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RESEARCH ARTICLE

Dioscorea alata L. Tubers Improve Diabetes through Anti-hyperglycemia, Anti-inflammation, Ameliorate Insulin Resistance and Mitochondrial Dysfunction

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Abstract

BACKGROUND: *Dioscorea alata* L. tubers (DA) are suspected to prevent diabetes mellitus (DM) because they have a low glycemic index. However, only a few reports about the anti-diabetic effect of DA were reported up to date. This study aims to analyze the effect of DA consumption on several diabetic biomarkers through *in vitro*, *in vivo*, and *in silico* analysis.

METHODS: *In vitro* experiments were conducted by observing the anti-inflammatory activity of DA extract, steroidal saponins (SDA) isolated from DA, and diosgenin in lymphocyte cell cultures. The tumor necrosis factor (TNF)-α and interferon (IFN)-γ percentages were analyzed by flow cytometry. *In vivo* study involved 24 healthy adolescents that were given a boiled DA 10 hours post-prandial. The blood sugar levels were measured at 0, 30, 60, and 120 min after treatment. Furthermore, the *in silico* study was carried out by analyzing the active compounds and predicting the biological activity, the target proteins, and interactions of target proteins with compounds contained in DA.

RESULTS: DA extract, SDA isolated from DA, and diosgenin at 50 μg/mL significantly reduced pro-inflammatory cytokines TNF-α and IFN-γ in lymphocyte cell culture. The blood glucose levels in the DA group were lower at 30 and 60 min after treatment. Based on the *in silico* study, the anti-diabetic activity of DA was speculated to be attributed to the mechanisms of anti-hyperglycemia, prevention of mitochondrial dysfunction, anti-inflammation, and treated insulin resistance. Several proteins included in the DM pathway became the protein target of compounds contained in DA.

CONCLUSION: DA potentially have an anti-diabetic activity through several mechanisms.

KEYWORDS: hyperglycemia, inflammation, insulin resistance, yam

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Introduction

Diabetes mellitus (DM) is one of the major well-being issues around the world. According to the International Diabetes Federation (IDF), the number of DM patients in 2011 was approximated at 366 million individuals. In 2021, the IDF recorded 537 million adults aged 20-79 years, or 1 in 10 people living with DM worldwide. Indonesia is in the fifth position, with 19.47 million people living with DM. With a population of 179.72 million, the prevalence of DM in Indonesia is 10.6%.(1) The prediction ten years ago that the number of DM patients would reach 350 million by 2025 was far exceeded.(2) In 2030, this number is anticipated

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to reach 552 million worldwide (1) and 79.5 million in Southeast Asia (3). More than half of the world's population who suffers from DM are in Asia, especially in India, China, Pakistan, and Indonesia.(2)

DM is a metabolic syndrome characterized by high blood glucose levels and insufficient insulin function.(4) In DM patients, the blood glucose levels are 126 mg/dL or higher. Insulin insufficiency can be caused by impaired or deficient insulin production by the β cells pancreas or caused by the cells being less responsive to insulin.(5) DM involves a highly complex pathway, including chronic inflammation and lipid metabolism.(6,7) Indeed, DM can cause complications and disorders of various organs such as heart failure, fatty liver, and kidney failure. Therefore, research on the prevention and treatment of DM needs to continue to evolve.

Eating carbohydrates with a low glycemic index (GI) can prevent the risk of people suffering from diabetes. Previous research has shown that subjects who consume high GI foods produce higher insulin retention than those who consume low GI foods.(8) A diet based on high GI foods that cause elevated blood glucose and insulin has been shown to cause and worsen inflammation.(9) In contrast, foods with a low GI break down slowly during digestion and are slowly assimilated.(10) Therefore, these foods have a slower impact on blood glucose levels and insulin response. Another study found that diabetic patients who ate a low GI diet had a 0.43% reduction in HbA1c levels and a 7.4% reduction in glycosylated protein levels.(11) Among overweight individuals, a low glycemic calorie-reduced diet has been shown to reduce inflammation.(9) Therefore, foods with a low GI are indispensable for patients with DM; good control of the GI is needed for DM patients so that blood sugar levels are controlled, and complications can be avoided.(12)

Foods with a low GI have GI scores ranging from 0-55%.(13) High-fiber foods have a low glycemic index, for example nuts, cereals, cassava (14), and yam (15). In addition, DA also have a low GI of less than 55%.(13) Previous study reported that boiled DA have a GI of (50.12±18.91)%, and roasted DA have a GI of (54.04±13.25)%.(16)

DA, or locally known *as yam*, is a prospective local food that can be used as a functional food and diversification, especially in Indonesia. DA contains high carbohydrates and protein but low sugar content (16), while *Dioscorea batatas* has an anti-diabetic effect in streptozotocin-induced DM rats (17). *Dioscorea* application decreased the blood glucose level in streptozotocin-induced DM rats.(18) Steroidal saponin (SDA) and diosgenin are the most important compounds in DA.(19) However, there has been a few studies on the anti-diabetic effect of DA up to date. Therefore, this study aims to analyze the influence of DA consumption on several diabetic biomarkers through *in vitro*, *in vivo*, and *in silico* analysis.

