LITERATURE REVIEW: GREEN TEA EXTRACT POTENCY (*Camellia sinensis)* in LOWERING GLYCEMIC INDEX OF A WISTAR RAT

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ABSTRACT

The aim of the research is to determine the potency of green tea extract (*Camellia sinesis*) to decrease blood glucose levels in diabetes mellitus model Wistar rats. A systematic review based on PRISMA-P 2020 with a literature search strategy using Google Scholar, PubMed, Science Direct, and Scopus databases. Literature quality was assessed using the JBI Critical Appraisal Checklist which produced 12 comprehensive potential literatures based on selection with inclusion and exclusion criteria. Analysis of 12 journals found that there were 11 studies that showed a significant decrease in blood glucose levels after administration of green tea extract (*Camellia sinensis*). Green tea extract (*Camellia sinensis*) has the potential to reduce blood glucose levels in Wistar strain rats with diabetes mellitus model. The mechanism underlying the antihyperglycemic effect is proven to be due to the protective and antidiabetic effects of the compounds contained in green tea extract, especially the flavonoid group, namely catechins. The minimum dose of green tea extract observed to cause an antihyperglycemic effect in experimental animals was 40 mg/kgBW.

Keywords: Camellia sinensis; Diabetes mellitus; Wistar rat

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INTRODUCTION

Diabetes mellitus was ranked the 9th leading cause of death in the world in 2019[(1)]. Based on the 2019 Basic Health Research Results (RISKESDAS) report by the Ministry of Health, the prevalence of DM in Indonesia is 6.2% of the population. DM patients in Indonesia are expected to increase from 10.7 million in 2019 to 16.7 million in 2045, this makes Indonesia in seventh position on the planet in 2019 and eighth position on the planet in 2045 [(2)].

DM therapy currently used can be in the form of drug therapy and non-drug therapy [(3)]. DM therapy is a persistent and rooted treatment that requires relatively expensive costs and becomes an economic burden if carried out in the long term for sufferers. These social and economic factors often cause the patient to fail in undergoing therapy, causing various side effects. Therefore, it is necessary to think about alternative healing that is effective, inexpensive, and easy to obtain. As many as 69.6% of the Indonesian population utilize traditional medicine in overcoming a disease because it is affordable and accepted to have slightly lower side effects [(4)].

In recent years, secondary metabolites in plants have attracted the attention of experts to be used as a source of medicinal agents. Continuous research and testing are carried out in various countries on various spices that are commonly used to treat certain diseases [(5)]. A number of studies have shown that the combination of the use of traditional herbal

medicines with chemical drugs has a good antihyperglycemic effect in experimental DM rats. The study utilized green tea extract (*Camellia sinensis*) in DM rats and suggested that green tea might potentially reduce blood glucose levels.

Indonesia as an agricultural country is an example of a country where the largest tea provider is able to contribute to the growing ability to drink tea for DM therapy [(6)]. Tea is a drink commonly consumed daily in various parts of the world [(7)]. Several pharmacological studies show that green tea can function as a sedative, cell strengthening, antimutagenic, and prevent cancer-causing, and can prevent heart defects. Epidemiological investigations indicate that regular consumption of tea can reduce blood glucose levels. The substance of the synthetic mixture contained in green tea is very complex. It contains alkaloids, saponins, tannins, proteins, amino acids, polyphenols, and flavonoids [(8)].

Based on the description above, it can be seen that the extract may be able to suppress blood sugar levels by various mechanisms. This study was conducted to explore the potential of green tea extract in suppressing blood sugar levels in Wistar rats through the Systematic Literature Review methodology by utilizing existing research data so as to provide more recent, valid, and comprehensive facts.

MATERIALS AND METHODS

a. Materials

The research design used in this study is the Systematic Literature Review or SLR. Data collection was carried out according to the PRISMA 2020 (Preferred Reporting Items for Systematic Reviews and Meta-analysis 2020) method. The literature search begins by conducting an initial search on databases in the form of Google Scholar, Scopus, Science Direct, and PubMed. The keywords used were "green tea extract" OR "green tea extract" OR "Camellia sinensis" AND "diabetes" OR "diabetic" AND "Wistar rats" OR "Wistar rats" OR "Rattus norvegicus Wistar strain".

b. Method

Search keywords must be in accordance with the research question and pay attention to synonyms or alternative words based on the Population, Intervention, Comparison, and Outcome (PICO) method. The PICO used were P: Wistar rats with diabetes mellitus model: I: Green tea extract (Camellia sinensis), C: There is no comparison in this study; O: Decreased blood glucose levels.

