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THE EFFECT OF HIBISCUS SABDARIFFA LINN.ETHANOLIC EXTRACT ADMINISTRATION ON RAGE, GLP-1 AND GLP-1R EXPRESSION IN HEART TISSUE OF DIABETIC MICE

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ABSTRACT

Diabetes mellitus (DM) is a multifactorial disease associated with hyperglycemia and an increased risk of complications in the cardiovascular system. During hyperglycemia, there is a significant decrease in GLP-1 and GLP-1R expression, which have been known to have protective effects on the cardiovascular system. Increased RAGE expression due to hyperglycemia can also lead to inflammation, contributing to the pathogenesis of diabetes complications, specifically cardiovascular diseases. Administration of Hibiscus sabdariffa Linn. (HSL) can improve cardiovascular function, partly by increasing GLP-1 expression and suppressing RAGE expression. The aim of this study is to investigate the effect of HSL administration on RAGE, GLP-1, and GLP-1R expression in the heart. This study is an experimental study using 9-to-11-week-old DDY mice divided into four groups: control (K), DM control (KDM), positive control DM with quercetin (KQ), and DM with HSL at a dose of 400 mg/kgBW (DM-HSL). The experimental animals received treatment for four weeks.The analysis of GLP-1R and RAGE expression was conducted using RT-PCR, while GLP-1 expression were obtained using the ELISA method. The results of this study indicate a decrease in RAGE expression and an increase in GLP-1 and GLP-1R expression in the hearts of diabetic mice. Quercetin administration was also found to decrease RAGE expression, increase GLP-1 expression, and raise GLP- 1R expression in the hearts of diabetic mice. These findings suggest that HSL administration has the potential to protect the cardiovascular system in diabetes, which is related to the activity of GLP-1 and GLP-1R and RAGE.

Keywords: *Hibiscus Sabdariffa Linn; Diabetes Melitus; Heart; RAGE, GLP-1R, GLP-1*

INTRODUCTION

Diabetes mellitus (DM) is one of the major health problems worldwide.[1] DM is a primary metabolic disorder that currently affects more than 250 million people globally. It is estimated that DM prevalence will increase to 350 million by the year 2030.[2]According to the 2018 Riskesdas data, there was a 6.9% increase in DM cases in Indonesia from 2013 to 2018, reaching 8.5%. The age group most affected was in the range of 55-64 years and 65-74 years. Additionally, it was found that the prevalence of female DM patients in Indonesia was higher (1.78%) than that of males (1.21%).[3] If not properly managed, DM can lead to various long-term complications, one of which is cardiovascular issues.[3] The prevalence of diabetes has significantly increased due to aging, obesity, and lifestyle choices, with cardiovascular complications of diabetes being a major cause of death among diabetic patients.[4] In Indonesia, DM with cardiovascular complications was the third-highest cause of death according to the 2014 Indonesian's Sample Registration System.[5]

Diabetes is known as a heterogeneous group of disorders with common elements of hyperglycemia and glucose intolerance due to insulin deficiency, impaired insulin effectiveness, or both. Generally, it can be classified into two types. Type 1 diabetes is caused by damage to pancreatic β cells, resulting in insulin secretion deficiency, and it commonly occurs in younger patients. Type 2 diabetes is characterized by decreased sensitivity of target tissues to the metabolic effects of insulin and generally affects older patients.[6]

Chronic hyperglycemia is one of the main characteristics of diabetes. Continuous exposure to elevated glucose levels has been recognized as one of the primary factors causing diabetes complications.[7] Chronic hyperglycemia leads to non-enzymatic covalent binding of carbohydrates, such as glucose, to proteins and lipids in a process known as glycation. Short-term glycation products can join to form cross-linked structures known as advanced glycation end products (AGEs).[8] AGEs have been implicated in the pathogenesis of diabetes vascular complications and diabetic cardiomyopathy through interaction with the receptor for AGE (RAGE).[4] The interaction between AGE and RAGE forms a positive feedback loop that transforms acute inflammatory stimuli into sustained cellular dysfunction, subsequently amplifying tissue damage. Thus, the regulation of the RAGE signaling pathway has emerged as a promising target for treating diabetic cardiomyopathy and vascular complications.[9]

Preventing cardiovascular complications in diabetes should be a primary goal when choosing a treatment strategy. The treatment options for patients with diabetes are complex, not only because of the available therapeutic choices but also due to various factors that need to be considered when selecting the appropriate treatment (efficacy, weight reduction, cardiovascular risk or complications, and side effects).10 Currently, it is well recognized that Glucagon-like peptide-1 (GLP-1) provides cardiovascular protective effects, especially in reducing endothelial dysfunction.