Methods

Preparation of DA Extract (DAE) and SDA for *in vitro* Experiment

To extract steroid and phenolic compounds in DA more optimally, 70% ethanol solution was used for the production of DAE by the maceration method. The ethanol extracts were filtered and evaporated in a rotary evaporator; then, the resulting powder was used as a DAE for the subsequent assays. The SDA was isolated from DAE using column chromatography. Meanwhile, diosgenin (3β,25R)-spirost-5-en-3-ol, powder was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Isolation of SDA in DA

Isolation of SDA compounds in DA was carried out by using the bioassay-guided separation method. The 70% ethanol extract of DA was fractionated using column chromatography with silica G60 as a stationary phase (Cat. No. 1.07733 Merck, Kenilworth, NJ, USA) and chloroform, chloroform/methanol, and methanol as mobile phases. The active fraction was re-chromatographed using a column with silica G60 as a stationary phase and hexane : chloroform as the mobile phase. Each stage of compound separation was monitored by Thin Layer Chromatography (TLC) with butanol : ethanol : water = 6:2:3 and butanol : acetic acid : water = 2:1:1 as eluents. The TLC test results were added with the combination of Liebermann-Burchard reagent, acetic anhydride, and sulfuric acid (1:1:1:1). The steroidal saponin preparations were detected using a UV lamp on a silica G60 plate (Cat. No. 1,05748.0001, Merck) in butanol-ethanol-water (2:4:4). Formation of purple spots indicated the positive results.

Lymphocyte Cell Culture

Healthy BALB/c mice were dissected, and the spleen was isolated in Laminar Air Flow. Lymphocyte was isolated from spleen in sterile PBS. The homogenate of lymphocyte cell was resuspended with 10 mL PBS and then centrifuged at 2500 rpm, 4°C, for 5 min. Cells were resuspended with 1 mL RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. A total of 7.5 x 10^3

cells/mL was seeded in 24 well plates and then incubated for 24 h, 37°C in a 5% CO_2 incubator. Cells were then treated with 50 µg/mL of DAE, SDA, or diosgenin overnight. Cells were harvested by pipetting each well plate and transferring them into tubes. The tubes were then centrifuged at 2500 rpm, 10°C for 5 min, resuspended with 1 mL of PBS, and continued with the immunostaining procedure. The *in vitro* experiment has received ethical clearance/approval by the Research Ethics Committee from Brawijaya University Malang (No. 144-KEP-UB).

Immunostaining and Fluorescence Activated Cell Sorting (FACS) Analysis

The immunostaining procedure and FACS analysis were performed based on literature with some modifications.(6) Cells were stained with fluorescein isothiocyanate (FITC)-conjugated anti-mouse CD4 and incubated for 20 min. Furthermore, cells were added with 50 µL cytofix-cytoperm and were incubated for 20 min in a dark room. Then, they were added with 500 µL washperm and were centrifuged at 2500 rpm, 4°C, for 5 min. Cells were resuspended with intracellular antibodies phycoerythrin (PE)-conjugated anti-tumor necrosis factor (TNF)-α and PECy5-conjugated anti-interferon (IFN)-γ. Then, each sample was analyzed using a flow cytometer (FACSCalibur; BD Biosciences, Franklin Lakes, NJ, USA).

Preparation of DA for *in vivo* Experiment

DA were purchased from Yogyakarta traditional market. A total of 3 kg of DA were boiled over medium heat for 30 min with fresh water. Boiled DA were left at room temperature and were ready to be given to subjects.

Subjects Recruitmentent

Subjects with normal fasting blood glucose, as much as 24 subjects, were recruited. The sampling technique used was purposive sampling, with inclusion and exclusion criteria determined by the researcher as follows. The inclusion criteria of the subjects in this study were male; healthy; aged 20-25 years; no history of food allergies; no family history of DM, hypertension, and cholesterol; willing to be research respondents, and filling out the informed consent. Meanwhile, the exclusion criteria were subjects that had fasting blood sugar values above 126 mg/dL.

The *in vivo* experiment has been approved by the Ethical Committee of Health Research Ethics Committee, Faculty of Medicine and Health Sciences Universitas Muhammadiyah Yogyakarta (No. 187/EC-KEPK FKIK UMY/VI/2021).