The literature was then selected based on inclusion and exclusion criteria so as to obtain a comprehensive potential literature of 12 literatures. The literature that has met the inclusion and exclusion criteria is then assessed for quality using The Joanna Briggs Institute (JBI) Critical Appraisal Tools in accordance with the design of a quasi-experimental study. Data extraction and data synthesis were carried out afterward.

RESULT & DISCUSSION

Characteristics of different samples at the beginning of the experiment can affect the internal validity of a study. Differences in sample characteristics can lead to outcomes or effects that are not caused by the intervention given. Therefore, it is necessary to assess the characteristics of the sample to avoid selection bias in the study. The results of the assessment of the literature used show the characteristics of similar experimental animals with the total number of samples in 12 literature as many as 414 samples.

The largest number of samples was 56 samples in the study of Oudah et al., (2021), these samples were male Wistar rats which would later be induced by the chemical streptozotocin DM. While the minimum sample is 15 samples in the research of Peristiowati et al., (2015). The entire literature uses Wistar rats as research samples. The rat strain is a strain that is sensitive to the chemical induction of alloxan and streptozotocin. Wistar rats are susceptible to chemical induction of streptozotocin and alloxan due to the weakening of their antioxidant capacity and do not have strong

antioxidant properties to protect pancreatic -cells as found in humans [9].

The research analyzed did not show any difference in the treatment given outside the intervention in each group of the research sample. This is indicated by the similarity of the type of diet given, the similarity of the environment, and the similarity of other conditions determined in each study. The aim is to ensure that there are no different exposures given at the same time as the intervention in one or a group of samples that could affect the experimental results.

The literature studied used two DM-inducing chemicals, namely streptozotocin and alloxan. Pancreatic cells can be specifically ablated using streptozotocin or alloxan, chemicals that structurally mimic glucose and are selectively imported into cells. Depending on the dose of the drug, the entire mass of cells may be partially or almost completely ablated within a few days [10]. These chemicals are the most widely used substances as diabetogenic agents in DM research and are capable of causing damage to cells of the islets of Langerhans although their cytotoxicity is achieved through different pathways [9]. However, currently, the use of streptozotocin is more effective because it is more selective against cells of the islets of Langerhans than alloxan which can cause severe damage to other cells that express GLUT 2 [11].

The entire literature uses male rats in the study. This is in accordance with the selection of the DM rat model which is more often found in male rats because they are more susceptible to DM than females due to the estrogen hormone which can affect insulin sensitivity and secretion [12].

There are various types of solubles used in the green tea extraction process in several examinations. Various solvents will affect the natural action of the plant release. Different techniques that can be used in green tea extraction can also affect the antidiabetic mechanism due to differences in the composition of the extracts [13]. The 12 literatures studied used four types of green tea extract, namely aqueous extract, ethanol extract, methanol extract, and ethyl acetate extract. The dose and duration of administration used in each literature varies in different ranges.

A total of 6 kinds of literature using water extract of green tea use doses in the range between 50-15000 mg/KgBW/day given orally. While the other three literatures made by Abolfathi et al., (2012), Atia et al. (2020) and Fiorino et al., (2012) used concentrations in the range of 1.5% to 7% and were proven to basically lower blood glucose levels. In 4 literature using green tea ethanol extract, the dose used was in the range between 20-800 mg/kgBW. Research by Shokri et al. (2015) is the only study that uses methanol as a solvent in the green tea extraction process at doses of 50, 100, and 200 mg/kgBW. Research Biswas et al. (2017) used the ethyl acetate fraction in green tea methanol extract at a dose of 100 mg/kgBW for 28 days.

Green tea extract has the potential to be a supportive therapy in addition to the definitive therapy that has been given. The potential hypoglycemic effect of green tea extract is dose-dependent [14].