GLP-1 is an incretin hormone secreted postprandially by L cells in the intestine.[11] GLP-1 is released during food ingestion to counteract postprandial hyperglycemia by increasing insulin secretion from pancreatic cells and reducing glucose expenditure and glucagon secretion.[12] In patients with type 2 diabetes, the incretin effect is found to be reduced by 53% compared to the normal group.[13] The action of GLP-1 is mediated through the GLP-1 receptor (GLP-1R), which is a member of the G-coupled protein family. GLP-1R is widely expressed outside the pancreas, including in the cardiovascular system; in blood vessels, smooth muscle cells of blood vessels, endothelial cells, and in the liver.[14]

Activation of GLP-1R can directly reduce inflammation in organs and cell types expressing GLP-1R.[15,16] Dipeptidyl peptidase-4 (DPP-4) is an enzyme that degrades GLP-1, causing GLP-1 to only last a few minutes in circulation. Circulating GLP-1 levels can be maintained by inhibiting the DPP-4 enzyme and increasing GLP-1 secretion in the ileum, providing cardiovascular protective effects.[11] According to a study by Harbrik et al., the infusion of GLP-1 for 5 weeks in 12 patients with diabetes and heart failure improved the left ventricular function.[17] Study by Poornima et al. demonstrated that intraperitoneal infusion of GLP-1 for 3 months in mice had direct cardiovascular effects, including maintaining cardiomyocyte viability, improving heart function, and inducing vasodilation.[18]

Many herbs have been proven to have potential for treating diabetes. *Hibiscus sabdariffa* Linn / HSL (roselle) has been shown to have high antioxidant activity.[19]A study by Kartinah et al. revealed that *Hibiscus sabdariffa* Linn increases GLP-1 secretion and the amount of GLP-1 bound to its receptor.[20] *Hibiscus sabdariffa* Linn is rich in organic acids such as citric acid, ascorbic acid, pectin, and polyphenols, including anthocyanins, phenolic acids, flavonoids, and others.[21] According to Andraini et al.'s study in 2014, a dose of 400 mg/kgBW of *Hibiscus sabdariffa* Linn lowered fasting blood glucose levels and insulin resistance in diabetic mice.[22] One of the phenolic compounds in *Hibiscus sabdariffa* Linn is quercetin. Several studies have shown that quercetin, through its antioxidant and anti-inflammatory activities, can prevent various diabetes complications, including cardiovascular complications.[23,24] A study by Fan et al. found that quercetin can inhibit the DPP-4 enzyme, thus maintaining GLP-1 levels.[24] However, studies on the influence of *Hibiscus sabdariffa* Linn in increasing GLP-1 in the heart is currently limited. Therefore, this study aims to determine whether *Hibiscus sabdariffa* Linn can affect the expression of RAGE, GLP-1R, and GLP-1 levels in the hearts of diabetic mice. This study is part of a broader study titled "Potential of *Hibiscus sabdariffa* Linn to Improve Cognitive Function in

Diabetic Mice Through GLP-1 Signaling."

METHODS

SUBJECT

This study is an *in vivo* study involving male DDY mice, including a total of 24 healthy mice, approximately 9 weeks old, with an initial weight of 20-30 grams. They were then randomly divided into four groups: the control mice group (K), the control diabetic mice group (K-DM), the diabetic mice group with quercetin administration (KQ), and the diabetic mice group with *Hibiscus sabdariffa* Linn 400 mg/kgBW administration (DM-HSL).

Induction of Diabetes Melitus

The induction of diabetes mellitus in the experimental animals was carried out by feeding them a High Fat Diet (HFD) (19% fat, 24% protein) for 3 weeks, followed by intraperitoneal injections of streptozotocin (STZ) administered twice within a one-week interval, each time with a dose of 40 mg/kgBW. After the first STZ injection, blood glucose levels were checked 72 hours later. If the fasting blood glucose level was < 150 mg/dl, a second STZ dose was given after one week with the same dosage of 40 mg/kgBW. Blood glucose levels were then measured every week until the end of the study.