DA Treatment

The weight of all subjects was measured. Subjects were randomly divided into 2 groups: the control and DA group. Each group consisted of 24 healthy subjects. Subjects had fasted for 10 hours prior to treatment administration. Then, the control group was given 2.5g/kg BW glucose consumption, while the DA group was given 2.5g/kg BW boiled DA. Blood glucose levels were measured at fasting condition, and 30, 60, and 120 min after administration of glucose or boiled DA.(15) The measurement method used to examined blood glucose levels was Glucose oxidase (GOD) – Peroxidase (POD).

Data Analysis for *in vitro* and *in vivo* Study

In vitro data were analyzed using BD CellQuest PRO software (Becton, Dickinson and Company, San Jose, CA, USA). The results of *in vivo* and *in vitro* presented in the figures represent at least three independent experiments. The values are presented as mean±standard deviation (SD). The differences of *in vitro* data were analyzed using ANOVA, followed by Tukey's multiple comparison test. While, the differences of *in vivo* data were analyzed using paired t-test. The statistical significance was set at $p<0.05$ and $p<0.01$.

Analysis of The Compound Contained in DA Through An *in silico* Study

The bioactive compounds of DA were obtained computationally from study literature (20) and databases from Knapsack (*http://www.knapsackfamily.com/KNApSAcK/*). After finding the compounds of DA, ID code and simplified molecular-input line-entry system (SMILE) obtained from the PubChem database (*https://pubchem.ncbi.nlm.nih.gov/*) were collected as sample analysis. The sample was then analyzed for its role as a drug using the Lipinski Rule of Five parameters. Lipinski parameters were as follows: the compound did not have a molecular weight of more than 500 Daltons; did not donate more than 5 hydrogen bonds; accepted less than 10 hydrogen bonds; had LogP value <5; and has molar refraction of 40–130. Each active compound must not violate Lipinski's rules more than two points out of 5 Lipinski parameters.(21)

Quantitative structure-activity relationship (QSAR) analysis of active compounds by Way2Drug

The biological activity of each active compound was predicted and determined. A QSAR analysis was carried out to determine the similarity of the compounds and the database using Way2Drug/PASS online (*www.way2drug.com/PASSOnline*). The score shown by the webserver varies

from 0-1, indicating the accuracy of the analysis. QSAR is an *in silico* analysis that focuses on the similarity of the input structure compared to the database.(22) Parameters analyzed were potentiality related to anti-diabetic.

Protein Target Analysis

Protein targets that can interact with active compounds were predicted using Similarity Ensemble Approach (SEA) Target (*https://sea.bkslab.org/*), which used similarity approach to predict the interactions with target proteins. The minimum cut-off used was 0.57, as suggested in the literature.(23)

Protein-Protein Interaction

The target protein obtained from the target protein analysis was then continued for an analysis of their interaction using STRING DB V.11 (*https://string-db.org/*) with a high confidence score of 0.7 as input for Homo sapiens organisms. After that, 14 target proteins were obtained, and then the betweenness centrality (BC) of each protein using Cytoscape version 3.8.2 was analyzed. The protein from SEA target with the highest value BC was chosen as the target protein. BC was an analysis to see the role of the most dominant protein in the pathway that is being analyzed. STRING DB was used to see protein interactions for further analysis in Cytoscape version 3.8.2 (Institute for Systems Biology, Seattle, WA, USA), a program for pathway visualization and analysis.

Results

DAE and SDA Reduce the Number of Pro-inflammatory Cytokines in Lymphocytes Cell Culture in BALB/c Mice

Based on Figure 1, the DAE potentially decreases the relative number of pro-inflammatory cytokine TNF-α and IFN-γ, as well as its SDA and diosgenin. SDA and diosgenin were used because these 2 compounds are the major compounds found in DA. Interestingly, the SDA has stronger effects on suppressing the pro-inflammatory cytokines than DAE and diosgenin at the same dose (50 μg/mL). To prove its anti-hyperglycemic activity, we conducted an *in vivo* study.

Characteristics of Subjects

In vivo research was conducted on 24 healthy subjects with normal fasting blood glucose levels for each group. The characteristics of the subjects, including age, weight, height, body mass index, and blood pressure, were presented in Table 1. Based on the characteristics of subjects in Table 1, it can be seen that most subjects (79.17%) did not smoke, 86% regularly exercised, 83 % had moderate activity, 58% had a regular diet, and 96% had adequate rest time.

DA Stabilizes Blood Glucose Levels in Healthy Subjects

Before consuming DA, subjects' blood glucose levels were normal, ranging from 70-130 mg/dL. After consuming DA, the subjects' blood glucose levels will rise from this limit but are still less than 140 mg/dL after 2 hours. With fasting for ten hours, normal blood glucose levels are supposedly less than 100 mg/dL. The blood glucose level of all subjects after 10 hours of fasting was around 80 mg/dL (Table 2). In control groups, who were fed with 25 mg/kg BW glucose, the blood glucose level of subjects increased to 132.37±20.46 mg/dL after 30 min, 97.54±29.53 mg/dL after 60 min, and 68.58±12.91 after 120 min.

