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RESEARCH ARTICLE

Effect of Black Garlic Extract on Blood Glucose, Lipid Profile, and SGPT-SGOT of Wistar Rats Diabetes Mellitus Model

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Abstract

Dyslipidemia is one of the complications of diabetes mellitus (DM) that can lead to elevated total cholesterol, TG, and LDL. Increased SGOT-SGPT indicates damage of hepatic cells. Black garlic extract (Allium sativum L.), which contains polyphenol, flavonoid, and anthocyanin is thought to improve blood glucose, lipid profile, and SGPT-SGOT in human. This study aimed to observe the effect of black garlic extract on blood glucose, lipid profile, and SGPT-SGOT of Wistar Rats Diabetes Mellitus Model. This study was held in 2017 at Biomedical Lab Medical Faculty of Muhammadiyah Malang University. This was a true experimental study with post test only control group design. The rats were assigned to a positive control group and 3 treatment groups consisting of 6 experimental animals each and treatment was given for 25 days. Blood glucose lipid profile, and SGPT-SGOT levels were measured by a spectrophotometer. Data were analyzed using one-way ANOVA, Post Hoc Bonferroni, and Linear Regression tests. Differences were between different groups of rats (p=0,000) with a significant difference seen between all treatment groups and the control group based on the Post Hoc Bonferroni test results (p=0.000). Black garlic extract reduced blood sugar by 91.4%, total cholesterol by 79.1%, TG by 69.5%, LDL by 81.3%, SGPT by 91.4%, and SGOT by 70.6%. This effect is seen across doses starting from 1.5 mg/200gBW to the highest dose of 6 mg/200gBW. It can be concluded that Black garlic extract (Allium sativum L.) affects blood glucose, lipid profile, SGPT, and SGOT positively in male wistar (Rattusnorvegicus) rats diabetes mellitus model by decreasing blood sugar, lipid profile, SGPT, and SGOT levels.

Key words: Black garlic extract, blood glucose, diabetes mellitus, dyslipidemia, lipid profile, SGPT-SGOT

Pengaruh Ekstrak *Black Garlic* (*Allium sativum. L*) Jenis Solo Peroral terhadap Kadar Gula Darah, Profil Lipid, dan SGPT-SGOT pada Tikus Wistar Jantan (*Rattusnovergicus*) Model Diabetes Melitus

Abstrak

Dislipidemia adalah salah satu komplikasi diabetes melitus (DM) yang dapat berpengaruh terhadap peningkatan kolesterol total, trigliserid (TG), dan *low-density lipoprotein* (LDL). Peningkatan *serum glutamic oksaloasetat transaminase* (SGOT) dan *serum glutamic pyruvic transaminase* (SGPT) mengindikasikan kerusakan pada sel hepar. Ekstrak bawang putih hitam (*Allium sativum L.*) mengandung polifenol, flavonoid, dan *anthocyanin* yang dipercaya dapat memperbaiki gula darah, profil lipid, dan SGPT-SGOT pada manusia. Penelitian ini dilaksanakan pada tahun 2017 di Laboratorium Biomedik Fakultas Kedokteran Universitas Muhammadiyah Malang dan menggunakan desain eksperimental *post test* kelompok kontrol. Kelompok kontrol positif dan 3 kelompok perlakuan yang terdiri dari 6 hewan coba di setiap kelompok selama 25 hari. Gula darah, profil lipid, dan kadar SGPT-SGOT diukur menggunakan spektrofotometer. Data dianalisis menggunakan uji ANOVA satu arah, uji Bonferroni *post hoc*, dan uji regresi linear. Uji ANOVA satu arah menunjukkan ada perbedaan antarkelompok perlakuan tikus (p=0,000). Hasil uji Bonferroni *post hoc* menunjukkan perbedaan signifikan (p=0,000) di semua kelompok perlakuan dan kontrol. Bawang putih hitam berpengaruh 91,4% terhadap penurunan gula darah, 79,1% terhadap penurunan kolesterol total, 69,5% terhadap penurunan TG, 81,3% terhadap penurunan LDL, 91,4% terhadap kadar SGPT dan 70,6% terhadap kadar SGOT. Dosis yang memiliki efek yang signifikan sudah mulai terlihat pada dosis awal yaitu 1.5 mg/200 gBB sampai pada dosis tertinggi 6 mg/200 gBB. Dapat disimpulkan bahwa ekstrak bawang putih hitam (*Allium sativum L.*) terbukti mempengaruhi kadar gula darah, profil lipid, dan SGPT-SGOT tikus Wistar jantan model diabetes melitus (*Rattusnorvegicus*) yaitu menurunkan gula darah, profil lipid, dan SGPT-SGOT.

