The Effectiveness of Black Garlic (Allium sativum L. Fermentation) on Blood Glucose Levels in Streptozotocin-Induced Diabetic Rats

Sheila Rifqa Kamila[a], Indri Safitri Mukono[b], Danti Nur Indiastuti[c]

[a]Medical Program, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
[b]Department of Physiology and Medical Biochemistry, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
[c]Department of Anatomy, Histology, and Pharmacology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

Abstract

Background: Diabetes Mellitus (DM) is a major health issue worldwide, with hyperglycemia being the primary symptom. Uncontrolled hyperglycemia in diabetes mellitus can lead to a variety of complications. This research aims to analyze the effectiveness of black garlic in reducing blood glucose in diabetic rats induced by streptozotocin. **Methods**: A randomized pretest-posttest-only control group design was used in this experimental study. Twenty-four male Wistar Rats were divided into six groups randomly, that is a normal group (N) without any treatment, a negative control group (C-) with streptozotocin induction, a positive control group (C+) with streptozotocin induction plus administration of metformin 45mg/kg BW and treatment groups (T1, T2, and T3) with streptozotocin induction plus administration of black garlic at doses of 72 mg/200gBW/day, 144 mg/200gBW/day, and 288 mg/200gBW/day respectively. The study was conducted for 14 days of treatment. Blood glucose was measured with a glucometer, and statistical analysis was performed using SPSS 26 by paired t-test. **Results**: Based on the Comparability analysis, giving black garlic for 14 days showed significant differences in blood glucose levels before and after treatment in groups C-, C+, T1, T2, and T3 with P<0,05. Based on the different averages of the data, in group C+, there was a significant increase in blood glucose levels, while in groups C-, T1, T2, and T3, there was a significant decrease in blood glucose levels. **Conclusion**: Black garlic significantly lowers blood glucose levels in white male rats (Rattus norvegicus) diabetes mellitus model induced by streptozotocin.

Keywords: Diabetes Mellitus; Black Garlic; Blood Glucose Levels; S-Allyl Cysteine; Metformin

1. Introduction

Diabetes Mellitus is a major health issue worldwide. According to the International Diabetes Federation (IDF), there were approximately 537 million adults (20-79 years old) with diabetes in 2021, with a prevalence of up to 10.5% of the global population of the same age; this number is expected to rise to 783 million by 2045. Diabetes will also take the lives of approximately 6.7 million adults worldwide in 2021 [5].

Diabetes Mellitus is a metabolic disease with a cluster of carbohydrate, lipid and protein metabolism disorders, with chronic hyperglycemia as the main symptom. Diabetes can be caused by a defect in insulin secretion, insulin effectiveness, or both. When insulin secretion decreases, the body cannot maintain glucose levels physiologically. Insulin resistance raises glucose levels in the liver while reducing glucose absorption in other organs. If this pattern persists, diabetes mellitus will occur [9].

Diabetes management, both pharmacological and non-pharmacological, aims to maintain blood glucose levels under control. Inadequate blood glucose control can lead to diabetes mellitus complications such as kidney disorders (nephropathy), eye disorders (retinopathy), nerve disorders (nephropathy), peripheral arterial disease, heart disease, stroke, and lower extremity ulceration [3]. The most commonly used pharmacological treatments for diabetes mellitus are anti-diabetic medications and exogenous insulin. However, Long-term administration of certain anti-diabetic drugs at high doses, on the other hand, can cause some side effects.

Black garlic is fresh garlic fermented at high temperatures (60–90°C) and high humidity (80–90%). Black garlic is dark brown and has a sweet taste with a chewy, jelly-like consistency. Due to changes in allicin levels, black garlic does not have the same robust flavour as fresh garlic [7]. The GSAC (γ-glutamyl-S-allyl cysteine) content in fresh garlic is converted into SAC (S-allyl cysteine), a more stable bioactive component and the main bioactive ingredient in black garlic, during the heating process. The SAC level in black garlic is five to six times higher than in fresh garlic. SAC can reduce levels of free radicals in the body and has anti-diabetic potential. Kim et al., 2017 discovered that SAC could lower blood glucose levels and repair damage to pancreatic β-cells in the islets of Langerhans in type 2 diabetes rat models [8]. In another study published in 2009 by Seo et al., SAC on black garlic increased insulin and its sensitivity while tending to lower blood glucose levels in mutant rat models of diabetes mellitus[11]. SAC reduces free radical toxicity, which may enhance insulin secretion's signalling cascade through reduced stimulation of p38 mitogen-activated protein kinase (p38 MAPK). This situation results in increased expression of GLUT4 and reverses insulin resistance [1].

