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Original Research Article

Effect of Ginger (*Zingiber officinale*), Cinnamon (*Cinnamomum verum*), Cloves (*Syzygium aromaticum*), and their Combination on Blood Glucose, Superoxide Dismutase, and Malondialdehyde Levels in Diabetic Rat

Gita S. Prihanti[1], Badzlina[2], Maharani A. Tsani[2], Nesrin Zaharah[2], Irma N.A. Khasanah[2], Noviana D. Lestari[1]

[1]*Medical Education Study Program, Faculty of Medicine, Muhammadiyah Malang University, St. Bendungan Sutami, Malang 65145, East Java, Indonesia*
[2]*Faculty of Medicine, Muhammadiyah Malang University, St. Bendungan Sutami, Malang 65145, East Java, Indonesia*

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ABSTRACT

Diabetes mellitus is characterized by high blood glucose levels known as hyperglycemia. In addition, diabetes is associated with decreased Superoxide Dismutase and increased Malondialdehyde levels. Alternative treatments for diabetes mellitus may involve the use of natural ingredients. This study aimed to evaluate the effect of Ginger (*Zingiber officinale*), Cinnamon (*Cinnamomum verum*), Cloves (*Syzygium aromaticum*), and their combination on blood glucose, superoxide dismutase (SOD) and malondialdehyde (MDA)levels in a rat model of diabetes mellitus. Diabetes was induced in the rats by a single intraperitoneal dose of streptozotocin (55 mg/kg BW). The diabetic rats were divided into five groups; DM which served as the control (Diabetic rats without treatment), A1 (Diabetic rats treated with *Zingiber officinale* extract), A2 (Diabetic rats treated with *Cinnamomum verum* extract), A3 (Diabetic rats treated with *Syzygium aromaticum* extract), and A4 (Diabetic rats treated with a combination of the three spices). The treatments were orally administered once daily for 14 days. On day 15, all the rats were euthanized, and blood samples were collected. The blood samples were analyzed for the concentrations of glucose, superoxide dismutase (SOD), and malondialdehyde (MDA). The results showed that the administration of A3 significantly reduced blood glucose level, the administration of A1 and A4 significantly reduced MDA level compared to the control. Therefore, these spices have the potential to be used as an alternative medicine for the treatment of diabetes mellitus.

Keywords: *Zingiber officinale*, *Cinnamomum verum*, *Syzygium aromaticum*, Blood glucose, Superoxide dismutase, Malondialdehyde.

Introduction

Diabetes Mellitus (DM) is a metabolic disorder characterized by hyperglycemia an elevated blood glucose levels exceeding the normal range,[1] resulting from abnormalities in insulin secretion (due to dysfunction of β cells of islet), insulin resistance, or both.[2]This condition stems from either the inability of the pancreas to produce insulin or the body's inability to utilize insulin.[3]

The International Diabetes Federation (IDF) reported in 2021 that the number of diagnosed diabetes patients worldwide is up to 537 million. This number is projected to increase to 643 million people by 2030 and to 783 million people by 2045.[4] In Indonesia, in 2021, the number of diabetes patients was estimated to be 19,465,100 individuals among the adult population aged 20-79 years. Meanwhile, the total adult population aged 20-79 years is 179,720,500. When calculated based on these numbers, the prevalence of diabetes in the age group of 20-79 years is 10.6%.[5]

*Corresponding author: E-mail: sekar@umm.ac.id
Tel: +62 341 552443

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Several studies have shown that β cell dysfunction is caused by prolonged exposure to hyperglycemia and increased levels of free fatty acids or a combination of both. Prolonged exposure to hyperglycemia or increased free fatty acids can elevate reactive oxygen species (ROS).[1] Under normal conditions, the antioxidant system functions to neutralize exogenous radicals[6].

One of the organs that can experience oxidative damage is the pancreas.[7] The death of pancreatic cells leads to the accumulation of intracellular ROS. This results in an increase in superoxide anion radicals (O_2^-) and damage to the enzyme superoxide dismutase (SOD), which functions to counteract free radicals. A decrease in SOD levels in combating free radicals can lead to an increase in blood O_2^- levels, which can result in complications in the form of microvascular nephropathy and retinopathy, as well as macrovascular complications in the form of atherosclerotic cardiovascular diseases such as coronary heart disease (CHD), cerebrovascular disease, and peripheral artery disease. These are the leading causes of death in diabetic patients.[8] High levels of ROS can also lead to the formation of aldehyde molecules, known as malondialdehyde (MDA), which is the end product of the reaction between ROS and polyunsaturated fatty acids (PUFA). Therefore, MDA is often used as a biomarker of cellular damage in oxidative stress conditions.[9]

