HYPOGLYCEMIC EFFECTIVENESS TEST OF GREEN TEA LEAF ETHANOL EXTRACT INDUCED BY DEXAMETHASONE

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ABSTRACT

Diabetes mellitus (DM) is a chronic metabolic disease caused by abnormal insulin secretion characterized by increased blood glucose levels. Long-term use of drugs also causes side effects. Alternative therapies are needed with potential and mechanisms of action that are close to the body's physiological processes in controlling blood glucose so as to help reduce the development and complications of diabetes mellitus, and can be obtained at an affordable price. This study aims to test the effectiveness of green tea leaf extract in lowering blood sugar levels in male mice induced with dexamethasone. The type of research used is an experimental method with a Pretest-Posttest Control Group Design. This study tested the hypoglycemic effect of ethanol extract of green tea leaves on mice induced with dexamethasone 2.5 mg/kgBW of mice orally. The study began with the collection of green tea leaf samples, the manufacture of simplicia, and testing the hypoglycemic effect for 15 days of research. Ethanol extract of green tea leaves has a hypoglycemic effect on white mice induced by dexamethasone. EEDTH dose of 1.25 g/KgBW in rats showed better hypoglycemic potential compared to glibenclamide in a rat model induced by dexamethasone.

Keywords*: Diabetes melitus; Camellia sinensis* L.*; maceration; in-vivo; mice*

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease caused by abnormal insulin secretion characterized by increased blood glucose levels (Anggraini & Kusuma, 2019). Continuous hyperglycemia will produce excessive free radicals that play a role in diabetes complications (Husfa et al., 2020). Diabetes mellitus is the third leading cause of death in people aged 30-70 years after cancer and cardiovascular disease (Zanaria et al., 2019). The prevalence of diabetes for all age groups worldwide is estimated at 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to increase from 171 million in 2000 to 366 million in 2030 (Wild et al., 2004). The prevalence of DM in Indonesia in 2023 reached 1.7%, while in North Sumatra itself it was 1.4%. Type 2 DM is the type of DM with the highest prevalence (50.2%) compared to other types of DM such as type 1 DM (16.9%) and gestational (2.6%). The prevalence of type II DM in North Sumatra itself

reached 59.6%. Diabetes mellitus is one of the non-communicable diseases included in the Sustainable Development Goals (SDG's), Renstra Kemenkes 2020-2024, SPM in the health sector, health transformation and Germas (Republic of Indonesia, 2023).

Long-term diabetes treatment costs a lot of money. The use of conventional drugs for a long period of time also causes adverse effects. Based on this explanation, alternative therapies are needed with potential and mechanisms of action that are close to the body's physiological processes in controlling blood glucose so that they help reduce the onset of progression and complications of diabetes mellitus, and can be obtained at affordable prices (Zanaria et al., 2019). Many types of plants contain active compounds that can inhibit carbohydrate hydrolysis and glucose absorption, regenerate cells so as to increase insulin release, inhibit aldose reductase and control glucose levels. Synthetic and natural antioxidant compounds from various plants can control glucose levels and inhibit diabetes complications. Active polyphenol compounds in plants have antioxidant and hypoglycemic activities (Padua et al., 2022). One of
the medicinal plants that is widely used in lowering blood glucose levels in the community is green tea leaves.

Tea plants (Camellia sinensis) are perennial plants that are widely distributed in Southeast Asia, India, South China, Northwest Laos, Thailand, and Burma. *Camellia sinensis* is reported to contain about 4000 bioactive compounds, one third of which are polyphenols. Polyphenols are secondary metabolites of plants and generally function in protection from ultraviolet radiation or attacks from pathogens. In the last decade, there has been much interest in the potential health benefits of plants that contain polyphenols as antioxidants. Consumption of a polyphenol-rich plant diet provides protection from cancer, cardiovascular disease, diabetes, osteoporosis and neurodegenerative diseases (Husfa et al., 2020). Green tea from Sidamanik is known to help reduce the risk of obesity due to the presence of bioactive compounds such as flavonoids, tannins, catechins, and theaflavins (Zalukhu and Lubis, 2020; Josephine, Retnaningsih and Hendryanti, 2022). Green tea from Sidamanik Tea Plantation is positive for phenol, flavonoid, tannin, alkaloid, saponin, steroid/terpenoid and glycoside compounds. With IC50 results for wet tea of 31.99 ppm and dry tea of 10.31 ppm, it proves that dry tea has higher bioactivity than wet tea (Dian Pratiwi and Nasyrah Qolby, 2024). Previous studies have shown that administration of black tea and

green tea extracts can reduce glucose levels in mice induced by streptozotocin (STZ) (Tang et al., 2013). In vitro, green tea extract (IC50 44.79 µg/mL) has a greater inhibitory potential for the alpha glucosidase enzyme compared to black tea (IC50 54.86 µg/mL) (Holidah et al., 2018).