Kata kunci: Diabetes mellitus, dyslipidemia, ekstrak bawang hitam, gula darah, profil lipid, SGPT-SGOT

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Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia due to damages in insulin secretion, work, or both.[1] The prevalence of diabetes mellitus according to WHO 2016 is 43% at >70 years old and more than 90% of these patients suffer from DM type II[2].

There are a lot of micro- and macro-vascular complications which are caused by DM, including coronary heart diseases, stroke, kidney diseases, diabetic retinopathy, and neuropathy . The presence of disorders in the lipoprotein metabolism (dyslipidemia), which is one of the components of metabolic syndrome, is the major contributor to macrovascular complications.[3] This interruption of lipoprotein metabolism results in an increased total cholesterol, triglyceride. and low-density-lipoprotein (LDL) levels, which is atherogenic in nature, has a high susceptibility to oxidation, and is the most common factor of death in diabetic patients.[4]

Antidiabetic pharmacological therapy is a major treatment in diabetes. The most common oral antidiabetic drugs are sulfonylurea and biguanid group. However, the long-term use of these drugs bring some side effects such as allergic reactions, nausea, diarrhea, central nervous system disorders, and hematological symptoms such as agranulocytes and leukopenia.[5]

Garlic is known to have a strong odor and is considered to have the ability to prevent and cure various diseases including DM. The type of processed garlic that is mostly used is black garlic. Black garlic is produced by heating garlics to 70 °C for 21 days. The antioxidant content in garlic processed products is higher than that of unprocessed garlic.[6] The total content of polyphenol in black garlic (25.81–58.33 mg GAE/g) is 2–4 times higher than in raw garlic, which is only 13.91 mg GAE/g.[7]

This study aimed to show the effects of black garlic on blood glucose, lipid profile, SGPT, and SGOT of male wistar (*Rattusnorvegicus*) rats for diabetes mellitus model.

Methods

This study was conducted in 2017 at the Biomedical Lab of Faculty of Medicine, Muhammadiyah Malang University under the ethical clearance number 825-KEP-UB. Materials and instruments used were black garlic extract (*Allium sativum L.*), alloxan, BR-1 (animal feed), aquadest, formalin, 96% alcohol, chloroform, glucometer, modified feeding tube, equipment for measuring blood glucose, lipid profile and SGPT-SGOT, surgical instruments. The experimental animals used were male wistar rats (*Rattusnorvegicus*) aged 2–3 months, weighing 150–200 grams with healthy conditions and active movement characteristics, clear eyes, and thick, slippery, shiny, and clean fur.

The rats were kept for one week in a plastic cage with a wire cover of iron. Each cage contained 6 rats. During the process, rats were fed according to the standard feeding requirement of 12-20 gr per day with drinking water and clean environment that was achieved by changing the chaff every three days.[7]

Black garlic was extracted using the maceration method. The doses applied in this study were: 1.5 mg/200gBW/day (dose 1), 3 mg/200gBW/day (dose 2), and 6 mg/200gBW/day.[13]

After the adaptation period, the rats were divided into 4 groups of 6 rats. Every group received the assigned treatment at the same time at 4.00 PM for 25 days. These groups wereAlloxan-induced rats receiving 160 mg/kgBW alloxan+ BR-1 + aquades (group 1, positive control), Alloxan-induced rats receiving 160 mg/kgBW alloxan + 1.5 mg/200gBW/day black garlic extract + BR-1 + aquades (Group 2, P1), Alloxan-induced rats receiving 160 mg/kgBW alloxan + 3 mg/200gBW/day black garlic extract + BR-1 + aquades (group3, P2), Alloxan-induced rats 160 mg/kgBW alloxan + 6 mg/200gBW/day black garlic extract + BR-1 + aquades (group 4, P3).
The effective dose of alloxan used for intraperitoneal diabetic rats was 160 mg/kgBW.[8]

Results

The results of blood glucose monitoring were listed in Table 1. The control grouptreated by alloxan without black garlic extract showed the highest blood glucose level compared to the other group with 660.7 mg/dL, indicating that alloxan could increase the blood sugar level to above the normal limit of <200 mg/dL in rats.