The results of a systematic review generally show that green tea extract (Camellia sinensis) can significantly reduce blood glucose levels or act as antihyperglycemic in Wistar rats. Research by Abolfathi et al. (2012) stated that the administration of green tea extract was more stable than the use of a single compound (pure epigallocatechin gallate), because the complex mixture of various compounds in green tea extract worked synergistically as herbal medicine to provide beneficial effects.

Hafshah's research (2020) shows the phytochemical test results of qualitative green tea extracts that there are alkaloid compounds, saponins, tannins, phenolics, flavonoids, terpenoids, steroids, and glycosides in 10 grams of green tea extract. Catechins are the most dominant polyphenolic compounds in green tea extract. One of the catechin derivatives, namely Epigallocatechin gallate (EGCG) is a compound with the highest concentration (48 to 55%) which has great potential as antihyperglycemic through

various mechanisms [15]. The research of Sundarman et al. (2013) stated that there are 2 mechanisms underlying the hypoglycemic effect of green tea extract, namely by reducing blood glucose levels by increasing glucose uptake into tissues or by stimulating pancreatic beta cells to release more insulin.

The first mechanism underlies the antihyperglycemic ability of green tea extract, namely through inhibition of pancreatic -amylase and -glucosidase enzymes in the small intestine, thereby suppressing glucose absorption in the small intestine. This enzyme plays a role in the breakdown of carbohydrates into glucose in the digestive tract. Processing of sugar means making simpler compounds, to be specific as monosaccharides with the aim that it tends to be consumed by the veins through the mucosa of the small digestive tract. Restriction of pancreatic -amylase compounds cause the hydrolysis cycle of polysaccharides to become disaccharides inhibited and inhibition of -glucosidase causes the hydrolysis of -1,4 glucosyl oligosaccharide units does not occur so that the absorption of monosaccharides in the small intestine is reduced [16]. The inhibition effect of the two enzymes is caused by OH group in flavonoid substances that form hydrogen bonds with specific amino acids at the enzyme active site [17].

A journal made by Hafshah (2020) describes polyphenols and triterpenoids intensifying assumes a role in further developing insulin emission by restoring pancreatic beta cells, inhibiting pancreatic beta cell phosphodiesterase, and expanding Ca2+ uptake in pancreatic beta cells which invigorates insulin exocytosis. Research Atia et al. (2020) stated that green tea extract can block ATP K+ channels which causes membrane depolarization and opens Ca2+ channels. This will cause an increase in intracellular concentrations and then stimulate the release of insulin from pancreatic beta cells.

The research of Ladeira et al. (2021) and Hafshah (2020) mention green tea extract as an antidiabetic agent capable of influencing many signaling pathways. EGCG can increase tyrosine phosphorylation activity at insulin receptors, Insulin Receptor Substrate-1 (IRS-1), Phosphoinositide 3-Kinase (PI3K), Mitogen-Activated Protein Kinase (MAPK), and decrease the expression of the Phosphoenolpyruvate Carboxykinase (PEPCK) gene. EGCG has been shown to inhibit glucose production through gluconeogenesis and glycogenolysis mechanisms in cells by activating AMPK. Adenosine Monophosphate Protein Kinase (AMPK) is a protein kinase that plays an important role in cellular energy status and systemic energy balance. AMPK can be activated through several pathways and acts as the main regulator in cellular metabolism which is activated when cellular energy is depleted and has a key role in controlling metabolism [18].

When energy availability decreases, a decrease in cellular ATP concentration and an increase in AMP trigger changes in the AMPK complex. AMPK then promotes ATP production by activating catabolic pathways and inhibiting synthetic pathways within cells. In liver cells, AMPK blocks the synthesis of fatty acids and triglycerides and inhibits gluconeogenesis by blocking cAMP activation. AMPK is also involved in the regulation of insulin secretion by pancreatic beta cells and is able to increase the sensitivity of cells to insulin.