Extract Hibiscus Sabdariffa Linn. and Quercetin

Hibiscus sabdariffa Linn extract was orally administered using a gavage with a dose of 400 mg/kgBW once a day for 4 weeks (28 days). Before administration, the mice were weighed to determine the appropriate dosage. Quercetin in isolate form was given orally using a gavage with a dose of 40 mg/kgBW[25] once a day for 28 days.

Sampling Technique

Several molecular parameters will be examined, including RAGE expression using RT- PCR, GLP-1R expression using RT-PCR, and GLP-1 levels using ELISA.

ELISA

Tissue from each group was stored at -80°C until ELISA examination was carried out. GLP-1 levels were measured using the BIOENZY ELISA kit (BZ-08149350) according to the method specified by BIOENZY.

RT-PCR

Total RNA extracted from the hearts of diabetic mice using the Quick-RNA MiniPrep Plus kit (Zymo Study). The cDNA synthesis process was performed using the ReverTra ACETM qPCR

RT Master Mix with gDNA Remover, following the instructions provided by the kit. RT-PCR was conducted using the SensiFAST SYBR Hi-Rox Kit (Bioline). The thermal cycle was set at 95°C for 15 minutes, followed by 40 cycles at 95°C for 20 seconds, 58°C for 30 seconds, and 72°C for 30 seconds. Primers for the RAGE and GLP-1R genes, as well as the mice housekeeping gene GAPDH, were designed based on existing sequences from NCBI. These primers were then subjected to BLAST analysis. After the primer blast on the NCBI website, the selected primers for gene expression analysis were as follows: Mice RAGE primer:[26] Forward primer: 5'- GTCACAGAAACCGGCGATGA-3', Reverse primer: 5'-AGGAAGTGCCTCAAGGAGGA-3',
Mice GLP-1R primer.[71] Forward primer: 5'- TCAGAGACGGTGCAGAAATG-3', Reverse primer: 5'-CAGCTGACATTCACGAAGGA-3', Mice GAPDH primer.[72] Forward primer: 5'- GAAGCCCATCACCATCTT-3', Reverse primer: 5'-CAGTAGACTCCAACGA-3'
In this study, GAPDH was used as the housekeeping gene due to its good stability, making it suitable for checking genes in mice hearts.

STATISTICAL ANALYSIS

Data analysis in this study was using one-way ANOVA in SPSS 26. The analysis began with testing for normality and homogeneity, and if the data were normally distributed, parametric test would be performed. If the data were not normal, nonparametric Kruskal-Wallis test would be used.

RESULT

The mean values of relative RAGE gene expression for the control group were 1.01±0.161;DM group: 1.69±0.074; HSL group: 1.171±0.079; Quercetin group: 1.267±0.130 (Figure.1). Normality and homogeneity tests showed that the RAGE gene expression data were normally distributed and homogeneous ($p > 0.05$). Data were analyzed using one-way ANOVA. The results of the one-way ANOVA indicated a significant difference between groups ($p=0.001$). Further post-hoc testing using Duncan's test showed a significant difference in relative RAGE gene expression between the control, DM, and HSL/quercetin groups, indicating an increased relative RAGE gene expression in the DM group compared to the control group. The RAGE gene expression with HSL administration was lower than with quercetin administration in the DM condition, though not statistically different. Overall, this suggests an influence of HSL and quercetin administration on the relative RAGE gene expression with a significance level of $p < 0.05$.

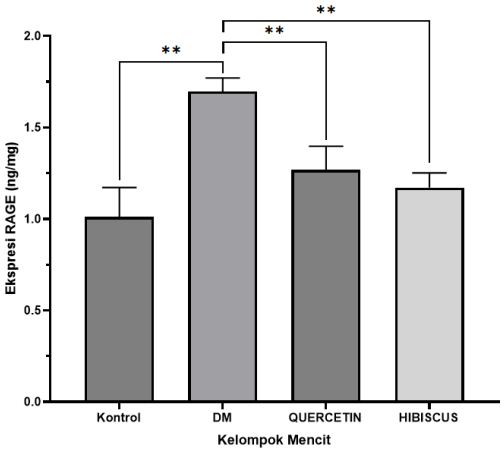

Figure.1 Comparison of RAGE expression in the hearts of mice after 4 weeks of administration of HSL at a dose of 400 mg/kgBW and quercetin. N= 6 mice/group, One way ANOVA test (p<0.05). **p<0.01