Total cholesterol levels are shown in Table 2 with the control group demonstrated the highest total cholesterol level compared to the other groups of 153.91 mg/dL, indicating that alloxan administration could increase total cholesterol levels above the normal threshold of 10–54 mg/dL in rats. Meanwhile, the LDL level in the control group was the highest compared to other

Table 1 Blood Glucose Levels in All Groups

Blood Glucose (mg/dL)	Treatment						Mean
	1	2	3	4	5	6	
Control	663.16	663.7	659	643.5	670.4	664.7	660.7
P1	613.2	615.8	614.3	618.5	614.7	611.9	614.7
P2	579.7	580.2	576.9	577.9	576.3	575.9	577.8
P3	290.6	293.2	287.8	285.4	285.5	290.6	240.4

Table 2 Total Cholesterol Levels in All Groups

Total Cholesterol (mg/dL)	Treatment						Mean	Std Deviation
	1	2	3	4	5	6		
Control	152.49	155.82	157.72	153.92	145.84	157.72	153.92	±4.46
P1	97.39	90.26	89.79	87.89	79.81	87.89	88.83	±5.64
P2	48.46	53.21	47.98	54.16	49.41	46.56	49.96	±3.04
P3	41.33	32.30	33.25	39.90	38.00	37.58	37.06	±3.59

groups of 142.16 mg/dL (Table 3), indicating that alloxan administration could increase LDL levels above the normal threshold of 7–27.2 mg/dL in rats.

The results of triglyceride level measurement are shown in Table 4 with the control group presented the highest triglyceride level when compared to other groups (91.21 mg/dL), indicating that alloxan administration could increase rat triglyceride levels to above the normal threshold <150 mg/dL.

The SGPT and SGOT levels for all groups are presented in Table 5 and Table 6, respectively. The control group presented the highest SGPT and SGOT levels when compared to other groups, i.e. 18.39 U/L and 157.48 U / L, respectively. This shows that that alloxan administration could increase SGPT and SGOT levels in rats to above the normal threshold of 40.8-50 U/L and 73.6–208.4 U/L, respectively.

Discussion

The control group in this study presented the highest blood sugar levels of 660.7 mg/dL that is due to the toxic biochemical effects of alloxan that disturb pancreatic beta cells and diabetogenic

Table 3 LDL Levels in All Groups

LDL (mg/dl)	Treatment						Mean
	1	2	3	4	5	6	
Control	141.09	145.61	142.99	140.86	141.33	145.13	142.16
P1	77.2	76.48	78.62	76.96	77.43	75.3	77.08
P2	38.24	39.19	37.29	40.38	37.05	35.39	37.76
P3	20.19	21.62	22.8	20.9	22.57	23.8	22.09

Table 4 Triglyceride Levels in All Groups

Triglyceride Level (mg/dl)	Treatment						Mean
	1	2	3	4	5	6	
Control	91.11	89.94	91.67	90.89	90.78	92.89	91.21
P1	40.21	39.85	42.11	43.56	44.22	44.67	42.43
P2	36.22	37.33	37.67	33.33	35.16	38.00	36.28
P3	25.33	28.67	26.00	22.67	27.11	25.89	25.94

Table 5 SGPT Levels in All Groups

SGPT (U/L)	Treatment						Mean
	1	2	3	4	5	6	
Control	18.31	17.93	17.98	18.29	19.39	18.44	18.39
P1	11.66	11.56	10.55	10.89	11.62	10.93	11.20
P2	8.33	8.40	8.76	8.35	8.66	9.10	8.60
P3	3.33	3.35	3.98	4.10	4.02	3.66	3.74

Table 6 SGOT Levels in All Groups

SGOT (U/L)	Treatment						Mean
	1	2	3	4	5	6	
Control	143.32	148.32	154.98	171.65	163.32	163.32	157.48
P1	64.99	63.33	66.66	68.33	63.33	61.66	64.71
P2	38.33	35.00	37.00	39.00	36.66	40.00	37.66
P3	21.66	23.33	20.00	25.00	24.00	21.67	22.60

β cells which may relate to alloxan-induced redox cycles and ROS formation. The formation of cytotoxic ROS is the result of cyclic reactions between alloxan and reduction product, dialuric acid, that is formed by autoxidation, resulting in superoxide radicals, hydroxyl radicals, and H_2O_2. ROS could take over DNA in pancreatic β cells, hence damaging the cells. In addition, alloxan can also cause rupture of subcellular organelles including rough endoplasmic reticulum and golgi complex. The inner and outer membranes of mitochondria become looser. These changes are irreversible and is considered to be the property of cellular necrosis. In the final phase, a permanent diabetes hyperglycemic condition may complement the complete degranulation and non-intact beta cells [8].

Statistical analysis using one-way ANOVA resulted in a significant value with p=0.000 (p<0.05). This means that at least 2 groups were significantly different. Post-Hoc Bonferroni test was then performed to identify which groups were significantly different. This test resulted in significant result (p<0.05), indicating differences between groups. Therefore, it can be concluded that the three doses of black garlic in P1, P2, and P3 groups were able to decrease the blood glucose level in alloxan-induced *Rattusnorvegicus* rats. This result iss consistent with that of a previous research that stated black garlic contains high total antioxidant polyphenol and flavonoid. Polyphenol and flavonoidcan reduce oxidative stress and prevent the destruction of pancreatic beta cells by inhibiting the chain reaction of converting superoxides into superoxide hydrogen. This is done by donating hydrogen atoms to bind to free radicals and disposing of them through the excretion system.[9]

The linear regression test in this study resulted in R^2=0.914, meaning that black garlic extract decreases the blood sugar level of in alloxan-induced male rats.