Regarding the characteristics of this animal model, high blood sugar levels can be caused by several pharmaceutical preparations, one of which is dexamethasone. Dexamethasone is one example of a group of synthetic glucocorticoids, which are commonly used to treat various inflammatory diseases, bronchial asthma, eczema, urticaria and various other allergic diseases. Chronic use of dexamethasone due to lack of public knowledge of glucocorticoid preparations can trigger DM in the future. Previous studies have shown that there was an increase in blood glucose levels in the dexamethasone-induced group, a decrease in the insulin sensitivity index and a decrease in body weight of mice (Hanim et al., 2018). Based on this explanation, this
study aims to test the effectiveness of green tea leaf extract in reducing blood glucose levels in male mice induced with dexamethasone.

METHOD

Types and Design of Research

The type of research used is experimental methods. The research design used is The Pretest-Posttest Control Group Design (Ratminingsih, 2010). This study tested the hypoglycemic effect of ethanol extract of green tea leaves on mice (Mus musculus) induced with dexamethasone 2.5 mg/kgBW of mice orally. The study began with the collection of green tea leaf samples, the manufacture of simplicia, and testing the hypoglycemic effect for 15 days of research. This research has obtained Research Ethics approval from the Health Polytechnic of the Ministry of Health of Medan with the number: 01.0796/KEPK/POLTEKKES KEMENKES MEDAN 2018.

Research Location

Green tea leaf samples were obtained from the Sidamanik area, Pematang Siantar. The location of the extract manufacture and testing of hypoglycemic effectiveness were carried out at the Phytochemistry Laboratory and Pharmacology Laboratory, Department of Pharmacy, Poltekes Kemenkes Medan, Jl. Airlangga No. 20 Medan.

Sampling Method

Green tea leaf samples were taken using purposive sampling. The samples tested in this study were young and fresh tea leaves obtained from Sidamanik, Pematang Siantar. Meanwhile, the grouping of test animals was carried out using random sampling.

Tools and materials

The tools used in this research were beakers, measuring cups, glucometers and Easy Touch® test strips, flannel cloth, stirring rods, measuring flasks, mortar and stamper, electric balance, 1 ml oral needle, 1 ml syringe, thermometer, animal scales. , water bath. The materials used in this study were ethanol extract of green tea leaves (Camellia sinensis L.), mice, dexamethasone, 70% alcohol, CMC Na, distilled water, Glibenclamide.

Extract Preparation

Young green tea leaves that have been collected, cleaned, drained and dried until dry and not exposed to direct sunlight which is indicated by being brittle when broken, then weighed. A total of 300 grams of green tea powder is put into a container plus 3 liters of 70% ethanol, the tea leaf powder is soaked for 6 hours while stirring occasionally then let stand for 18 hours,

then filtered and the process is repeated with the same type and amount of solvent as much as

1.5 L. Strain and squeeze collect the filtrate obtained, settle for two days and take the filtrate. Evaporate the ethanol at low temperature with a rotary evaporator until a thick extract with a constant weight is obtained.

Testing the Hypoglycemic Effect of Green Tea Leaf Water Extract (EEDTH) Preparation of test animals

The test animals used were healthy male mice (*Mus musculus*) weighing 20-30 g, aged 2-3 months. The number of mice used in the study was 24 mice. Before being used, the test animals were acclimatized for 7 days under the conditions of the Pharmacology laboratory, Department of Pharmacy, Poltekkes Kemenkes Medan. The mice were placed in a cage with a temperature of 25±2oC, with lighting for 12 hours and without lighting for 12 hours. The test animals also received sufficient food and drink. After 7 days, healthy mice were selected, marked by stable or increasing body weight and did not show strange behavior.

Grouping of Test Animals

The test animals in this study were divided into 4 groups, consisting of an induction group (given a CMC Na 1% suspension carrier), a comparison (glibenclamide 0.0065%), Green tea leaf ethanol extract (EEDTH) at dose of 1.25 g/KgBW and 2.5 g/KgBW of mice.

Procedure

The hypoglycemic effectiveness test of EEDTH was carried out for 15 days using a preventive method, namely administering the induction preparation together with the test extract preparation. Blood glucose levels were measured at T0, T5, T10, and T15 days of the study. Before the experiment, all mice were fasted for 8 hours (not fed, but still given standard drinks) to measure the initial blood glucose levels (T0). The blood glucose level of mice (T0) which was in the normal range would then be given induction treatment with dexamethasone 2.5 mg/kg BW orally according to the treatment of each group. Fasting blood glucose level measurements were then carried out every 5 days, namely at T5, T10, and T15 of the research day.