The GLP-1 protein expression in the heart were measured using the ELISA method. The Shapiro-Wilk test results indicated that the data had a normal distribution with a p-value > 0.05. Subsequently, the data were tested using the parametric one-way ANOVA. The results of the one- way ANOVA showed a significant difference in GLP-1 protein expression in the heart among the groups with a p-value of 0.006. Further analysis was conducted using the post-hoc Duncan test. The control group (4080 ng/mg) showed a significant difference from the DM group (2941.8 ng/mg) (Figure.2) . This indicates a decrease in GLP-1 expression in the DM group compared to the control group. There was an increase in GLP-1 expression in the groups with HSL administration (3618.7) and quercetin (3614.0) compared to the DM group. The GLP-1 expression after quercetin administration and the GLP-1 expression after HSL administration showed no statistically significant difference.

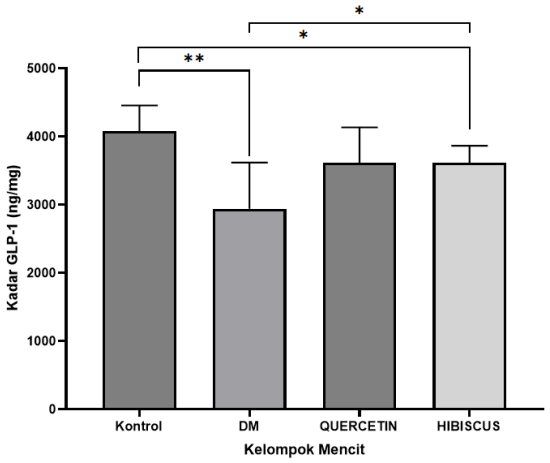

Figure.2 Comparison of GLP-1 levels in the hearts of mice after 4 weeks of administration of HSL at a dose of 400 mg/kgBW and quercetin. N= 6 mice/group, One way ANOVA test (p<0.05). *p< 0.05; **p<0.01

The results of GLP-1R expression were tested using real-time PCR. Normality and homogeneity tests showed that the data were not normally distributed and not homogeneous ($p < 0.05$), therefore statistical analysis was done using the Kruskal-Wallis test. The Kruskal-Wallis test showed a difference between groups ($p = 0.002$), and this was followed by the Mann-Whitney test. The Mann-Whitney test indicated that the expression of the GLP-1R gene in the DM group (0.12) was lower than in the control group (1.101), statistically significant with $p = 0.004$ (Figure.3). This indicates a decrease in GLP-1R in the DM group. With HSL and quercetin administration, GLP- 1R expression was higher than in the DM group with $p < 0.05$. The GLP-1R levels after quercetin administration and the GLP-1R levels after HSL administration showed no statistically significant difference.

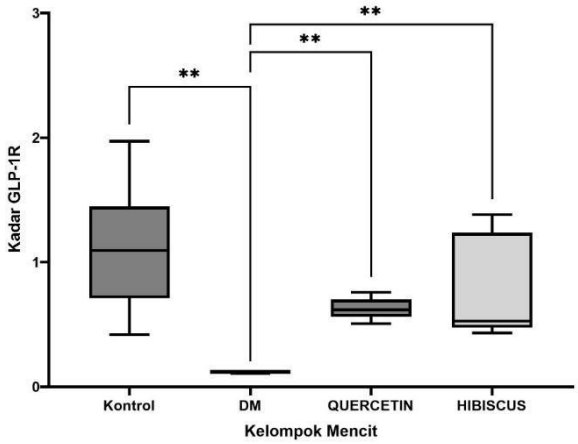

Figure.3 Comparison of GLP-1R expression in the hearts of mice after 4 weeks of administration of HSL at a dose of 400 mg/kgBW and quercetin. N= 6 mice/group. Data shows the Kruskal Wallis test (p=0.02), followed by the Mann-Whitney test (p<0.05). **p<0.01

DISCUSSION

In this study, it was found that in the control group or under normal conditions, RAGE expression was still detected, although when compared to the DM group, the levels were relatively low. In a healthy state, RAGE is expressed at low levels in various differentiated mature cells. When cells undergo glycosylation, macrophages require RAGE assistance to recognize and engulf these cells. However, in pathological conditions related to inflammation such as vascular diseases, cancer, nerve degeneration, and diabetes, RAGE is expressed in higher amounts.29