This study aimed to analyze black garlic for decreasi1ng blood glucose levels in a rat diabetic model that induced streptozotocin.

2. Method

This study is an experimental research with a pretest-posttest group design. The Rattus norvegicus model of diabetes mellitus induced with Streptozotocin was used in the experiment and obtained from the biochemistry laboratory at Airlangga University, Surabaya. The experimental animals used were Rattus norvegicus (235 ± 32 g, 10 ± 1 week) of 24 animals divided into 6 groups which were divided into the Normal group (N), which was the group of rats without treatment, the Negative Control group (C-), which was the group of diabetic rats without treatment, Positive Control (C+), which was the group of diabetic rats and given metformin at a dose of 45 mg/kg BW, Treatment 1 (T1) which was the group of diabetic rats and given chopped black onion at a dose of 72 mg/200grBW/day, Treatment 2 (T2) which was the group of diabetic rats and given chopped black onions at a dose of 144 mg/200grBW/day, and Treatment 3 (T3), which was the group of diabetic rats and given chopped black onions at a dose of 288 mg/200grBW/day.

The black garlic used in this study was made from fresh garlic that was determined to be Allium sativum L. by herbal laboratories directly associated with the regional health ministry. This garlic was produced by fermentation for 21 days at a temperature of 70 degrees Celsius. The black garlic was then mashed and diluted with distilled water to yield doses of 72 mg/ml, 144 mg/ml BW, and 288 mg/ml.

The rats were acclimatized for seven days prior to treatment. The rats were streptozotocin-induced on the eighth day, and blood glucose levels were measured three days later to ensure the rats had diabetes mellitus and they were ready to be treated. Blood glucose levels were measured after 14 days of treatment by taking a blood sample from the rat's tail. A glucometer was used to assess fasting blood glucose levels in this experiment. This research procedure was revised and approved by the health research ethics committee of the Faculty of Medicine, Airlangga University. In this study, data was processed using Microsoft Excel and SPSS version 26. The paired t-test was used in this study to analyse if there were significant differences in the rats' blood sugar levels before and after treatment.

3. Result

The 24 male Wistar rats (Rattus norvegicus) in this study were divided into six groups. The highest post-test mean was in group C- (132.75±12.419), the group of diabetic rats that were not given any treatment. The lowest post-test mean was in group T2 (81.50 ± 5.744), the group of diabetic rats that were given garlic at a dose of 144 mg/kg BW. The results of the data analysis are presented in table 1.

Table 1. characteristics of blood glucose level data(n=24)

Group	Pre-test (mg/dl)	Post-test (mg/dl)
Normal		
Mean±SD	120.0±8.832	132.75±12.419
Median	119.94	133.00
(Min-Max)	(110-131)	(119-146)
C-		
Mean±SD	414.25±9.912	567.50±17.785
Median	383.50	567.50
(Min-Max)	(278-421)	(549-586)
C+		
Mean±SD	496.60±45.735	175.75±150.957
Median	486.0	108.0
(Min-Max)	(453-561)	(86-401
T1		
Mean±SD	475.20±52.141	260.80±100.770
Median	506	286
(Min-Max)	(400-517)	(120-373)
T2		
Mean±SD	450.50 ± 41.461	81.50 ± 5.744
Median	447.50	84
(Min-Max)	(404-503)	(73-85)
T3		
Mean±SD	454.25±47.758	216.75±167.704
Median	463.50	162.50
(Min-Max)	(390-500)	(90-452)

Data on blood glucose levels were data with a numerical scale and had homogeneous data variants. This study was conducted in 6 groups in pairs. Because the difference in data was normally distributed, we used the parametric test for the comparability analysis—the paired t-test for compared pre-test and post-test data. Comparability analysis is presented in table 2.