Interestingly, in the DA group, the blood glucose levels of the subjects were relatively stable and remained normal during each observation time: 81.92±9.75 mg/dL after 30 min, 73.54±11.35 after 60 min, and 68.79±7.87 after 120 min. DA may have the possibility as a candidate for anti-diabetic traditional medicine.

Active Compounds Contained in DA Based on *in silico* Study

The screening results for active compounds in DA obtained 41 compounds. After being analyzed with Lipinski parameters, 21 compounds were obtained, as shown in the

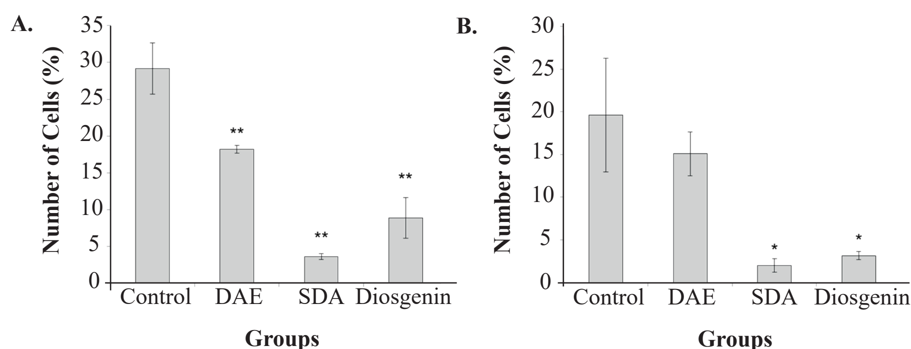

Figure 1. The DAE decreases the relative number of TNF-α and IFN-γ pro-inflammatory cytokines as well as SDA and diosgenin. A: $CD4^+TNF\text{-}\alpha^+$; B: $CD4^+IFN\text{-}\gamma^+$.

Table 1. Characteristics of the subjects.

Variable	Value
Age (years old), mean±SD	22.08±0.65
Weight (kg), mean±SD	67.70±12.89
Height (cm), mean±SD	168.43±6.01
Body mass index, mean±SD	23.76±4.14
Blood pressure, n (%)	
Systole	124.33±9.53
Diastole	81.42±9.54
Smoke, n (%)	
Yes	5 (20.83%)
No	19 (79.17%)
Exercise, n (%)	
Yes	21 (87.50%)
No	3 (12.50%)
Regularly Exercise, n (%)	
Yes	18 (85.71%)
No	3 (14.29%)
Physical Activity, n (%)	
High	2 (8.33%)
Moderate	20 (83.34%)
Low	2 (8.33%)
Dietary habit, n (%)	
Regular	14 (58.33%)
Irregular	10 (41.67%)
Resting time, n (%)	
Enough	23 (95.83%)
Less	1 (4.17%)

Table 3. The detected compounds were classified as steroidal saponins, triterpene saponins, phytosterols, glyoxylic acid oxide, flavonoids, anthocyanins, essential nutrients, proteins, saponins, diterpenoids, lactones, and phytosteroid sapogenins.

Prediction of The Activity of DA Active Compounds

Further, we screened with QSAR analysis whether the DA had an anti-diabetic compound. The selected mechanisms are mechanisms related to DM, including anti-inflammatory, β-glucosidase inhibitors, TNF-α inhibitors, anti-hypercholesterolemia, immunosuppressants, insulin promoters, nucelar factor-kappaB (NF-κB) inhibitors, and kinase inhibitors. The results of the QSAR analysis obtained 4 anti-diabetic compounds contained in DA with the highest predictive score (Figure 2A). The first compound was leucocyanidin (0.40), which had a role in anti-inflammatory, anti-hypercholesterolemia, immunosuppressant, and insulin promoter mechanisms. Naringenin (0.32) had a role in anti-inflammatory, β-glucosidase inhibitor, and immunosuppressant mechanisms. Peonidin (0.31) had a role in anti-inflammatory, TNF-α inhibitor, anti-hypercholesterolemia, and insulin promoter mechanisms. The last one was allantoin (0.29), which had a role in hypercholesterolemia and immunosuppressant mechanisms. Furthermore, several anti-diabetic activities in DA showed the highest predictive score of its main role in treating hyperglycemia (1.30), followed by preventing mitochondrial dysfunction (0.40), inflammation (0.33), and treating insulin resistance (0.21). This score was obtained based on the total score of DA active compounds activity related to anti-diabetic (Figure 2B).

