Investigation of ginger (Zingiber officinale) aqueous extract as an anti-diabetic in vitro

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Abstract.
Diabetes mellitus is a condition of metabolic imbalance, indicated by a high level of blood glucose (hyperglycemia) resulting from a reduction of insulin secretion, action, or both. People with diabetes suffer from a lack or deficiency of insulin or insulin resistance. The metabolic imbalances are often not satisfactorily corrected using conventional medicines and even cause some side effects, which can be detrimental. Research on herbal medicines for the treatment of diabetes is urged by the need to reduce unwanted side effects common with conventional medicines/treatments used in glucose regulation. This study aims to investigate the antidiabetic effect of Ginger (Zingiber officinale) aqueous extract in improving the glucose uptake in mouse tissues in vitro. This study is a true experimental research design with a posttest-only control group design. There were three groups of mice in this study: the control group, which were only given plain water; the second group of mice with 5% aqueous ginger extract and the last group were given 25% aqueous ginger extract. All groups were given treatment for four consecutive weeks, then dissected their cardiac muscle, skeletal muscle, pancreas, and liver tissues to analyze the glucose uptake. The result showed that both the ginger aqueous extract groups were able to increase the glucose uptake of the mice. In conclusion, this research has shown that aqueous ginger extract may have improved the glucose uptake in most tissues of the mice in the groups. Therefore, ginger could have great potential as an alternative way in the treatment of diabetes type 2.

Keywords: ginger, diabetes, DM, antidiabetic, traditional

1. Introduction
Diabetes mellitus is a condition of metabolic imbalance, indicated by a high level of blood glucose (hyperglycemia) resulting from a reduction of insulin secretion, action, or both.[1-3] Generally, diabetes mellitus is divided into two types based on the etiology: Type 1 (T1D) and Type 2 diabetes (T2D). In type 1 diabetes, the pancreas cannot produce insulin as the pancreatic β-cells of the islets are destructed; therefore, regular insulin injections are essential in maintaining blood glucose levels in the body.[1,3] In T2D, insufficient insulin is produced by the pancreas, or the tissues have become insulin resistant and do not adequately maintain glucose homeostasis. The management of T2D includes decreased calorie intake, increased exercises, oral medication, and insulin administration.[3,4] The characteristic of a person with diabetes is hyperglycemic, affecting such complications in the eyes, kidneys, nerves, heart, and blood vessels. In addition, congenital disabilities, foot problems, and finger curvatures have been associated with diabetes.[4-6]
In managing both diabetes type 1 and type 2, it is essential to maintain a healthy lifestyle to avoid risk factors such as obesity, hypertension, and cardiovascular diseases. This can be achieved by controlling food intake and increasing physical activity. In addition, pharmacological measures may be necessary. Exogenous insulin injections and subcutaneous insulin pumps are required in type 1 diabetes. Antidiabetic drugs available to control hyperglycemia include sulphonylureas, biguanides, alpha-glucosidase inhibitors, and thiazolidinediones for DM1. [5,7] However, few studies have shown that these antidiabetic medications may have several complications. [8] Sulphonylureas have been associated with hypoglycemia, beta-cell apoptosis, and damaged epithelial cells in rats.[9,10]

Moreover, the use of metformin has been debatable due to the lactic acidosis effect. Thus, it is not a drug of choice in the treatment regimen of some diabetes patients.[11] An investigation of the adverse effects of long-term use of metformin in patients with chronic kidney disease (CKD) found that there should be dose adjustment of metformin based on the CKD patients' threshold such as serum creatinine or glomerular filtration rate. Furthermore, the use of anti-diabetic medication may eventually lead to CKD. The research found that 39.7% of adult patients with T2D in the United States of America from 1999-2004 developed some degree of CKD. Therefore, advancements in antidiabetic medication are urgently needed.[12-14]

There is an increased interest in the use of traditional medicines for the management of diabetes. Indeed, the traditional understanding of medicines may be the basis of developing newer, safer medicines. Every culture has its indigenous traditional medicines, passed down the generations from ancestors to manage several health issues such as diabetes. Ginger is a rhizome plant that is widely spread in Asia. Based on data from FAO in 2002, Indonesia is a country that produces the third largest ginger after India and China. Ginger, which belongs to the Zingiberaceae family, is a rhizome plant that is very popular as a spice and medicinal ingredient. The rhizome is in the form of fingers that bulge in the middle of the segment. The dominant spicy taste is caused by a ketone compound called zingerone.