In this study, it was found that the expression of RAGE in the diabetes group was higher than in the control group. In a state of hyperglycemia, non-enzymatic covalent binding of carbohydrates, such as glucose, to proteins and lipids occurs in a process known as glycation. The glycation products formed in the short term can combine to form cross-linked structures known as Advanced Glycation End Products (AGEs). These modified proteins and lipids can bind to the cell surface receptor for AGEs (RAGE) present in macrophages and endothelial cells, triggering a series of events where the formation of Reactive Oxygen Species (ROS) and activation of nuclear factor- kappaB (NF-κB) lead to the production of pro-inflammatory cytokines. NF-κB also regulates the expression of RAGE, creating a vicious cycle that leads to further synthesis of ROS and cytokines. AGE/RAGE signaling is involved in the pathogenesis of microvascular and macrovascular complications of diabetes through the induction of oxidative stress. Modified plasma proteins can then bind to AGE receptors on various cells, including endothelial cells and macrophages, leading to the production of receptor-mediated oxygen free radicals. Chronic hyperglycemia in uncontrolled diabetes increases AGE formation, which also results in increased RAGE signaling, oxidative stress, and inflammation. Individuals with diabetes-related macrovascular complications have an eightfold increase in RAGE mRNA expression compared to non-diabetic control patients, and significantly higher serum AGE levels have been observed in diabetic patients with vascular complications.[8,30]

Administration of *Hibiscus sabdariffa* Linn. (HSL) can reduce hyperglycemia and hyperinsulinemia. Diabetes promotes AGE formation and lipid peroxidation, while HSL significantly reduces these increases. In the study by Peng et al., it was revealed that HSL inhibits the receptor for AGEs (RAGE), which is increased in type 2 diabetes hearts.[31] In line with this study, a decrease in RAGE expression was found in diabetic mice treated with HSL. This is because HSL extract contains anthocyanins. These results are consistent with previous study stating that anthocyanins, a type of flavonoid, can inhibit the formation of AGEs.[32]

This study found that the ability of HSL to reduce RAGE levels was lower compared to quercetin. This may be due to the HSL extract used containing various compounds other than flavonoids, such as saturated and unsaturated fatty acids,

proteins, carbohydrates, minerals, organic acids (citric acid, hydroxycitric acid, hibiscus flower, oxalate, and ascorbate), anthocyanins, and polysaccharides. With the variety of contents in the HSL extract, increasing the dose also increases the activity of other ingredients, reducing the effect of flavonoids.[32]

Anthocyanins are a type of polyphenol flavonoid within the antioxidant group. The mechanism by which anthocyanins are indicated as inhibitors of AGEs (Advanced Glycation End Products) formation is through inhibiting the binding of AGEs to their receptor (RAGE) and inhibiting monosaccharide auto-oxidation, preventing lipid peroxidation, and inhibiting the polyol pathway.[33] In this pathway, intracellular hyperglycemia occurs. In a state of hyperglycemia, glucose is metabolized by aldose reductase (AR) into sorbitol. The first enzyme in this pathway, AR, is a cytosolic enzyme that reduces glucose to sorbitol with the help of the co-factor NADPH. The second enzyme, sorbitol dehydrogenase (SDH), with the co-factor NAD+, further converts sorbitol into fructose.[6]

Under normal glucose levels, AR has a low affinity for glucose. However, in hyperglycemic conditions, increased enzymatic activity results in the conversion of glucose into fructose, accompanied by a simultaneous decrease in NADPH. The activity of the polyol pathway can lead to oxidative stress. Oxidative stress arises due to high glucose levels, increasing AR activity to reduce glucose, requiring more NADPH. This leads to increased NADPH consumption to reduce glucose, resulting in reduced NADPH levels. In normal conditions, NADPH is needed to regenerate the important intracellular antioxidant glutathione called GSH, leading to a decrease in GSH levels. AR activity in the cell will reduce GSH levels, potentially causing or worsening intracellular oxidative stress. Thus, inhibition through this pathway can not only suppress AGE formation but also reduce oxidative stress.6