Protein Target Analysis and Protein-Protein Interactions

To understand more about the potential bioactivity of the 21 selected compounds in Tabel 4, protein target prediction analysis was carried out using SEA Target, and protein-protein interactions (PPIs) analysis using STRING. The results of this analysis (Table 4) showed that 14 diabetes-related proteins (5 proteins from SEA Target and 9 proteins from STRING) became potential targets for interaction with the 21 selected compounds contained in DA. However, only protein from SEA target with the highest BC, was chosen to be the target protein. Whereas protein from STRING was used as enrichment analysis to clarify the whole mechanism that happened in DM pathway.

Based on Table 4, the three highest BC values were found in INS (0.52), SCAP (0.46), and SREBF2 (0.28). INS and SCAP had the highest BC values because their

Table 2. The blood glucose level (mean±SD) of subjects.

Blood Glucose Level	Groups		*p*-value
	Control	DA	
Fasting	80.29±8.96	80.08±10.61	$p>0.05$
30 min after treatment	132.37±20.46	81.92±9.75	$p<0.05$*
60 min after treatment	97.54±29.53	73.54±11.35	$p<0.05$*
120 min after treatment	68.58±12.91	68.79±7.87	$p>0.05$

*There was a significant difference between groups.

Table 3. Active compounds from DA after screened by Lipinski rules.

Compounds	Category	Drug-likeness Score				
		Mol. Mass (500 Da)	LogP (<5)	Hb Donor (<5)	Hb Accep (<10)	Mol Refraction (40<A<130)
Gracillin	Steroidal saponin	248.32	2.39	0	3	68.63
Prosapogenin	Triterpene saponin	604.81	3.82	5	8	164.52
γ-sitosterol/Fucosterol	Phytosterol	414.71	7.24	1	1	133.23
Allantoin	Glyoxylic acid oxide	158.12	-1.03	5	6	44.36
(+)-Catechin	Flavonoid	290.27	0.83	5	6	74.33
Peonidin	Anthocyanin	301.27	0.76	4	6	80.64
Pelargonidin	Anthocyanin	271.24	0.73	4	5	74.15
Leucopelargonidin	Anthocyanin	290.27	0.66	5	6	73.47
Leucocyanidin	Anthocyanin	306.27	0.07	6	7	75.5
Cyanidin	Anthocyanin	287.24	0.56	5	6	76.17
Dihydroquercetin	Flavonoid	304.25	0.51	5	7	74.76
Naringenin	Flavonoid	272.25	1.84	3	5	71.57
Chalcone naringenin	Flavonoid	272.25	1.83	4	5	74.34
Dihydrokaempferol	Flavonoid	288.25	0.99	4	6	72.73
Choline	Essential nutrient	104.17	-1.38	1	1	29.69
Mucin	Protein	210.14	-2.43	6	8	39.15
Chlorogenic acid	Polyphenol	354.31	-0.39	6	9	83.5
Sapogenin	Saponin	400.64	6.21	0	2	120.91
Myricetin	Flavonoid	318.24	0.79	6	8	80.06
Diosbulbin B	Diterpenoid lactones	344.36	1.98	0	6	83.83
Diosgenin	Phytosteroid sapogenin	414.62	5	1	3	121.59

interactions were the link between protein interactions on the right (SRBEF2, NR1H2, NR1H3, and INSIG2) with proteins on the left (IGF1, F2, F2R, IGF2, IGF1R, INSR, AKT1, and IRS 1) (Figure 3). Then, SREBF2 had the third highest BC value because it was the link between the interactions of NR1H2 and NR1H3 with INSIG2 and SCAP.

Moreover, according to the results of further PPIs analysis, 14 diabetes-related proteins have resulted in pathways based on the KEGG database. These pathways include regulatory pathways for type-2 DM, lipolysis in adipocytes, adipocytokine signaling, and pluripotent stem cell signaling. Each color in the protein node has a specific meaning, as presented in Table 5, blue: regulation of lipolysis in adipocytes, red: type-2 diabetes mellitus, green: adipocytokine signaling pathway, yellow: pluripotent stem cell regulatory signaling pathway. As shown in Figure 3B, IGF1 and its receptor (IGF1R) play a role only in pluripotent stem cell regulatory signaling pathways. INS and its receptor (INSR) play a role in regulating lipolysis in adipocytes and pathogenesis of type-2 DM. Insulin receptor substrate 1 (IRS1) regulates lipolysis in adipocytes, adipocytokine signaling pathway, and type-2 DM. Meanwhile, AKT1 plays a role in the pluripotent stem cell regulatory signaling pathway, regulation of lipolysis in adipocytes, and adipocytokine signaling pathway.