Research has suggested that ginger may improve blood sugar in the long-term for Type 2 diabetes. In addition, ginger is rich in gingerols, which is the major active compound of ginger that can increase the uptake of glucose into the muscle cells. Thus mimics the action of insulin, in the maintenance of blood sugar levels. Indeed, researchers have also reported that the active compound extracted from ginger may interact with serotonin receptors and reverse their effect on insulin secretion. Subsequently, ginger aqueous extract was studied for the hypoglycemic ability in diabetic induced rats, which showed that the aqueous ginger extract may have decreased the blood glucose levels up to 35% and about 10% increase of insulin levels found in the plasma. Another research also studied the effect of ginger powder on insulin resistance and glycemic index in patients with type 2 diabetes mellitus, which demonstrated that the use of 3 grams of ginger per day for 8 weeks significantly reduced the glycemic index. However, consumption of 2 grams of ginger daily for 8 weeks was not significant in lowering fasting blood glucose. Interestingly, a small dose of ginger may also delay the onset and progression of cataracts; which is one of the sight-related of long-term diabetic complications, in diabetic-induced mice.[12-14]

2. Materials and Methods
This study is a true experimental research design with a posttest-only control group design. There were three groups of mice in this study: the control group, which were only given plain water; the second group of mice with 5% aqueous ginger extract and the last group were given 25% aqueous ginger extract. All groups were given treatment for four consecutive weeks, then dissected their cardiac muscle, skeletal muscle, pancreas, and liver tissues to analyze the glucose uptake.

2.1. Preparation of Ginger (Zingiber officinale) Aqueous Extract
Ginger aqueous extracts were prepared at room temperature by soaking 100g of ginger powder in 1 litre distilled water for ten days. The aqueous extract was filtered using Whatman no. 1 filter paper. The extract was then centrifuged at 7000 G for 15 minutes to remove finer particles and stored at -20°C.
2.2 Animal experiments and preparation of animal tissues

Twelve adult male Swiss mice that weighed approximately 18-22g were prepared. The mice were kept at a constant temperature of 21°C in a light-controlled environment (light off at 7 pm and on at 7 am every day) and had constant access to standard rodent chow and tap water.

Mice were divided equally into three study groups, which is the control group, the 5% aqueous ginger extract group and the 25% aqueous ginger extract group. All mice were given their respective treatments daily, during the experiment that went for four consecutive weeks. Mice were euthanized by CO2 asphyxiation, and the pancreas, heart, abdominal muscle tissue, and liver tissue were removed without delay and placed in ice-cold phosphate-buffered saline (PBS, pH 7.4). Cardiac muscle, skeletal muscle, pancreas, and liver tissues were dissected into sections of approximately 2mm length, 2mm width, 2mm thickness, and weighed. Tissues were rinsed in PBS immediately before incubation. Only negative control was used in this study, which is sufficient to validate the performance of our experimental set-up and also to set the baseline value of our aqueous ginger extract.

2.3 In vitro bioassay - Tissue culture:

Five pieces of roughly similar tissues were placed in each well of a 48 healthy tissue culture plate. Each well contained 1ml DMEM. DMEM glucose was prepared at 5mM to mimic normal glycemic conditions. Skeletal muscles, cardiac muscles, pancreas and liver tissues were incubated with DMEM. The incubation was conducted at 37°C for 3 hours in a humidified atmosphere with 5% CO2. Immediately following the incubation, the tissue samples were weighed and stored at -20°C until analysis.[15,16]

2.4 Glucose analysis

Tissue culture were thawed to room temperature, and was analyzed for glucose using a DADE clinical analyzer (DADE-XL, USA) according to the manufacturer's instruction.[17,18]

2.5 Data Analysis

Tissue weights were used to determine metabolic uptake of concentrations. The data from tissue culture glucose secretion and uptake were statistically analyzed using a general linear model procedure in SAS statistical software (SAS Institute Inc. Cary, NC, USA), and evaluated by ANOVA One Way followed by the post hoc test Least Significant Difference (LSD). Values obtained were presented at mean ± standard deviation (±STD), and considered significant at p ≤ 0.05.

3. Results

|                      | Cardiac Muscle (mg/dL/g tissue) | Skeletal Muscle (mg/dL/g tissue) | Pancreas Tissue (mg/dL/g tissue) | Liver Tissue (mg/dL/g tissue) |
|----------------------|---------------------------------|---------------------------------|---------------------------------|-----------------------------|
| **Control Negative** | 310.71 (±1.25)                  | 578.76 (±2.28)                  | 88.16 (±0.18)                   | 5064.86 (±0.85)             |
| **5% Aqueous Ginger Extract** | 290.26 (±0.61)                  | 450.67 (±1.24)                  | 75.33 (±0.47)                   | 4990.67 (±0.94)             |
| **25% Aqueous Ginger Extract** | 275.40 (±0.99)                  | 467.13 (±0.65)                  | 71.50 (±0.42)                   | 5000.03 (±0.81)             |
|                      | p=0.000*                        | p=0.000*                        | p=0.000*                        | p=0.000*                    |

**Note:** *95% confidence interval*
As shown in Table 1, most of the tissues showed glucose absorption as indicated by the reduced glucose level in the ginger extract treated tissues compared to the control group. The cardiac muscles tissues demonstrated a progressive decline in the glucose levels in the control group (310.71 mg/dL/g tissue), the 5% aqueous ginger extract (290.26 mg/dL/g tissue) and the 25% aqueous ginger extract (27.40 mg/dL/g tissue). Similar trend was seen in the pancreas tissue, where the 5% aqueous ginger extract and the 25% aqueous ginger extract showed glucose uptake when incubated with DMEM medium, marked by decreased glucose level compared to the control group. Although both skeletal muscle and liver tissue showed slightly higher glucose levels in the 25% aqueous ginger extract groups than in the 5% aqueous ginger extract groups, the glucose uptake of these tissues were not denied as compared to the control group.