The binding of AGEs to their receptor RAGE can activate the pro-inflammatory transcription factor NF-κB and regulate the expression of a large number of cytokine genes, including TNF-α, IL-1β, and RAGE itself. This process is responsible for acute and chronic inflammation in various cell types. In the study by Lu et al., the administration of flavonoids inhibited the binding of AGEs to RAGE by inhibiting the activation of the NF-κB transcription factor, preventing further inflammatory processes.34 The groups of mice treated with Hibiscus sabdariffa Linn. (HSL) and quercetin can activate the cAMP/PKA signaling pathway by increasing GLP-1 levels. cAMP inhibits RAGE expression by converting mRAGE into sRAGE, cutting the cytosolic and transmembrane domains, preventing the binding of AGEs and RAGE.35

In hyperglycemic conditions, proteins, lipids, and nucleic acids undergo non-enzymatic glycation to form AGEs. AGEs mediate macrovascular and microvascular complications such as cardiovascular disease, diabetic nephropathy, retinopathy, and neuropathy. Quercetin, through its antioxidant, anti-inflammatory, and hypoglycemic activities, prevents diabetes complications.36 In accordance with this study, diabetic mice treated with quercetin showed lower RAGE expression compared to mice with diabetes. A study by Osmad et al. found that a single dose of quercetin (400 mg) inhibits α-glucosidase activity and reduces postprandial hyperglycemia in type 2 diabetes.37 Additionally, oral administration of quercetin (250 mg/day) increased antioxidant status in type 2 diabetes. It is known that the binding of AGEs to the RAGE receptor can induce oxidative stress and inflammation, leading to cardiovascular complications.38 Li et al. found that quercetin can reduce AGE production by inhibiting 50.5% glyoxal and 80.1% methylglyoxal, which are important reactive dicarbonyl precursors of AGE.39 AGEs are stable end products formed by the reaction of dicarbonyl compounds methylglyoxal (MGO) and glyoxal (GO) with amino acids in proteins during glycolysis. Moreover, quercetin has been found to decrease RAGE expression and suppress oxidative stress by blocking the binding between AGE and RAGE, protecting cells from injury.38

GLP-1 is an intestinal hormone that contributes to glucose homeostasis, synthesized by proglucagon and secreted from neuroendocrine cells in response to nutrition. GLP-1 regulates glycemia by increasing insulin and decreasing glucagon secretion simultaneously, protecting pancreatic β cells from apoptosis and regulating gastric emptying.40 GLP-1 secretion is disrupted in diabetic patients. Furthermore, several studies have shown the beneficial effects of GLP-1 on cardiovascular function.41 GLP-1 reduces ischemic damage and decreases the size of myocardial infarction in mice myocardial infarction experiments, and recent data indicate that GLP-1 administration improves endothelial function in diabetic mice.42 Additionally, several studies have shown that GLP-1 suppresses oxidative stress in endothelial cells and diabetic mice. GLP-1 can provide direct protection against oxidative stress in the aorta of diabetic mice.43

In this study, the DM group had lower GLP-1 expression than the control group. This is consistent with the study by Nielsen et al., which found a 53% decrease in GLP-1 levels in diabetic patients compared to healthy groups.44 This is believed to be due to chronic hyperglycemia in diabetes. In normal conditions, when consuming glucose, the increase in glucose in the body causes L cells in the intestine to increase GLP-1 secretion. However, due to the chronic increase in glucose levels (> 72 hours), there is an increase in glycated serum (GS) production. GS is a toxic diabetes product that can reduce the enzyme involved in the post-translational process of proglucagon into GLP-1 in intestinal cells, namely prohormone convertase 1/3 (PC 1/3).40 Chronic hyperglycemia can also lead to Glucolipotoxic conditions that inhibit GSK-3β activity, inhibit cAMP-response element-binding protein (CREB) phosphorylation, and decrease β-catenin regulation, which is an essential mechanism for GLP-1 biosynthesis and can result in reduced GLP-1 production and secretion.41

