan pada model tikus hiperkolesterolemia dibandingkan dengan pemberian *Simvastatin* 1.8 mg/kgBB/hari.

Kata Kunci: *Centella asiatica, Simvastatin,* Antidiabetes, Glukosa Darah Puasa, Hiperkolesterolemia.

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INTRODUCTION

The advancement of the times and technology has led to significant changes in human lifestyles, including in Indonesia. Lifestyle factors such as dietary habits, smoking, and low physical activity have become the main causes of chronic diseases like diabetes mellitus. Diabetes mellitus is a chronic metabolic disease characterized by hyperglycaemia or elevated blood glucose levels, which can cause serious damage to the heart, blood vessels, eyes, kidneys, and nerves (CDC, 2024). A total of 463 million people worldwide suffered from diabetes mellitus, which increased to 537 million people in 2021, causing 6.7 million deaths. The International Diabetes Federation (IDF) predicts that the incidence of diabetes mellitus will rise to 643 million people worldwide by 2030 and 783 million people by 2024 (IDF, 2019; IDF, 2021). Indonesia ranks seventh in the world for the highest incidence of diabetes mellitus, with 10.7 million sufferers in 2019, and it rose to fifth place with 19.5 million sufferers. This number is expected to increase to 28.6 million sufferers by 2045 (IDF, 2019; IDF, 2021). Diabetes mellitus is closely related to pancreatic function, especially with the cells located in the islets of Langerhans, one of which is the beta (β) cell that produces insulin (El-Gohary & Gittes, 2018; Sneddon et al., 2018). Various factors such as genetics, infections, and free radicals can significantly contribute to beta cell damage, reducing insulin production, and ultimately leading to hyperglycaemia, the main characteristic of diabetes mellitus (Pathak et al., 2019).

Hypercholesterolemia is a condition where the concentration of cholesterol in the blood increases above normal limits (>200mg/dL). Through mechanisms of insulin resistance, pancreatic beta-cell dysfunction, chronic inflammation, and lipid metabolism disorders, hypercholesterolemia can contribute to hyperglycaemia, eventually leading to diabetes mellitus (Samuel & Shulman, 2016).

Many people use synthetic drugs to treat diabetes mellitus and hypercholesterolemia. However, the long-term use of these drugs can cause harmful side effects. Therefore, it is necessary to search for alternatives from natural ingredients that are expected to have a lower risk of side effects compared to synthetic drugs (Putri et al., 2017).

One traditional plant found in Indonesia that can be used as medicine is *Centella asiatica* (Pegagan). *Centella asiatica* extract has many uses, including as an antioxidant and antidiabetic agent. Pegagan can significantly suppress blood sugar levels compared to Glibenclamide in rats induced with alloxan (Chauhan et al., 2010). Ethanol extract of *Centella asiatica* leaves at a dose of 500 mg/kgBW/day for 5 weeks has been proven to reduce body weight and triglyceride levels in hypercholesterolemia rat models (Nafiisah et al., 2023; Gaos, 2023). Therefore, researchers are interested in conducting further studies to determine the antidiabetic effects of *Centella asiatica* extract and compare it with the first-line drug for hypercholesterolemia, Simvastatin.

METHODS

This research was conducted from July to September 2023. *Centella asiatica* plants were obtained from the Banyumas area, then identified and extracted at the Faculty of Biology, Universitas Jenderal Soedirman. The acclimatization process, induction of hypercholesterolemia, and administration of the extract to the test animals were conducted at the Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta.

This study used a true experimental design with a post-test only control group design on rats (Rattus norvegicus). The inclusion criteria were male Sprague Dawley rats, aged 2-3 months, weighing 150-200 grams, healthy, and active. The exclusion criteria were sick and deceased rats during the study. There were 4 groups in this study with 8 rats in each group. The groups were as follows: normal control (K1), which consisted of healthy rats; negative control (K2), which consisted of hypercholesterolemia model rats; positive control (K3), which consisted of hypercholesterolemia model rats given simvastatin at a dose of 0.18 mg/kgBW/day; and the treatment group (K4), which consisted of hypercholesterolemia model rats given *Centella asiatica* extract at a dose of 500 mg/kgBW/day.

Before the study began, 32 rats were acclimatized for 7 days with standard maintenance and an oral standard diet. The rats were kept in cages of the same size for each rat. All protocols related to the test animals were approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Jenderal Soedirman, with approval number 096/KEPK/PE/VII/2023.