Data Analysis

The results of the blood glucose level measurements were then summarized using Ms. Excel and analyzed statistically using ANOVA (Analysis of Variance) at a 95% confidence level ($\alpha = 0.05$) with the help of the SPSS program.

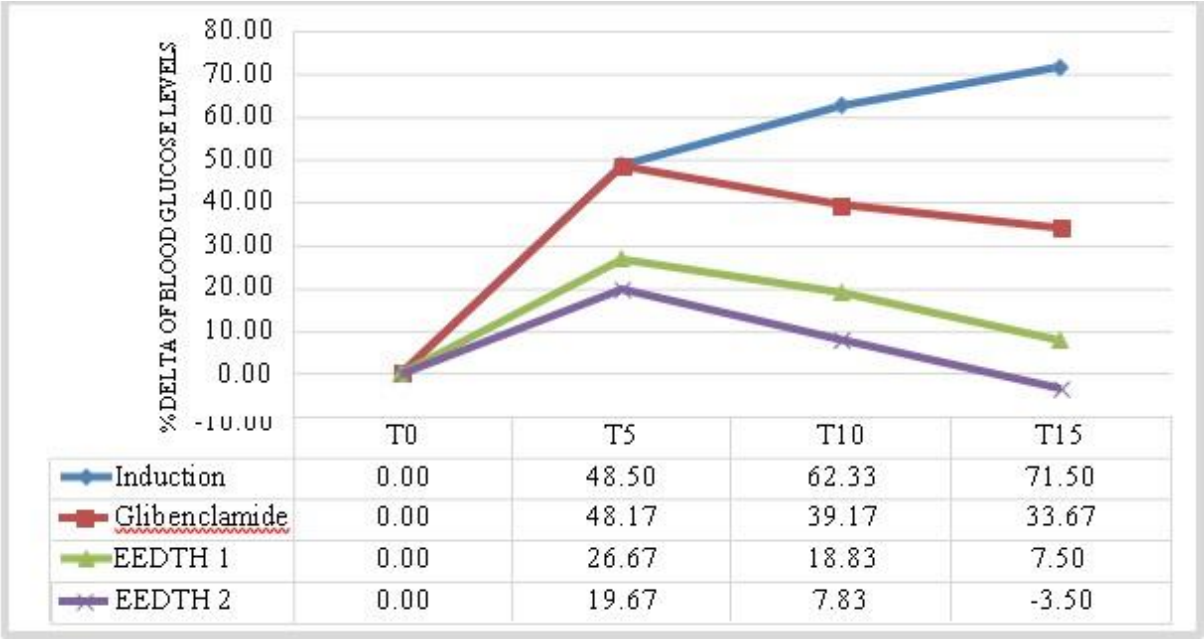
RESULTS AND DISCUSSION

Green tea leaf extraction was carried out using 70% ethanol solvent with the maceration method. Based on the extraction results, the extract yield was 13.16%.

Table 1. Results of measuring blood glucose levels of test animals

Group	T0 ± SD	T5 ± SD	T10 ± SD	T15 ± SD
Induction	87.67 ± 9.95	136.17 ± 8.47	150 ± 10.79	159.17 ± 7.39
Glibenclamide	85.67 ± 9.85	133.83 ± 3.92	124.83 ± 3.76	119.33 ± 2.73
EEDTH 1	85.33 ± 8.14	112 ± 5.22	104.17 ± 5.98	92.83 ± 10.94
EEDTH 2	88.83 ± 6.91	108.5 ± 4.76	96.67 ± 3.08	85.33 ± 3.56

All test animals at T0 measurement showed blood glucose levels within the normal range of blood glucose levels in mice. These results indicate that the test animals used were in good health and met the researcher's criteria. Measurements at T5 to T10 showed an increase in blood glucose levels in all test groups, while at T15, the EEDTH 1 and EEDTH 2 groups showed changes in blood glucose levels towards normal levels in mice (Table 1). This study is in line with previous studies which showed that administering ethanol extract of tea leaves can significantly reduce blood glucose levels in white mice (Hafshah & Simanjuntak, 2020; Katno, Dian Anistyani, 2008).