The management of diabetes mellitus is still primarily based on pharmacological therapy using standard medications with or without insulin, but it has been reported to have various side effects.[10] Indonesia, being a tropical country, has a rich heritage of traditional medicinal plants that have been used for generations. Based on previous research, Indonesian spices like ginger (*Zingiber officinale*), cinnamon (*Cinnamomum verum*), and cloves (*Syzygium aromaticum*) are rich sources of antioxidants.[11-15] Gingerol, shigeol, and zingerone are major constituents of ginger, and have been found to have anti-hyperglycemic

properties. Cinnamaldehyde, a major constituent of cinnamon has antioxidant and insulinmimetic properties. Cloves contain eugenol, another antioxidant with hypoglycaemic properties.[12-15] Lowering blood glucose levels reduces ROS, thus preventing oxidative stress, and ultimately reduces tissue damage.[12]Therefore, this study aimed to evaluate the effect of some medicinal spices on blood glucose, SOD, and MDA levels in diabetic rats.

Materials and Methods

Preparation of Extracts

Three hundred grams (300 g) each of *Zingiber officinale*, *Cinnamomum verum*, *Syzygium aromaticum* were sliced, dried, and then ground into powdered form. The materials are obtained from the UPT Herbal Materia Medica Laboratory in Batu, East Java Provincial Health Office, with number No. 074/ IIIC/ 1027/ 2018. The powdered plant samples were macerated sequentially with 90% ethanol, 80% ethanol, and methanol at room temperature for 24 h withoccasional stirring. The extracts were filtered, and the marc were re-maceration twice. The combined filtrates were concentrated using a rotary evaporator at reduced pressure to obtain concentrated extracts.

Animal and Experimental Design

Induction of Diabetes

A total of 24 male rats (*Rattus norvegicus*) were used for the study. The rats were fasted for 4 h, after which they were administered a single intraperitoneal dose of streptozotocin (STZ) (Bioworld, USA) at 55 mg/kg body weight to induce diabetes.

The diabetic rats were divided into six groups of four rats each, and were treated as follows:

a. Group DM: Diabetic rats not treated with extracts (control).
b. Group A1: Diabetic rats administered *Zingiber officinale* extract at a dose of 100 mg/kg BW for 14 days
c. Group A2: Diabetic rats administered *Cinnamomum verum* extract at a dose of 100 mg/kg BW for 14 days.
d. Group A3: Diabetic rats administered *Syzygium aromaticum* extract at a dose of 100 mg/kg BW for 14 days.
e. Group A4: Diabetic rats administered combination of the extracts at 100 mg/kg BW for 14 days.

Analysis of Blood Glucose, Superoxide Dismutase (SOD), and Malondialdehyde (MDA) Levels

On day 15, all the rats were sacrificed by chloroform euthanasia. Subsequently, blood samples were collected from the hearts and centrifuged at 4500 rpm for 10 min at 23°C to obtain serum. The serum was analyzed for blood glucose, and SOD using a semi-automatic machine(HORIBA ABX SAS, B.P 7290, France). The MDA levels were measured using spectrophotometric method with TBARS as the reagent. The serum was reacted with trichloroacetic acid (TCA) and thiobarbituric acid (TBA), and the absorbance was measured using a spectrophotometer at a wavelength of 532 nm.

Ethical Approval

The study was approved by the Ethical Commission of Muhammadiyah Malang University with registration number No: E.5.a/228/KEPK-UMM/VII/2018.

Statistical Analysis

Statistical analysis was performed using SPSS version 16 for Windows. The data underwent one-way analysis of variance (ANOVA) followed by Turkey's posthoc test to determine significant differences among the treatments.P value ≤ 0.05 was regarded as significant.

Results and Discussion

A single intraperitoneal dose of streptozotocin was used to induce diabetes in the experimental animals. Rats were confirmed diabetic if their blood glucose were≥200 mg/dL. In this study, the average blood glucose level in the diabetic rats was 489 mg/dL. The diabetic rats were then treated with ginger, cinnamon, and clove extracts separately and in combination. The results indicated that the administration of clove extract at 100 mg/kg (Group A3) significantly reduced blood glucose level compared to the control (DM, the untreated diabetic rats) (Figure 1).