Hypercholesterolemia induction was carried out in groups 2, 3, and 4 after the acclimatization process. Induction was performed for 14 days using 2% cholesterol and 0.2% cholic acid administered once a day. Induction was considered successful if the total cholesterol level in the blood exceeded 130 mg/dL (Alaydrus et al., 2020). *Centella asiatica* extract was prepared by maceration using 96% alcohol. Simvastatin and *Centella asiatica* extract were administered after successful hypercholesterolemia induction. Simvastatin at a dose of 0.18

mg/kgBW/day was given for 5 weeks in group 3, and *Centella asiatica* extract at a dose of 500 mg/kgBW/day was given in group 4. Hypercholesterolemia induction and administration of Simvastatin and *Centella asiatica* extract were performed via gastric gavage. After 5 weeks of treatment with *Centella asiatica* extract and simvastatin, the rats were fasted for 12 hours, and blood samples were taken to measure fasting blood glucose using the GOD-PAP method (Glucose Oxidase–Peroxidase Amino-antipyrine).

The data obtained were tested for normality using the Shapiro-Wilk test and for homogeneity using Levene's test. Subsequently, a one-way ANOVA test was performed, followed by a post hoc Bonferroni test.

RESULTS

Table 1. Total Cholesterol Levels of Test Animals (mg/dL) After Induction

Group	Mean ± SD
K1	75,00±2,46
K2	201,92±4,68
K3	205,47±4,00
K4	202,65±2,67

Table 2. Results of One-Way ANOVA Test for Fasting Blood Glucose (FBG) Levels (mg/dL) in Each Group

Group	Mean ± SD	*p value*
K1	70,85±1,89	<0,01
K2	151,82±2,91	
K3	92,34±1,86	
K4	83,89±2,17	

After inducing hypercholesterolemia by administering 2% cholesterol and 0.2% cholic acid for 2 weeks in groups 2, 3, and 4, the results showed that group 1 had cholesterol levels within the normal range (<130 mg/dL), which could be used as a healthy control. Rats in groups 2, 3, and 4 had total cholesterol levels above the normal range, making them suitable as hypercholesterolemia model rats.

The results of the univariate and bivariate analysis of fasting blood glucose (FBG) can be seen in Table 4.1. Group 2 had the highest average FBG level above the normal range (80-100 mg/dL), at 151.82±2.91 mg/dL, followed by group 3 with 92.34±1.86 mg/dL, group 4 with 83.89±2.17 mg/dL, and group 1 with 70.85±1.89 mg/dL. In the normality and homogeneity tests, the data in this study were normally distributed and homogeneous, allowing for further analysis with One-Way ANOVA. The One-Way ANOVA analysis showed a p-value of <0.01 (<0.05), indicating a significant difference in FBG levels between the

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groups (Table 1). Furthermore, based on the post hoc Bonferroni test, each study group had a p-value of <0.01, indicating significant differences between each group.

DISCUSSION

This study used *Centella asiatica*, commonly known as Pegagan, which was obtained from Banyumas, Central Java. The plant identification was previously conducted at the Faculty of Biology, Universitas Jenderal Soedirman. The results confirmed that the plant used was indeed Centella asiatica.

The extraction of *Centella asiatic*a leaves was carried out using the maceration method with 96% ethanol as the solvent. Extraction is a process of separating two or more desired chemical components by adding a solvent to dissolve these components (Suryanto, 2012). The solvent penetrates the cell walls and enters the cell cavities containing the active compounds (Nurhayati, 2011).

This study used 32 male white rats (Rattus norvegicus) as test animals because they tend to easily adapt, are easier to handle and maintain, have a high reproductive capacity, and hormonally, male rats are more stable as they do not experience estruses and pregnancy phases, which can interfere with the final results of a study. Their production and reproduction characteristics tend to be similar to other mammals, making them easier to handle during experimental treatment (Azhari et al., 2016).

In this study, the induction of hypercholesterolemia using 2% cholesterol and 0.2% cholic acid in groups 2, 3, and 4 was declared successful with total cholesterol levels >130 mg/dL. Administration of *Centella asiatica* extract and simvastatin successfully reduced total cholesterol levels, while group 2 showed an increase in cholesterol levels until the end of the study. This reduction in cholesterol occurred due to the inhibition of the HMG-CoA reductase enzyme by *Centella asiatica* and Simvastatin, which prevents the conversion of HMG-CoA to cholesterol.