	T0	T5	T10	T15
Induction	0.00	48.50	62.33	71.50
Glibenclamide	0.00	48.17	39.17	33.67
EEDTH 1	0.00	26.67	18.83	7.50
EEDTH 2	0.00	19.67	7.83	-3.50

Picture 1. Percentage of delta blood sugar levels

Based on the Picture 1. shows that the induction group showed the highest increase in blood glucose levels compared to other test groups from T5-T15 of the study. This shows that the inducer used, namely dexamethasone suspension 2,5g/kgBW of mice, was able to increase blood glucose levels of mice. Glucocorticoids are widely used as anti-inflammatory and immunosuppressive agents. Excess endogenous and exogenous glucocorticoids impair insulin sensitivity, contributing to the development of metabolic syndrome, including insulin resistance, obesity, and hypertension. The incidence of this syndrome is closely related to increased mortality from cardiovascular disease (Qi et al., 2004). However, long-term use of glucocorticoids can cause undesirable metabolic complications, including the development of type 2 diabetes mellitus. This effect occurs due to the activation of glucocorticoid receptors in several tissues, resulting in interorgan interactions that increase hepatic glucose production and inhibit peripheral glucose uptake (Li & Cummins, 2022). Research on the diabetogenic effects of dexamethasone has been widely presented by previous studies. Islet cells from rodents showed decreased insulin secretion in response to various glucocorticoid compounds, both acute and chronic exposure (Jeong et al., 2001). In addition to glucocorticoids contributing to hyperglycemia and insulin resistance, there is an additional role for glucocorticoids in directly damaging pancreatic beta cells (Rojas et al., 2015).

Diabetes mellitus occurs when pancreatic beta cells experience disruption in the production of insulin which is known as a hormone that regulates blood glucose levels. As a result of decreased insulin production, glucose does not enter the cells but remains in the blood. Increased blood glucose levels signal to increase fluid intake in an effort to remove glucose from the body through urine. Other symptoms include increased thirst (polydipsia) and increased urination (polyuria). Cells become deprived of energy due to lack of glucose and signal the patient to eat, causing the patient to experience increased hunger (polyphagia). There are three types of diabetes mellitus. These are Type I, known as insulin-dependent (IDDM), where beta cells are destroyed by an autoimmune process; Type II, known as non-insulin- dependent (NIDDM), where beta cells produce insufficient insulin; and gestational diabetes mellitus (DM that occurs during pregnancy) (N. Azizah et al., 2022; Khotimah; et al., 2022).

The results of the normality (Shapiro Wilk) and homogeneity test of the percentage of changes in blood glucose levels using SPSS showed that the data were normally distributed and homogeneous ($p>0.05$). These results indicate that the data can be further tested using
parametric statistical analysis One Way ANOVA with Tukey's post hoc test. The percentage increase in blood glucose levels of mice in the extract group (T5) showed the lowest increase compared to exctract groups significantly ($p=0,001$). While the Glibenclamide group has begun to show its hypoglycemic effectiveness at T10 significantly ($p=0.001$) compared to induction group. The percentage change in blood glucose levels in the extract group showed that EEDTH 1 showed the same hypoglycemic potential as EEDTH 2 ($p=0,075$) at T10 and at T15 ($p=0,238$) and was superior to the glibenclamide group significantly at T10 and T15 ($p=0,001$). These results are in line with previous research which stated that regular use of green tea showed a significant decrease in glucose levels, fasting glucose levels and HbA1c levels (F. N. Azizah & Fitriani, 2022; Hidayah et al., 2023).

Polyphenols found in Camellia sinensis are catechins. Catechins in Camellia sinensis have the ability 100 times more effective than vitamin C and 25 times more effective than vitamin E in neutralizing free radicals. The types of catechins found in Camellia sinensis are epicathechin (EC), epicathechin gallate (ECG), epigallocathecin (EGC) and

epigallocathechin-3- gallate (EGCG). Catechins act as antioxidants and work against pro-oxidants by capturing Reactive Oxygen Species (ROS) such as singlet oxygen, superoxide radicals, hydroxyl radicals, nitrogen oxides, which are unstable oxygen compounds with unpaired electrons and easily react in tissues, thereby reducing damage to proteins, lipid membranes and nucleic acids. In addition to acting as antioxidants, catechins also act as antihyperglycemics, especially EGCG which has an effect similar to the work of insulin. Its role is to inhibit hepatic glucose production, control gluconeogenesis and regulate the gene expression process in insulin signal transduction and glucose uptake (Husfa et al., 2020).

CONCLUSION

Ethanol extract of green tea leaves has a hypoglycemic effect on white mice induced by dexamethasone. EEDTH dose of 1.25g/KgBW of mice showed better hypoglycemic potential compared to glibenclamide in the mouse model induced by dexamethasone. This result describe that the extract ethanol of green tea leaves potential as agent antidiabetic type II.

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