Another factor that may cause a decrease in GLP-1 expression in the group of mice with DM is the increase in ROS (Reactive Oxygen Species). Hyperglycemia is suspected to induce free radicals and damage the endogenous antioxidant defense system through several different mechanisms. Specifically, hyperglycemia induces inflammation by promoting the formation of AGEs, PKC, and hyperactive hexosamine and sorbitol pathways, triggering ROS formation and disrupting insulin signaling, leading to impaired insulin secretion.45 GLP-1 deficiency will occur due to disrupted insulin secretion. Insulin is known to stimulate GLP-1 synthesis through proglucagon gene expression. Lim et al. found in a chronic hyperinsulinemia mice model that insulin activates the phosphatidylinositol 3 kinase-Akt and MAPK kinase, ERK1/2 pathways in all insulin-secreting cells, stimulating proglucagon gene expression, and increasing GLP-1 secretion by up to 58%. Therefore, the decrease in insulin-mediated GLP-1 secretion in insulin resistance is due to a decrease in MEK-ERK1/2 activation.46

In this study, the HSL and quercetin groups were found to have higher GLP-1 expression compared to the DM group. This is consistent with previous study indicating that GLP-1 secretion is influenced by herbal plants. Yu et al. showed that the administration of Huang-Lian-Jie-Du- Decoction herbal medicine for 5 weeks in diabetic mice increased proglucagon mRNA for GLP-1 synthesis and GLP-1 secretion.47 One herbal plant commonly used as an alternative treatment for diabetes is Hibiscus sabdariffa Linn (HSL) or roselle.48 It is now known that HSL has the potential to inhibit dipeptidyl peptidase-4 (DPP-4) enzyme activity.49 DPP-4, also known as CD26 T cell antigen, is a multifunctional protein that, in addition to its catalytic activity, also functions as a binding protein and ligand for various extracellular molecules. DPP-4 is expressed in cells throughout the body and is also released from the membrane and circulates as a soluble protein in plasma. Numerous bioactive molecules can be cleaved by DPP-4 in vitro, including the incretin hormone GLP-1, which plays a crucial role in maintaining normal glucose homeostasis. DPP-4 has been proven to be a key enzyme that regulates its biological activity. This enzyme catalyzes and drastically reduces the biological activity of GLP-1, therefore the half-life of GLP-1 is short, only about 1-2 minutes. This pathway has been pharmacologically targeted through the development of DPP-4 inhibitors. DPP-4 is also believed to modulate the function of β cells as part of a paracrine system involving locally produced GLP-1 in the pancreatic islets.50

Another potential mechanism of HSL that can be used in the management of DM is the enhancement of insulin production. Administration of an HSL dose of 200 mg/kg body weight in DM and streptozotocin (STZ) induction can increase GLP-1 secretion, which will stimulate insulin production.51 GLP-1 binds to the GLP-1 receptor (GLP-1R) in pancreatic β cells and activates adenylate cyclase (AC), leading to the formation of cyclic adenosine monophosphate (cAMP), protein kinase A (PKA), mitogen-activated protein kinase (MAPK), and phosphoinositide 3- kinases (PI3K), which in turn stimulate insulin secretion in pancreatic β cells.20 HSL is a compound that contains polyphenols, and polyphenols are known to have antidiabetic effects.49 This is consistent with the findings of Fujii et al., showing that polyphenols can stimulate active GLP-1 secretion, thereby reducing postprandial hyperglycemia, promoting cell growth, and inhibiting cell apoptosis in pancreatic β cells. Higher GLP-1 expression after polyphenol consumption can enhance insulin sensitivity. The recently discovered G protein-coupled receptor 119 (GPR119) has been shown to mediate GLP-1 secretion by increasing cAMP levels in several GLP-1-secreting cell models. A 37% increase in active GLP-1 secretion was observed.52

Quercetin is a flavonoid known for its antidiabetic properties both in vitro and in vivo by improving oral glucose tolerance, as well as the function of pancreatic β cells to secrete insulin. Medicinal plants containing quercetin have been traditionally used for the treatment of diabetes, infections, and cancer. Recent study reveals that quercetin reduces the risk of cardiovascular disease by lowering hyperglycemia, high blood pressure, hyperlipidemia, and promoting weight loss. Some studies have shown that this flavonoid is beneficial in chronic hypertension, dyslipidemia, obesity, and type 2 diabetes. Quercetin has been proven to reduce blood glucose, liver glucose levels, enzyme levels, and serum cholesterol levels. In this study, the quercetin group was found to have higher GLP-1 levels than the DM group. In hyperglycemic conditions, proteins, lipids, and nucleic acids undergo non-enzymatic glycation, forming AGEs (Advanced Glycation End Products) that can lead to diabetes complications such as cardiovascular disease, nephropathy, retinopathy, and neuropathy. Quercetin has been found to inhibit the non-enzymatic glycation process and inhibit the binding of AGEs to their receptor. By inhibiting the binding of AGEs to their receptor, it can inhibit the expression of the DPP-4 enzyme and its release from proximal tubule cell culture, the main cell type expressing the DPP-4 enzyme. The release of the DPP-4 enzyme is known to degrade GLP-1.53,54 This is also in line with the study by Tahara et al., showing that ROS induced by AGEs-RAGE can stimulate the release of DPP-4 from endothelial cells, further reinforcing the detrimental effects of AGEs.55