PPIs were also performed to examine proteins that play a role in insulin signaling and insulin resistance (Figure 3B), red: proteins that play a role in insulin resistance, blue: proteins that play a role in insulin signaling (Table 5). Based on this, six proteins are known to play a role in insulin resistance, including INS, INSR, IRS1, AKT1, Nuclear Receptor Subfamily 1 Group H Member 2 and 3 (NR1H2 and NR1H3). Meanwhile, proteins that play a role in the insulin signaling pathway are INS, INSR, AKT1, and IRS1.

Furthermore, the predicted pathway from the results of the PPI analysis needs a false discovery rate (FDR) value as a computational value to show possible errors that result in different bioactivity and lead to different pathways as well. FDR value described the statistical approach used in multiple hypothesis testing to correct several FDR comparisons representing the false positive rate or the equivalent of a repeated calculated *p*-value. This means that

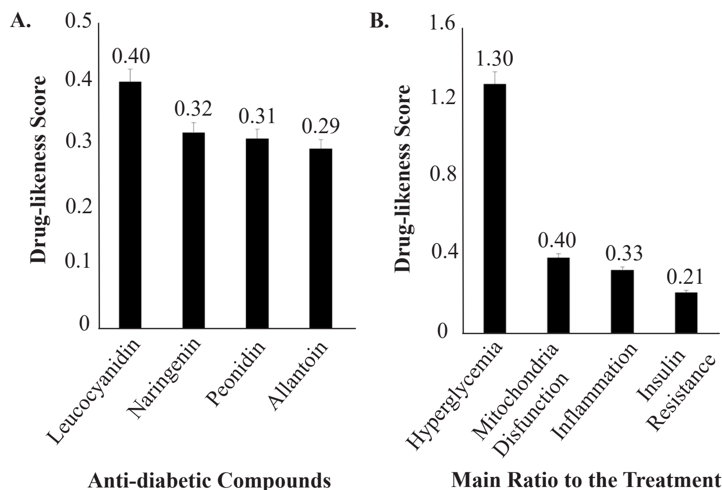

Figure 2. Prediction of anti-diabetic activity of the DA active compounds. The higher the drug-likeness score, the higher the activity potential, which will affect its efficacy as anti-diabetic.

the smaller the FDR value, the more likely the pathway will occur. A small FDR value indicates high accuracy. Based on FDR values in Table 5, all mechanisms described in Figures 3 have a high possibility.

Discussion

When type-2 DM starts to develop, the body becomes insensitive to insulin, resulting in insulin resistance and inflammation. Chronic inflammation can occur in various organs, as well as the pancreas. This condition causes more insulin resistance and vice versa. TNF-α has been identified as major regulator of inflammatory responses and known to be involved in pathogenesis of some inflammatory diseases including DM.(24) In addition, in type-2 DM, $CD4^+$ T cells tend to polarize to T helper 1 (Th1) and T helper 17 (Th17) cells in either peripheral blood or adipose tissue. Therefore, IFN-γ production from those T cells increases.(25) In this study, the DAE, its SDA, and diosgenin reduced the pro-inflammatory cytokine TNF-α and IFN-γ (Figure 1). It proved that DA and their compounds potentially have anti-inflammatory activity. The results of this study are supported by the previous study's findings that reported the anti-inflammatory activity of the Dioscorea genus, such as *Dioscorea trifida* (26) and *Dioscorea bulbifera* L. bulbis (27). Based on that, DA possibly have anti-hyperglycemic activity since these two mechanisms are closely related.(28) Hyperglycemia acutely increases circulating pro-inflammatory cytokine concentrations by an oxidative mechanism.(9)

Furthermore, in this study, we demonstrated that the consumption of DA stabilized the blood glucose level in healthy subjects compared to the control group (Table 2). The subjects were chosen randomly to prove whether the individual biological variations would affect the result or not. In addition, the subjects' activities during the sampling interval were controlled to minimize differences in the metabolic rate of each respondent. In the control group, glucose consumption increased blood glucose levels at 30 and 60 min after eating compared to fasting blood sugar. In contrast, blood glucose levels in the DA group at 30 and 60 min after eating tended to be stable compared to fasting blood sugar. Blood glucose levels in both groups at 120 min after eating decreased because the blood glucose had been absorbed. These anti-hyperglycemic effects may occur due to a low GI of DA. Foods with a high GI tend to increase blood glucose levels after consumption; therefore, they are not recommended for diabetic patients.(11) Foods with high

Table 4. Betweenness centrality score of proteins involved in DM pathway.

Name	Betweenness Centrality
INS[a]	0.515384615
SCAP[a]	0.461538462
SREBF2[b]	0.282051282
F2[b]	0.153846154
IGF1[a]	0.130769231
IGF2[a]	0.130769231
IRS1[a]	0.002564103
INSR[b]	0.002564103
F2R[a]	0
NR1H2[b]	0
IGF1R[a]	0
NR1H3[b]	0
AKT1[a]	0
INSIG2[a]	0

[a]STRING; [b]SEA Target.