The diabetic rat model showed low SOD level (30.44 U/mg). Administration of the extracts of various spices such as *Zingiber officinale*, *Cinnamomum verum*, *Syzygium aromaticum*, and their combination resulted in no significant difference in the SOD level after 14 days of treatment. However, it was observed that the administration of A3 showed a slight increasein SOD level compared to the control, although not statistically significant (Figure 2). Therefore, it could be assumed that the administration of A3 may increase SOD level to a small extent.

Induction of diabetes significantly increased the level of MDA. The rats After 14 days of treatment with the medicinal spices (*Zingiber officinale*, *Cinnamomum verum*, and *Syzygium aromaticum*) extracts, and their combination, it was observed that treatment with *Cinnamomum verum* extract (A2), and the combined extract (A4) significantly decreased MDA level from594 ng/mL to 509 ng/mL, and from 594 ng/mL to 514 ng/mL, respectively(Figure 3). This suggests that the administered dose of ginger and the combination of the three spices were effective in reducing MDA level to a significantly low value compared to the diabetic untreated rats(DM) after 14-day treatment period.

Diabetes is a multifactorial disease, not solely caused by high blood glucose levels. Oxidative stress is a factor that influences the occurrence of diabetes. Additionally, elevated levels of inflammatory metabolites can affect the severity of diabetes.[3]Oxidative stress has a negative impact on health as it can trigger cell damage, inflammation, and contribute to various diseases.[16] The body's antioxidant system, including the enzyme superoxide dismutase (SOD), plays a crucial role in maintaining a balance between free radicals and antioxidants. Efforts to reduce oxidative stress often involve increasing antioxidant intake through food or supplements.

An increase in oxidative stress can be characterized by elevated levels of malondialdehyde (MDA). High MDA levels in the body are often used as an indicator of oxidative damage. Excessive oxygen reactivity or free radicals can damage cells and tissues. This cellular damage can contribute to various diseases, including heart disease, diabetes, neurodegenerative diseases, and cancer.

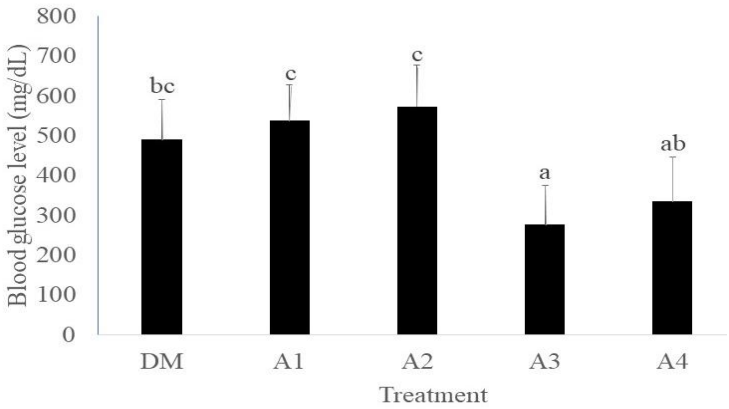

Figure 1: Effect of Extracts on Blood Glucose Level in Diabetic Rats. DM = Diabetes mellitus (Untreated), A1 = *Zingiber officinale*, A2 = *Cinnamomum verum*, A3 = *Syzygium aromaticum*, A4 = Combination of all three (A1, A2, and A3).
Values represent mean ± standard deviation (SD).Values with different lower case letters are statistically different atP-value ≤ 0.05.

This study utilized three natural ingredients, namely *Zingiber officinale*, *Cinnamomum verum*, *Syzygium aromaticum*, and a combination of all

three, for treating diabetes in rats. From the results obtained, the administration of *Syzygium aromaticum* significantly reduces blood glucose levels compared to the control group (untreated diabetic rats). Meanwhile, the administration of *Zingiber officinale* and the combination of all three significantly reduce MDA levels. In the case of SOD, the administration of these natural ingredients did not affect the SOD levels after 14 days of treatment. However, it was observed that the administration of *Syzygium aromaticum* appears to increase SOD levels, although not significant. The increase in SOD levels may be attributed to the active compound in *Syzygium aromaticum*, eugenol.