EGCG has been shown to inhibit PI3K and mTOR by binding to ATP-binding proteins. Protein kinase B (AKT) is the main protein affected by the effects of catechins. EGCG can activate the diacylglycerol kinase (DGK) pathway, thereby inhibiting the activation of protein kinase C beta (PKC-β) and improving diabetic conditions. The process is initiated by the interaction of EGCG with the 67-kDa laminin receptor (67LR), which is an EGCG receptor and is also capable of activating AKT. EGCG activates DGK-α via 67LR binding. Other catechin compounds found in green tea have an effect through the activation of the AKT pathway with different receptors if they cannot bind to the 67LR receptor. However,

the membrane receptors for other compounds are still unknown.

In the metabolic signaling pathway activation of serine/threonine kinase AKT/PKB is regulated by an insulin signaling cascade with activation of phosphatidylinositol-3-kinase. The insulin signal that activates AKT promotes the translocation of GLUT4-containing vesicles to the cell membrane, thereby increasing glucose uptake into muscle. Glucose Transporter 4 (GLUT4) is a specific protein that facilitates insulin-sensitive glucose transport.

In the journals made by Abolfathi et al., (2012), Atia et al., (2020), Biswas et al., (2017), Fiorino et al., (2012), Hafshah, (2020), and Peristiowati et al. al., (2015) stated that the antidiabetic effect of green tea extract is related to its antioxidant properties, which can counteract the toxic and pro-oxidant effects caused by the induction of streptozotocin or alloxan chemicals in Wistar strain rats, thereby preventing damage to the pancreas and decreasing insulin secretion. Damage to the structure of the pancreas is also associated with glucotoxicity due to the excessive uptake of glucose into pancreatic cells in hyperglycemic conditions. Increased glucose levels in the islets of Langerhans will encourage glycation reactions and mitochondrial electron transport chains that produce ROS and damage macromolecules beyond the limit of antioxidant capacity. The resulting oxidative stress will interfere with insulin secretion and synthesis and initiate a cellular cascade process that ultimately causes cytotoxicity and pancreatic cell death. The increase in antioxidant activity by green tea extract was obtained by inhibiting the process of oxidative stress by increasing antioxidant enzymes, namely glutathione s-transferase, glutathione peroxidase, glutathione reductase, superoxide dismutase, and catalase.

Research conducted by Biswas et al. (2017) showed that the ethyl acetate fraction of green tea leaf methanol extract resulted in a significant recovery in antioxidant enzyme activity in diabetic rats. The protective mechanism is due to an increase in antioxidant enzymes such as SOD and catalase. The increase in antioxidant enzymes was able to protect the damage to pancreatic cells mediated by ROS. In several journals studied also added antioxidants play an important role in inhibiting and neutralizing ROS. In research, Abolfathi et al. (2012) said that daily consumption of green tea extract on a regular basis can improve the antioxidant status of liver tissue in streptozotocin-induced diabetic rats.

EGCG can also protect pancreatic islet masses through Nitrite Oxide Synthase (NOS) guidance and inhibit the initiation of NF-κB (DNA coding protein). Activation of NFkB by ROS produces an inflammatory and autoimmune response in the pancreas. The anti-inflammatory mechanism is obtained through a decrease in TNF-α levels as a result of the inhibition of NFkB. NFkB becomes dynamic due to an increase in specialist ROS causing endothelial damage, microbial exposure, DNA damage, and actual stress. The ability of NFkB to control articulation quality that encodes proinflammatory cytokines and chemokines such as TNF-, IL1β, IL-6, and different proteins. The active compounds in green tea extract are able to inhibit the inflammatory process in the islets of Langerhans, as NFkB inhibitor which is important as inflammation and immunology main regulator in alloxan or streptozotocin-induced DM [(19)].

Another mechanism that underlies the effect of green tea extract in overcoming hyperglycemia is a decrease in cellular inflammation characterized by reduced inflammatory biomarkers. Glucolipotoxicity in DM induces inflammation through the activation of pro-inflammatory signaling pathways such as factor NFkB which stimulates various pro-inflammatory biomarkers. Once these biomarkers are activated, tissue-specific inflammation is induced. Inflammation that occurs in pancreatic beta cells will result in cell death and worsen disease progression.