Table 2. Blood Glucose Comparability Analysis (n=24)

Group	Blood Glucose levels (mg/dl)			p value
	Pre-test Mean±SD	Post-test Mean±SD	Diff Mean±SD	Paired T-test
N	120.0±8.832	132.75±12.419	-12.75±11.087	0.105
K-	414.25±9.912	567.50±17.785	-201.00±79.423	0.015
K+	496.60±45.735	175.75±150.957	320.75±185.315	0.041
P1	475.20±52.141	260.80±100.770	214.40±100.770	0.029
P2	450.50 ± 41.461	81.50 ± 5.744	369.00 ± 45.935	0.001
P3	454.25±47.758	216.75±167.704	237.50 ±131.799	0.037

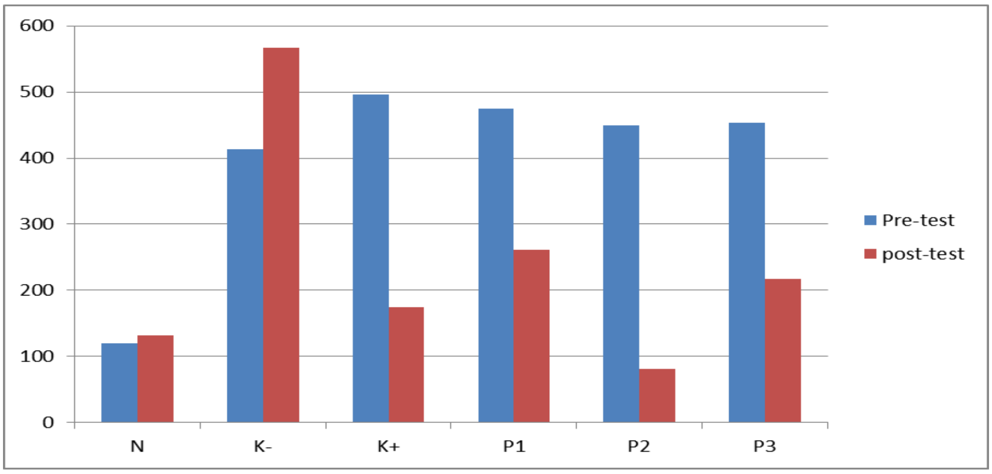

Fig. 1. Pretest and Posttest Mean Distribution

Based on the Comparability analysis, giving black garlic for 14 days showed significant differences in blood glucose levels before and after treatment in groups C-, C+, T1, T2, and T3 with P<0,05. Based on the average of the data we got, shown in fig 1, in group C+ there was a significant increase in blood glucose levels, while in groups C-, T1, T2, and T3, there was a significant decrease in blood glucose levels. The most effective group that decreased blood glucose levels in the treatment group was in group T2, which was given black garlic at a dose of 144 mg/200gr BW, the decrease in this group was almost the same as the decrease in group

C- which was given metformin at a dose of 45 mg/kg BW.

4. Discussion

Black garlic contains rich antioxidants, one of which is S-allyl cysteine (SAC). SAC can reduce free radicals, especially Reactive Oxygen Species (ROS), which endogenous antioxidants cannot absorb. In Eidi et al., 2006, SAC could manage lipid peroxidation better than glibenclamide and insulin, encouraging insulin secretion in vitro from beta cells isolated from normal mice [2]. SAC decreases blood glucose levels by activating the p38 MAPK pathway, stimulating insulin signaling, and raising GLUT4 expression [10].

The black garlic heating process in this study used a temperature of 70ºC for 35 days, and this procedure was based on the research of Sook et al. (2014), who stated that this procedure caused optimal physicochemical and antioxidant changes in black garlic. In this study, we used metformin as a comparison because metformin is the first-line oral antidiabetic pharmacological therapy in type 2 DM [13].

In this study, the negative control group encountered a significant increase in blood glucose levels before and after treatment ($p < 0.05$) because this group of diabetic rats was not given any additional therapy, which increased the blood glucose level. The positive control group encountered a significant lowering in blood glucose levels before and after treatment ($p<0.05$). In this group, metformin at a dose of 45 mg/kg BW lowered blood glucose levels by inhibiting Hepatic Glucose Production (HGP), which suppresses the process of gluconeogenesis [4].

Glucose levels of diabetic rats in the treatment group that was given black garlic at graded doses of 72 mg/200grBW/day, 144 mg/200grBW/day, and 288 mg/200grBW/day for 14 days had a p-value <0.05, which indicated a significant decrease in blood glucose level between before and after treatment. This study showed the same results as the study of Karlina 2020, that blood glucose levels in diabetic rats given black garlic at doses of 36 mg/200grBW/day, 72 mg/200grBW/day, and 144 mg/200grBW/day can reduce blood glucose level in 14 days.