The ability of eugenol in *Syzygium aromaticum* to reduce blood glucose level in this study is suspected to involve a complex set of interactions affecting insulin production, insulin sensitivity, glucose absorption, and pancreatic cell protection. Eugenol can inhibit the activity of α-glucosidase enzymes involved in breaking down complex carbohydrates into glucose. By inhibiting this enzyme, eugenol slows down glucose absorption in the intestine, which, in turn, reduces glucose spikes.[17]

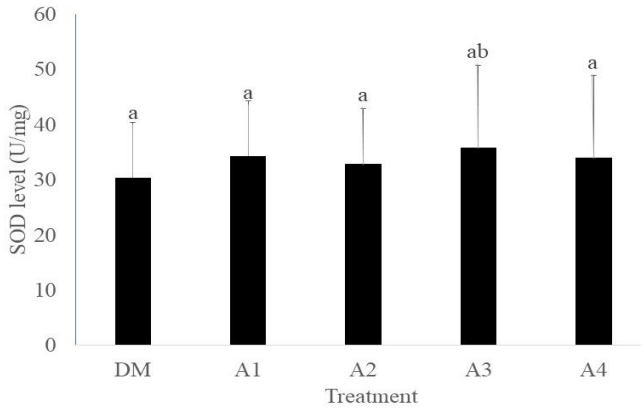

Figure 2: Effect of Extracts on Super Oxide Dismutase (SOD) Level in Diabetic Rats.
DM = Diabetes mellitus (Untreated), A1 = *Zingiber officinale*, A2 = *Cinnamomum verum*, A3 = *Syzygium aromaticum*, A4 = Combination of all three (A1, A2, and A3).
Values represent mean ± standard deviation (SD).Values with different lower case letters are statistically different atP-value ≤ 0.05.

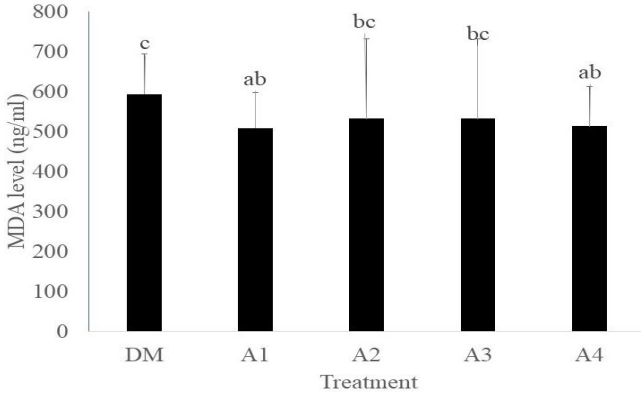

Figure 3: Effect of Extracts on Malondialdehyde (MDA) Level in Diabetic Rats.
DM = Diabetes mellitus (Untreated), A1 = *Zingiber officinale*, A2 = *Cinnamomum verum*, A3 = *Syzygium aromaticum*, A4 = Combination of all three (A1, A2, and A3).
Values represent mean ± standard deviation (SD).Values with different lower case letters are statistically different atP-value ≤ 0.05

Previous research suggests that eugenol is a strong antioxidant compound that may be employed in the treatment of metabolic diseases caused by oxidative stress. Pretreatment with clove essential oil has been shown to reduce oxidative stress as assessed by reduced malondialdehyde and increased SOD levels in rats.[18] Several factors may have influenced the results of this study, at small doses or concentrations, theactive compounds in the spices are present in small amounts and do not interfere with the expected results. However, at higher concentrations, the effects of these active compounds become more pronounced, potentially affecting and disrupting the expected responses.[19] Therefore, at higher concentrations, increased antioxidant responses may not necessarily be better, and blood glucose levels, which are influenced by antioxidants in this case, may also be disrupted.[19]

Additionally, there are other endogenous factors in rats that cannot be controlled, including genetics, hormones, stress conditions, varying immunity, and others. When rats are stressed, they tend to feel anxious, and their digestive system is affected, leading to excessive food and drink consumption. Rats also become hyperactive, attacking their surroundings, and more. If rat stress conditions persist, they may become depressed, with symptoms including lethargy, reduced food and drink intake, and indifference to their environment. This can be attributed to a weakened immune system in rats, making them susceptible to diseases and increasing the risk of mortality.[20]

Conclusion

Spices like ginger (*Zingiber officinale*), cinnamon (*Cinnamomum verum*), and cloves (*Syzygium aromaticum*) have a potential role as an anti-diabetic agent by controlling blood glucose, SOD, and MDA levels. Administration of clove decreased blood glucose level, whileginger and the combination of all three spices (ginger, cinnamon, and clove) significantly decreased the level of MDA. It is envisioned that the findings from this study have the prospectbeing translated into practical applications, such as the development of spice-based supplements or dietary recommendations to diabetic patientsthat will help in managing their condition more effectively.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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