Table 2. Multiple Comparisons of the glucose level found in cardiac muscle, skeletal muscle, pancreas, and liver tissue culture

| Treatment                        | Sig(Cardiac muscles) | Sig.(Skeletal muscles) | Sig(Pancreas tissue) | Sig(Liver tissue) |
|----------------------------------|----------------------|------------------------|----------------------|-------------------|
| LSD                              | Control              | 5% Aqueous Ginger Extract | .000* | .000* | .000* | .000* |
|                                  | Control              | 25% Aqueous Ginger Extract | .000* | .000* | .000* | .000* |
| 5% Aqueous Ginger Extract        | Control              |                          | .000* | .000* | .000* | .000* |
|                                  | 25% Aqueous Ginger Extract |                          | .000* | .000* | .000* | .000* |
| 25% Aqueous Ginger Extract       | Control              |                          | .000* | .000* | .000* | .000* |
|                                  | 5% Aqueous Ginger Extract |                          | .000* | .000* | .000* | .000* |

* p < 0.05

In addition, the One-way ANOVA statistical approach showed that there was a significant difference (p=0.000) between treatments of 5% aqueous ginger extract and the 25% aqueous ginger extract (Table 2). The least significance difference (LSD) test also indicated the treatment group was also significantly different from the negative control group (p= 0.000) (Table 2).

4. Discussions

Generally, oral antidiabetics and insulin can manage the level of blood glucose within a normal physiological range. However, it does not stimulate the pancreas to produce more insulin, and therefore, long-term diabetes patients often develop serious complications.[11,14,20] This highlights a need to find safer and more effective treatments.[20,21]

The effects of aqueous extracts of ginger as an antidiabetic are proposed through the mechanism of insulin secretion and action. It is suggested that ginger may have a single or multiple secondary metabolites that could act directly or indirectly in maintaining glucose homeostatic pathways of insulin secretion and action. Findings in other studies in vitro have shown that ginger may have a
stimulatory effect on insulin secretion as well as an insulin-like effect on the uptake of glucose.[22,23,24]

The glucose analysis in the cardiac muscle of the treated mice model compared to the control group (310.71 mg/dL/g tissue) with 5% aqueous ginger treatment (290.26 mg/dL/g tissue) and the 25% aqueous ginger treatment (275.40 mg/dL/g tissue) indicated glucose absorption. The glucose absorption in the cardiac muscle is facilitated by GLUT 1 and GLUT 4 proteins.[1,25] Therefore, there is a hypoglycemic effect contained in the aqueous ginger extract.

Ingested glucose could trigger a response from the liver in order to dispose of the excessive glucose.[25,26] GLUT 2 proteins are found in the liver and mobilized by insulin for the facilitated glucose diffusion. The result from the present study displayed glucose absorption in both 5% and 25% aqueous ginger extract treatments; hence, any changes found were mediated by a non-peptide structure, as this would have been denatured during the autoclaving process. Thus, the 5% ginger extract treatment contains a compound or protein similar to insulin to achieve a hypoglycemic state.[27]

The glucose sensor is located in the islet Langerhans of the pancreas. Indeed, the pancreatic β-cells expressed GLUT 2 proteins as the glucose transporter.[24] Both the 5% and the 25% ginger extract on the mice model showed glucose absorption. Thus, both the 5% and 25% aqueous ginger extracts contained an insulin-like compound.

In resting skeletal muscle, insulin mobilizes GLUT 4 protein for facilitated glucose diffusion.[27-29] It has been suggested that glucose regulation may have occurred at the transcriptional or translational level. Alternatively, cellular uptake of the active compound may require facilitated transport and be subjected to the kinetics of such a transport mechanism. Interestingly, the 5% ginger extract on the mice model showed better glucose absorption than the 25% ginger extract, although not significant. Therefore, the ginger extracts might contain an insulin-like compound that could mobilize GLUT 4 for glucose transportation. Based on these results, treatment using aqueous extracts of ginger might offer an alternative treatment to patients with T2D to maintain glucose homeostasis.[28-30]

5. Conclusions
In conclusion, this study has shown evidence of the hypoglycemic effects of the medicinal plant ginger (Zingiber officinale). It has demonstrated the possible mechanistic pathways, which mimics the act of insulin in triggering glucose uptake that contributed to the potentially antidiabetic effect of aqueous ginger extract. There could be through the phosphatidylinositol 3-kinase (PI3K, a lipid kinase)/AKT pathway or the Raf/Ras/MEK/MAPK (mitogen activated protein kinase) pathway. However, research is still necessary to isolate and identify the active metabolites responsible for the effects of hypoglycemic activity, as well as the exact mechanisms acquired in lowering the level of blood glucose.

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