The control group in this study presented the highest total cholesterol, LDL, and triglyceride levels of 153.92 mg/dL, 142.16 mg/dL, and 91.21 mg/dL, respectively which may be due to the effect of alloxan as a chemical compound that can cause damages to β-pancreas, followed by defects in insulin secretion that underlies the occurrence of insulin resistance. Insulin resistance leads to an interruption of lipoprotein metabolism, which was one of the metabolic syndrome components that increase free fatty acids (FFAs) in the blood. FFA caused oxidative stress, fatty liver, and cholesteryl ester transfer Protein (CETP) damages, leading to increased CETP activity. CETP is a plasma protein mediating the exchange of cholesteryl esters from HDL with triglyceride molecules from LDL, VLDL, and kilo microns, making VLDL rich in cholesterol, and HDL rich in triglycerides, or triglyceride-rich lipoprotein (TG). This leads to normal LDL. Increased CETP activity causes Apo A-1 to break away from triglyceride-rich HDL and this free ApoA-1 is cleared immediately from the plasma, reducing the ability of HDL to reverse cholesterol transport to VLDL and LDL. As a result, elevated levels of LDL in the blood is seen and LDL-rich triglycerides might undergo lipolysis into atherogenic small dense LDL. This small dense LDL is easily oxidized due to the present of some antioxidant vitamins that makes it more susceptible to oxidation than larger forms of lipoproteins.[11]

PAs shown in a previous study, polyphenol, flavonoid, and anthocyanin have the ability to inhibit oxidative stress by CETP inhibition using polyphenol and anthocyanin while flavonoid worsk on the accumulation of TG resulting in a decrease in LDL levels.[11] This is also seen in this study. The beneficial effects of polyphenol are rimarily attributed to antioxidant properties, as they may act as chain breakers or radical collectors depending on their chemical structure. Polyphenol may also trigger changes in signal pathways and subsequent gene expression.[12] Flavonoid can lower LDL levels through its ability as an antioxidant substance and will work to inhibit the accumulation of TG, resulting in decreased levels of LDL.[11]

The analysis using linear regression on total cholesterol showed that meaning black garlic extract decreased the cholesterol content of alloxan-induced male wistar rats(R^2=0.791). Meanwhile, the regression value for LDL was R^2=0.813, which also shows that black garlic extract has the effect of decreasing the LDL level of male Wistar rats. The same effect was also seen for triglyceride levels with R^2=0.695.

In terms of SGPT and SGOT levels, the positive control group presented the highest SGPT and SGOT levels of 18.39 U/L and 157.48 U/L, respectively. This was due to the effect of the ROS gained through alloxan induction that triggers the hepatic cells to release intracellular enzymes into the blood thus increasing SGPT and SGOT levels.[8]

Statistical analysis on SGPT and SGOT levels using One-Way Anova followed by Post-Hoc Bonferroni test has presented significant differences between groups with a conclusion that the three doses of black garlic in P1, P2, and P3 groups are able to decrease SGPT and SGOT levels in alloxan-induced wistar rats. This finding is supported by a previous study stating that black garlic that contains high total antioxidant polyphenol and flavonoid can reduce oxidative stress; hence, liver cell damage can be reduced and SGPT and SGOT levels decrease.[8,9]

Linear regression analysis in this study provides evidence that black garlic extract decreases the SGPT and SGOT levels (R^2=0.914 and R^2=0.716, respectively).

In summary, the results of this study indicated that black garlic extract can lower blood glucose, lipid profile (total cholesterol, LDL, triglyceride), SGPT, and SGOT in male wistar of DM type II model with higher doses of black garlic extract present the biggest decrease in blood glucose, lipid profile, SGPT level, and SGOT level. This results have to be interpreted with caution due to the fact that not all internal and external factors were examined. The internal factor that was not considered in this study was metabolism differences that affect the absorption of the extract, appetite that influence the amount of nutrient intake, and blood sugar level before treatment. Meanwhile, the external factor that was not included in the analysis was the crowded situation of the cage and the stress experienced during manipulation.

Black garlic extract (*Allium sativum L.*) has the ability to decrease blood sugar, lipid profile, SGPT level, and SGOT level in male wistar (*Rattusnorvegicus*) for diabetes mellitus model. The dose that creates a significant effect starts from 1.5 mg/200gBW.

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