Based on the results of the one-way ANOVA analysis, it was shown that the administration of *Centella asiatica* extract in hypercholesterolemia rat models significantly reduced fasting blood glucose (FBG) levels ($p < 0.05$) and showed significant differences. The statistical analysis of FBG levels in group 1 demonstrated a significant difference compared to the other groups, as evidenced by the post hoc Bonferroni test results.

The difference in mean FBG levels between group 1, the healthy control, and group 2, the negative control, was significant with $p < 0.05$ in the post hoc Bonferroni test. This is because the administration of 2% cholesterol and 0.2%

cholic acid can increase blood sugar through several mechanisms. High dietary cholesterol can trigger insulin resistance, a condition where body cells become less responsive to insulin, the hormone that regulates blood sugar levels. Cholic acid, a bile component, can exacerbate this condition by increasing fatty acid accumulation in the liver, contributing to insulin resistance. As a result, body cells cannot efficiently absorb glucose, leading to increased blood sugar levels (Jung et al., 2013).

A high-cholesterol and cholic acid diet can also increase the production of free radicals, causing oxidative stress and inflammation. Oxidative stress damages pancreatic beta cells, responsible for insulin production, thus reducing insulin secretion. Chronic inflammation also increases insulin resistance, reducing insulin's effectiveness in regulating blood sugar levels (Li et al., 2014; Hotamisligil, 2017). Additionally, increased cholesterol levels can cause mitochondrial dysfunction in pancreatic beta cells and other tissues essential for glucose metabolism. Mitochondrial dysfunction disrupts the energy production necessary for effective insulin secretion and other cellular functions, contributing to hyperglycaemia (Galbo et al., 2014).

Cholesterol and cholic acid in the diet can also cause fat accumulation in the liver (hepatic steatosis), which contributes to insulin resistance. The liver plays a crucial role in blood glucose regulation, and liver dysfunction due to fat accumulation can disrupt this process, thereby increasing blood sugar levels (Sanches et al., 2016).

Hypercholesterolemia rats in group 3 treated with simvastatin had higher FBG levels compared to those treated with *Centella asiatica* extract. Simvastatin is a drug used to lower cholesterol levels, but studies have shown that it can also contribute to lowering blood sugar levels through several mechanisms. Simvastatin has been shown to increase insulin sensitivity in peripheral tissues. This is due to simvastatin's effects in reducing inflammation and oxidative stress, which can interfere with insulin signalling (Li et al., 2017).

Simvastatin has strong anti-inflammatory properties by inhibiting the NF-κB pathway, which reduces the production of pro-inflammatory cytokines such as TNF-α and IL-6. This reduction in inflammation helps improve insulin signalling and increases glucose uptake by cells (Hotamisligil, 2017). Additionally, by reducing oxidative stress, simvastatin helps maintain beta-cell function and enhances insulin secretion (Jung et al., 2014).

Several studies have shown that simvastatin can indirectly increase the expression and activity of glucose transporter type 4 (GLUT4) in muscle and adipose tissue. GLUT4 is a protein that mediates glucose uptake into cells in response to insulin. Increased GLUT4 activity helps lower blood glucose levels (Chogtu et al., 2015).

Simvastatin also plays a role in modulating lipid metabolism. It can lower LDL cholesterol levels, which are often associated with insulin resistance and type 2 diabetes. Lowering LDL levels can help improve blood lipid profiles, indirectly enhancing glucose metabolism and reducing blood sugar levels (Sattar et al., 2014).

In this study, group 4, which consisted of hypercholesterolemia rats given *Centella asiatica* extract, had FBG levels closest to the healthy control compared to the group given simvastatin. Centella asiatica contains many active compounds, including asiaticoside, madecassoside, asiatic acid, and flavonoids. Asiaticoside is one of the main triterpenoids with strong anti-inflammatory and antioxidant properties. Asiaticoside can increase insulin sensitivity and reduce insulin resistance by enhancing the activity of enzymes involved in glucose metabolism and reducing oxidative stress in pancreatic beta cells, thereby improving beta-cell function and insulin secretion (James & Dubery, 2009).