The content of SAC in black garlic can activate the antioxidant response, which is marked by an increase in the nuclear factor erythroid-2-related factor 2 (Nrf2). This situation will reduce free radical levels, increasing insulin signaling through the p38 MAPK pathway and increasing GLUT4 translocation. It will be followed by an increase in glucose uptake and a decrease in blood glucose levels [12]. A significant difference due to the decrease in blood glucose indicates that the SAC in black garlic acts as an antioxidant by activating the Nrf2 gene. SAC will be suppressed free radicals and decrease blood glucose levels significantly.

5. Conclusion

Blood glucose levels of Wistar rats (Rattus norvegicus) induced by streptozotocin and given black garlic extract (Allium sativum L. significantly reduced blood glucose levels and was as significant as metformin at a dose of 45 mg/kg BW. The most effective dose of black garlic in lowering blood glucose levels is 144 mg/200grBW/day.

Acknowledgments

The author would like to thank all the staff at biochemistry laboratory Airlangga University for their assistance during data collection.

References

[1] Arain, A.A. 2018. Effects of S-allyl cysteine on insulin secretion: A proposed mechanism for its anti-hyperglycemic effects. Biomedical Journal of Scientific & Technical Research, 6(3).

[2] Eidi, A., Eidi, M. and Esmaeili, E., 2006. Antidiabetic effect of garlic (Allium sativum L.) in normal and streptozotocin-induced diabetic rats. Phytomedicine, 13(9-10), pp.624-629.

[3] Gregg, E. W., Li, Y., Wang, J., Burrows, N. R., Ali, M. K., Rolka, D., Williams, D. E., & Geiss, L. 2014. Changes in diabetes-related complications in the United States, 1990-2010. The New England journal of medicine, 370(16), 1514–152.

[4] Hunter RW, Hughey CC, Lantier L, Sundelin EI, Peggie M, Zeqiraj E, Sicheri F, Jessen N, Wasserman DH, Sakamoto K. 2018. Metformin reduces liver glucose production by inhibition of fructose-1-6-bisphosphatase. Nat Med, 24(9):1395-1406.

[5] IDF. (2021). International Diabetes Federation (IDF) Diabetes Atlas Tenth edition : International Diabetes Federation

[6] Karlina, D. 2020. 'Pengaruh Pemberian Bawang Hitam (Black Allium Sativum) Terhadap Kadar Gula Darah Pada Tikus Putih (Rattus Novergicus) Diabetes Mellitus'. Skripsi Thesis, Poltekkes Kemenkes Yogyakarta.

[7] Kimura, S., Tung, Y., Pan, M., Su, N., Lai, Y. and Cheng, K., 2017. Black garlic: A critical review of its production, bioactivity, and application. Journal of Food and Drug Analysis, 25(1), pp.62-70.

[8] Kim, J. H., Yu, S. H., Cho, Y. J., Pan, J. H., Cho, H. T., Kim, J. H., Bong, H., Lee, Y., Chang, M. H., Jeong, Y. J., Choi, G., & Kim, Y. J. 2017. Preparation of S-Allylcysteine-Enriched Black Garlic Juice and Its Antidiabetic Effects in Streptozotocin-Induced Insulin-Deficient Mice. Journal of agricultural and food chemistry, 65(2), pp.358–363.

[9] Petersmann, A., Müller-Wieland, D., Müller, U.A., Landgraf, R., Nauck, M., Freckmann, G., Heinemann, L. and Schleicher, E., 2019. Definition, Classification and Diagnosis of Diabetes Mellitus. Experimental and Clinical Endocrinology & Diabetes, 127(S 01), pp.S1–S2

[10] Rajani Kanth,V., Uma Maheswara Reddy,P., Raju T. N. 2008. Attenuation of streptozotocin-induced oxidative stress in hepatic and intestinal tissues of Wistar rat by methanolic-garlic extract. Acta Diabetologica, 45(4): 243-251.

[11] Seo YJ, Gweon OC, Lee YM, Kang MJ, Kim JI. 2019. Effect of garlic and aged black garlic on hyperglycemia and dyslipidemia in animal model of type 2 diabetes mellitus. Journ al of Food Science and Nutrition, 14, pp 1–7.

[12] Shi, H., Jing, X., Wei, X., Perez, R. G., Ren, M., Zhang, X., & Lou, H. 2015. S-allyl cysteine activates the Nrf2-dependent antioxidant response and protects neurons against ischemic injury in vitro and in vivo. Journal of neurochemistry, 133(2), 298–308.

[13] Sook, Choi, H.S Cha and Y.S Lee. 2014. Physicochemical and Antioxidant Properties of Black Garlic. Molecules, 19 : 11-23.