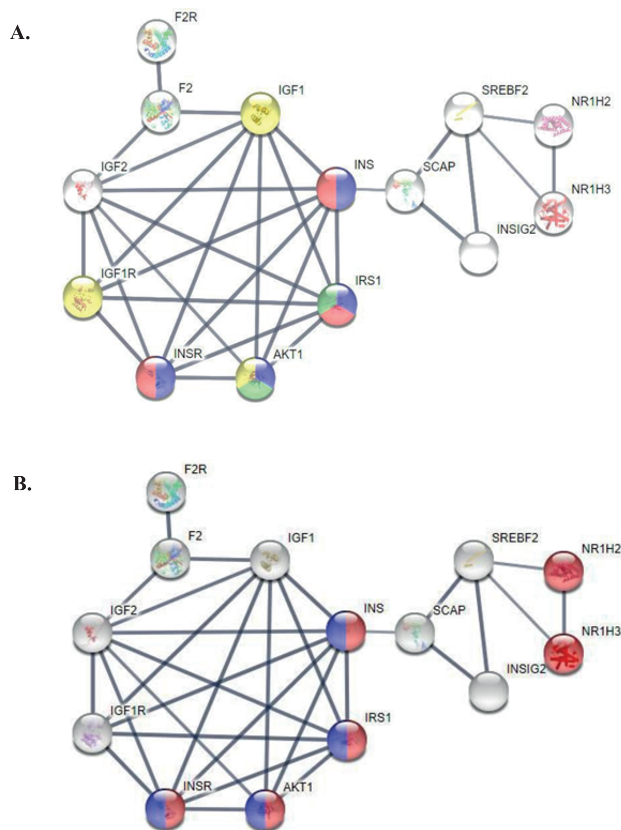

Figure 3. Protein-protein interactions associated with the diabetes mellitus pathway (A) and insulin signaling pathway (B).

GI not only rapidly increase blood glucose but also insulin responses following food consumption. In response to a fast and large spike in blood glucose, the pancreas releases a large amount of insulin, a hormone that removes glucose from the bloodstream. Meanwhile, foods with a lower GI can be used in a diabetic diet.(29) Boiled DA have a GI of 50.12%, where the GI is classified as low if it is <55%.(22) The low-GI diet is more effective in controlling glycated hemoglobin and fasting blood glucose than a higher-GI diet or control in patients with type-2 DM.(30) Foods with low GI may contribute to glycemic control compared to foods with high GI by promoting insulin sensitivity, reducing fluctuations in blood glucose levels, and reducing daily insulin requirements.(10)

In silico study was performed to explain the anti-diabetic mechanism of DA computationally in detail. There were 21 active compounds in DA (Table 3), and 4 compounds were found to have an anti-diabetic role. The score for preventing or treating hyperglycemia is 1.30, obtained from the activity score as anti-hypercholesterol, an inhibitor protein kinase, and a β-glucosidase inhibitor. Furthermore, mitochondrial dysfunction is known to cause DM through mutations in its genome (mtDNA). The predicted score of 0.40 indicates that the active compound from DA has the computational potential to overcome or prevent mtDNA mutations. The parameters used are activities of anti-inflammatory, immunosuppressant, NF-κB inhibitor, and protein kinase inhibitor. Then, the active compound from DA was also predicted to be anti-inflammatory, with a score of 0.33. The predictive score was obtained from anti-inflammatory activity, TNF-α and NF-κB expression inhibitors, and immunosuppressant activity. The last is preventing or treating insulin resistance with a predictive score of 0.21 (Figure 2).

In DM conditions, glucose production is altered in hepatocytes by dysregulation of the glucose and glycogen pathways, resulting in not only hepatic insulin resistance but also oxidative stress.(31) Oxidative stress that occurs in the hepatocyte will then impact the dysregulation of lipid metabolism, which causes the accumulation of triglycerides and cholesterol in the liver as well as in the serum. This condition further leads to hypercholesterolemia.(32) Hypercholesterolemia conditions will further aggravate diabetes and *vice versa*.(33) Anti-hypercholesterolemia activity of some active compounds plays a role in improving this condition. Moreover, hyperglycemia will increase the overproduction of reactive oxygen species (ROS) from the increased metabolic activity of diacylglycerol through protein kinase C and nicotinamide adenine dinucleotide

Tabel 5. FDR from every mechanism obtained.