Madecassoside is another triterpenoid found in *Centella asiatica*. This compound also has anti-inflammatory and antioxidant effects. Madecassoside can increase the expression and activity of GLUT4 in muscle and adipose tissue, helping to increase glucose uptake by cells and lowering blood glucose levels. Additionally, madecassoside is known to inhibit gluconeogenesis enzymes in the liver, contributing to reduced endogenous glucose production (Wu et al., 2016).

Asiatic acid is another bioactive component found in *Centella asiatica*. Asiatic acid can lower blood glucose by inhibiting the enzymes α-glucosidase and α-amylase, reducing glucose absorption from the intestines into the bloodstream. Furthermore, asiatic acid can induce the regeneration of pancreatic beta cells and increase insulin secretion (Chen et al., 2015).

Centella asiatica also contains various flavonoids that have antioxidant and anti-inflammatory effects. Flavonoids in *Centella asiatica* can reduce inflammation and oxidative stress in pancreatic beta cells, thus enhancing insulin secretion. Additionally, flavonoids can improve endothelial function and increase blood flow to the pancreas, supporting beta-cell function (Mahmoud et al., 2017).

The group treated with *Centella asiatica* extract performed better in lowering FBG levels compared to the group treated with simvastatin due to various factors. *Centella asiatica* extract contains multiple bioactive compounds such as asiaticoside, madecassoside, and asiatic acid. It also contains flavonoids, which are potent antioxidants. These active compounds effectively reduce oxidative stress and chronic inflammation, critical factors in the pathogenesis of diabetes and insulin resistance, thus lowering blood glucose more effectively than simvastatin (Hotamisligil, 2017; Wu et al., 2016; Chen et al., 2015; Sattar et al., 2014).

Madecassoside is known to increase the expression and activity of GLUT4 in muscle and adipose tissue, improving glucose uptake by cells and lowering blood glucose levels more effectively. Simvastatin does not have a significant direct effect on GLUT4 expression or activity (Jung et al., 2014).

CONCLUSION

Based on the results of this study, it can be concluded that the administration of *Centella asiatica* extract at a dose of 500 mg/kgBW can significantly reduce FBG levels in hypercholesterolemia rat models compared to the administration of simvastatin at 1.8 mg/kgBW/day.

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REFERENCES

1. American Diabetes Association. (2020). Diagnosis and classification of diabetes mellitus. Diabetes Care, 43(Suppl 1), S14-S31.
2. Azhari, D. M., Yuliet, Y., & Khaerati, K. (2016). Uji aktivitas serbuk jamur tiram putih (Pleurotus ostreatus (Jacq.) P. Kumm) terhadap kadar glukosa darah pada model hewan hiperkolesterolemia-diabetes. *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy)(e-Journal)*, 2(2), 96-102.
3. Chauhan, P. K., Pandey, I. P., Dhatwalia, V. K., & Singh, V. (2010). Anti-diabetic effect of ethanolic and methanolic leaves extract of Centella asiatica on alloxan induced diabetic rats.
4. Chogtu, B., Magazine, R., & Bairy, K. L. (2015). Statin use and risk of diabetes mellitus. *World journal of diabetes,* 6(2), 352.