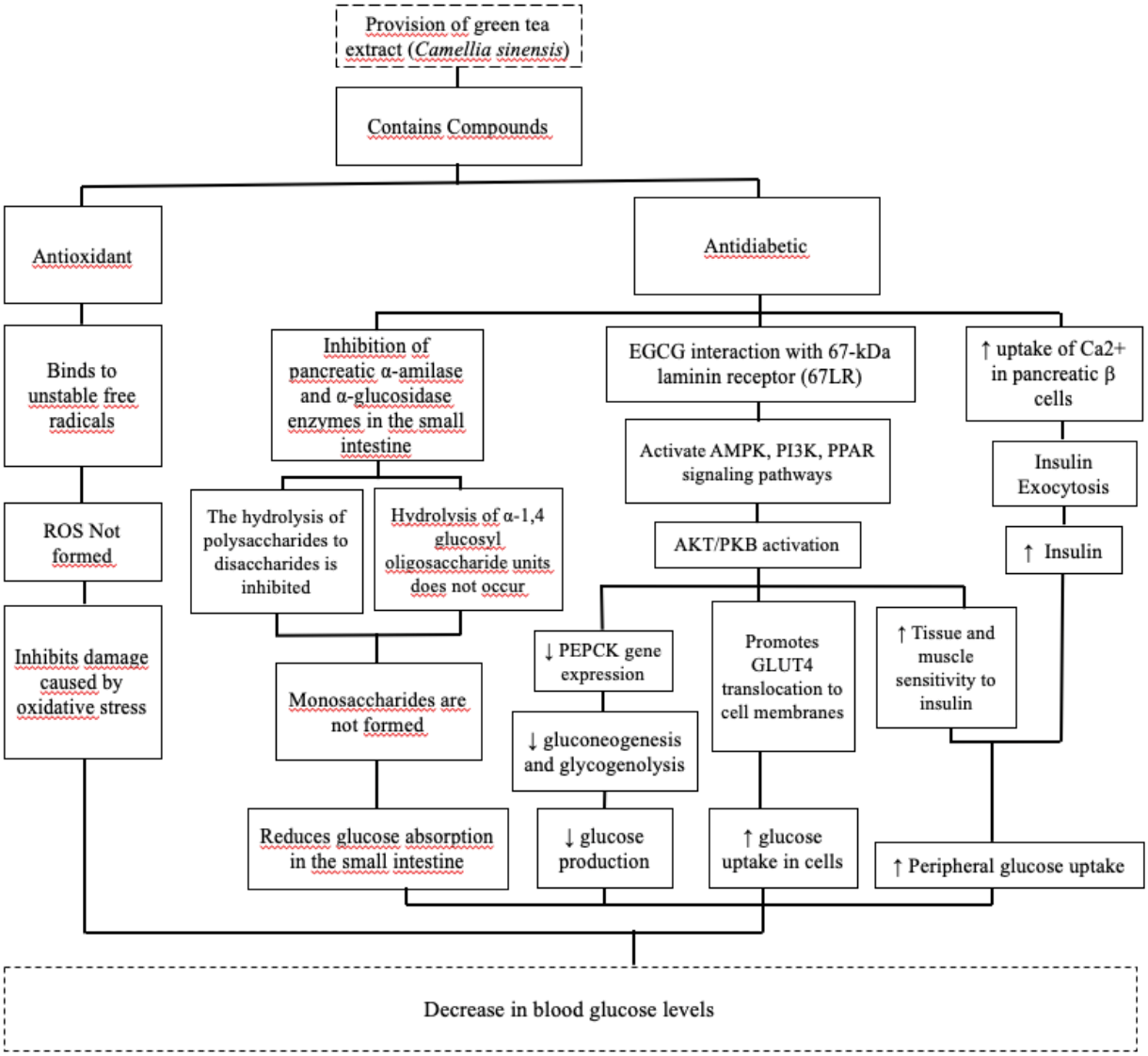

Figure 1. Antihyperglycemic activity mechanism of green tea extract

CONCLUSION

The literature shows that green tea extract (Camellia sinensis) has the potential to reduce blood glucose levels in Wistar rats with diabetes mellitus model. The mechanism underlying the antihyperglycemic effect is proven to be due to the protective and antidiabetic effects of the compounds contained in green tea extract, especially flavonoids, namely catechins. The minimum dose observed to cause the antihyperglycemic effect of green tea extract in experimental animals was 40 mg/kgBW.

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