Mechanism	FDR	Color	Description
Regulation of lipolysis in adipocytes	0.000000434	Blue	Figure 3
Type II diabetes mellitus	0.0000246	Red	Figure 3
Adipocytokine signaling pathway	0.0033	Green	Figure 3
Signaling pathways regulating pluripotency of stem cells	0.00043	Yellow	Figure 3
Insulin resistance	2.41E-09	Red	Figure 3
Insulin signaling pathway	0.0000121	Blue	Figure 3

phosphate (NADPH) oxidation.(34) Therefore, protein kinase inhibitor activity of DA has an essential role in preventing the increase in ROS in hyperglycemia. A recent study proved that *D. batatas* peel extract decreased the level of ROS.(35) *Dioscorea japonica* was reported to inhibit mitogen-activated protein kinase activation.(36) Moreover, in hyperglycemia conditions, the glucosidase enzyme plays a significant role. Glucosidase cleaves the glycosidic bond causing an increase in the blood glucose level. The presence of a glucosidase inhibitor will inhibit the activity and result in a decreased blood glucose level.(37)

Besides hyperglycemia, one of the signs of type-2 DM is insulin resistance. In insulin resistance, muscle, adipose, and liver cells do not respond well to insulin and cannot use glucose from the blood for energy. Another study stated that the pancreas would work hard to produce more insulin with the change of adipocytokines and a signal for the higher demand for insulin.(5) This condition will promote oxidative stress, endoplasmic reticulum (ER) stress, and mitochondrial dysfunction in pancreatic β cells. As a result, islet inflammation will occur and lead to reduced insulin secretion.(37) As explained before, the active compound from DA that can prevent mtDNA mutations will play an important role in this mechanism.

Since hypercholesterolemia also occurs during DM, adipose tissue is also affected because this tissue plays a role in storing excess lipids in the body. It will cause adipocyte hypertrophy, leading to adipocyte inflammation and insulin resistance.(5) Adipocyte inflammation will accelerate lipolysis. On the other hand, oxidative stress that occurs in the liver during DM will also promote inflammation and hepatic insulin resistance.(33) The inflammation that occurs in the pancreas, adipose, and liver simultaneously provokes chronic inflammation. Inflammatory cells such as macrophages and lymphocytes will be activated, infiltrate into the site of inflammation, and produce pro-inflammatory cytokines such as TNF-α and IFN-γ through NFκB activation.(5,38) In this state, the inflammation will be worse. A previous study has reported that the extract of DA possesses a potent anti-inflammatory effect by inhibiting TNF-α expression.(39)

D. japonica was reported to exhibit anti-inflammatory activity by inhibiting NF-κB.(36) In this study, active compounds of DA that have immunosuppressant, TNF-α, and NF-κB inhibitor activity will play an important role in this mechanism to inhibit the activation of immune cells and reduce inflammation. The reduction of inflammation will further improve insulin resistance. Besides, prevalent compounds of DA play a role in treating insulin resistance even though the predictive value is the smallest compared to other activities. Previous research proved that *D. batatas* ameliorate insulin resistance in high-fat diet (HFD)-induced mice.(40)

The last, this study showed that several molecular protein interactions with DA compounds (Table 5) that play a role during DM (Figure 4) include INS, INSR, IRS1, AKT1, NR1H2, and NR1H3. Those proteins have been found in mechanisms related to the pathogenesis of type-2 DM, regulation of lipolysis in adipocytes, adipocytokine, and insulin signaling pathways. They possibly become a target for the anti-diabetic compounds of DA. Although this study demonstrated the anti-diabetic mechanism of DA comprehensively, the *in silico* study should be supported with experimental animal research. Using an animal model with diabetes mellitus is important to confirm the results of this study. However, this study can be used as the fundamental for research on active compounds of DA.

Conclusion

DA extract, its SDA, and diosgenin potentially reduced pro-inflammatory cytokine TNF-α and IFN-γ *in vitro*, presenting anti-inflammatory effects. DA possibly had an anti-hyperglycemic effect due to their activity to stabilize blood glucose levels after eating in healthy people. An *in silico* study proved that the four highest active compounds in DA have anti-diabetic effects: leucocyanidin, naringenin, peonidin, and allantoin. The anti-diabetic activity was possibly obtained through mechanisms of anti-hyperglycemia, prevention of mitochondrial dysfunction, anti-inflammation, and treating insulin resistance. Several proteins in diabetes mellitus pathways (INS, INSR, IRS1, AKT1, NR1H2, and NR1H3) became the protein target of compounds in DA. PPIs proved that those targeted proteins play main roles in type-2 DM, regulation of lipolysis in adipocytes, insulin resistance, adipocytokine and insulin signaling pathways. Overall, DA may potentially have anti-diabetic activity through several mechanisms.

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Authors Contribution

SNNM, ST, IS, and MK were involved in planning and supervising the work. SNNM, ST and IS performed the measurements, processed the experimental data, performed the analysis, drafted the manuscript, and designed the figures. SNNM performed the calculations and statistical analysis. SNNM, ST, IS, and MK aided in interpreting the results and worked on the manuscript. All authors discussed the results and commented on the manuscript.

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