5. El-Gohary, Y., & Gittes, G. (2018). Structure of islets and vascular relationship to the exocrine pancreas. Pancreapedia: The Exocrine Pancreas Knowledge Base.
6. Galbo, T., Perry, R. J., Jurczak, M. J., Camporez, J. P., Alves, T. C., Kahn, M., *et al.* (2014). Saturated and unsaturated fat diets induce NASH and liver fibrosis by involving mitochondrial dysfunction. *Journal of Lipid Research*, 55(5), 983-999.
7. Hotamisligil, G. S. (2017). Inflammation, metaflammation and immunometabolic disorders. *Nature*, 542(7640), 177-185.
8. International Diabetes Federation. (2015*). IDF Diabetes Atlas 7th Edition*. Page : 9, 17, 28, 29, 51.
9. James, J. T., & Dubery, I. A. (2009). Pentacyclic triterpenoids from the medicinal herb, Centella asiatica (L.) Urban. *Molecules*, 14(10), 3922-3941.
10. Jung, U. J., & Choi, M. S. (2014). Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. *International journal of molecular sciences*, 15(4), 6184-6223.
11. Kanon, M. Q., Fatimawali, F., & Bodhi, W. (2012). Uji Efektivitas Ekstrak Kulit Buah Salak (Salacca zalacca (Gaertn.) Voss) Terhadap Penurunan Kadar Gula Darah Tikus Putih Jantan Galur Wistar (Rattus norvegicus L.) yang Diinduksi Sukrosa. *Pharmacon*, 1(2).
12. Li, J., Liu, Y., Zhang, L., et al. (2017). Simvastatin alleviates insulin resistance and inhibits inflammatory responses in adipose tissues of obese mice. *Adipocyte*, 6(2), 212-219.
13. Li, P., Oh, D. Y., Bandyopadhyay, G., Lagakos, W. S., Talukdar, S., Osborn, O, *et al.* (2014). The role of HSP70 in inflammatory and metabolic stress responses in macrophages and adipocytes. American Journal of Physiology-Endocrinology and Metabolism, 306(9),
14. Mahmoud, M. F., El-Baz, A. M., Elsayah, M., & Rashwan, A. (2017). Flavonoids protect against diabetes-induced nephropathy in rats. *Pharmaceutical Biology*, 55(1), 722-728.
15. Marianne, M., Yuandani, Y., & Rosnani, R. (2011). Antidiabetic activity from ethanol extract of Kluwih's leaf (Artocarpus camansi). *Jurnal Natural*, 11(2).
16. Pathak, V., Pathak, N. M., O'Neill, C. L., Guduric-Fuchs, J., & Medina, R. J. (2019). Therapies for type 1 diabetes: current scenario and future perspectives. *Clinical Medicine*

Insights: Endocrinology and Diabetes, 12, 1179551419844521.

17. Putri, C. A., Pradana, D. A., & Susanto, Q. (2017). Efek ekstrak etanolik daun bayam merah (Amaranthus tricolor L.) terstandar terhadap indeks massa tubuh dan kadar glukosa darah pada tikus sprague dawley yang diberikan diet tinggi lemak sebagai upaya preventif obesitas. *PHARMACY: Jurnal Farmasi Indonesia (Pharmaceutical Journal of Indonesia),* 13(02), 150-161.
18. Samuel, V. T., & Shulman, G. I. (2016). The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. *The Journal of clinical investigation*, 126(1), 12-22.
19. Sanches, S. C. L., Ramalho, L. N. Z., Augusto, M. J., da Silva, D. M., & Ramalho, F. S. (2015). Nonalcoholic steatohepatitis: a search for factual animal models. *BioMed research international*, 2015(1), 574832.
20. Sattar, N., Preiss, D., Murray, H. M., Welsh, P., Buckley, B. M., de Craen, A. J., *et al.* (2010). Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials. *The Lancet*, 375(9716), 735-742.
21. Sun, X., Ren, Z., Pan, Y., & Zhang, C. (2016). Antihypoxic effect of miR-24 in SH-SY5Y cells under hypoxia via downregulating expression of neurocan. *Biochemical and biophysical research communications*, 477(4), 692-699.
22. Suryanto, E. 2012. *Fitokimia Antioksidan*. Putra Media Nusantara Press, Surabaya.
23. Tulung, G. L., Bodhi, W., & Siampa, J. P. (2021). Uji efektivitas ekstrak etanol daun pegagan (Centella asiatica (L.) Urban) sebagai antidiabetes terhadap tikus putih jantan (Rattus norvegicus) yang diinduksi aloksan. Pharmacon, 10(1), 736-742.
24. World Health Organization. (2016). *Global report on diabetes*. Geneva: World Health Organization.
25. World Health Organization. (2023). *Diabetes*. Geneva: World Health Organization. Link https://www.who.int/health-topics/diabetes#tab=tab_1.
26. Sun, X., Ren, Z., Pan, Y., & Zhang, C. (2016). Antihypoxic effect of miR-24 in SH-SY5Y cells under hypoxia via downregulating expression of neurocan. *Biochemical and biophysical research communications*, 477(4), 692-699.
27. Zhu, X., Jing, L., Chen, C., Shao, M., Fan, Q., Diao, J., ... & Sun, X. (2015). Danzhi Xiaoyao San ameliorates depressive-like behavior by shifting toward serotonin via the

 https://doi.org/10.33508/jwm.v10i2.6031

downregulation of hippocampal indoleamine 2, 3-dioxygenase. *Journal of ethnopharmacology*, 160, 86-93.