Quercetin maintains glucose homeostasis by interacting with molecular targets in the small intestine, pancreas, skeletal muscle, adipose tissue, and liver. Studies on STZ-induced diabetic mice have revealed that quercetin can restore the disrupted expression of insulin receptor substrate- 1 (IRS-1) protein, resulting in increased insulin.56 Insulin is known to stimulate GLP-1 synthesis by activating the phosphatidylinositol 3 kinase-Akt and MAPK kinase, ERK1/2 pathways and stimulating proglucagon gene expression.46

The GLP-1 receptor (GLP-1R) belongs to the same family as the GIP and glucagon receptors. Typically, receptors pair with G proteins to interact with adenylate cyclase. These receptors are widely distributed in the pancreatic islets, brain, heart, kidneys, and the digestive system, including the stomach.57

In this study, it was found that the GLP-1R expression in the DM group were lower compared to other groups. This result aligns with Rajan et al.'s study, which indicates that chronic hyperglycemia can lead to the loss of GLP-1R from the cell surface and disruption of GLP-1R signaling.58 The GLP-1R signaling system is influenced by hyperglycemia with increased mRNA expression encoding inducible cAMP early repressor (ICER) and adenylyl cyclase 8, along with decreased PKA activity. Under low glucose levels, several GLP-1R units were found in the plasma membrane. Conversely, in high glucose levels, there was a decrease in PKA subunit expression, impacting the reduction of GLP-1R.58 In a study by Xu et al., a decrease in GLP-1R and GIP-R was observed in response to hyperglycemia, with a significant decrease in 90% of pancreatectomized hyperglycemic mice and isolated mice cultured in high glucose for 48 hours.59 GLP-1R signaling controls the physiological response to GLP-1 and is currently a primary target for therapy development due to its diverse potential beneficial effects on type 2 diabetes. These effects include glucose-dependent insulin secretion enhancement, increased insulin biosynthesis, protection of pancreatic β cells, improved peripheral insulin sensitivity, and increased weight loss.60,61 New treatments targeting pancreatic β cells through GLP-1R and GIP-R have been introduced in the last decade to treat type 2 diabetes, leveraging the benefits of increased insulin secretion and potentially extending the lifespan of pancreatic β cells. However, there is a reduced response of β cells to GLP-1R agonists in individuals with prediabetes and patients with type 2 diabetes.62

In this study, the effect of HSL in increasing GLP-1 in the presence of diabetes was observed. GLP-1 induces its effects through GLP-1R, although data on how HSL affects this receptor are still very limited. However, it is known that HSL contains flavonoids, and according to the study by Wootten et al., flavonoid administration63 for 17 weeks increased mRNA regulation of GLP-1R in the ileum. Similarly, according to a study by Yang et al., puerarin, a food isoflavone, can improve glucose homeostasis in obese diabetic mice and protect the survival of pancreatic β cells through the mechanism of increased GLP-1 receptor expression. This is associated with the ability of flavonoids to enhance GLP-1R through the activation of the cAMP/PKA-dependent ERK1/2 signaling pathway.64 Furthermore, the increase in GLP-1R expression is also associated with epigenetic processes in the promoter methylation of GLP-1R. It has been reported that flavonoids modulate DNA methylation by weakening the effect of DNA-methyltransferase (DNMT), inducing an overall reduction in DNA methylation. The exact mechanism of DNMT1 inhibition by flavonoids is still under study, but it may occur through direct enzyme inhibition, indirect enzyme inhibition, or the downregulation of DNMT1 expression and translation.63

In this study, the effect of quercetin in increasing GLP-1R in the DM group was also observed. GLP-1R expressed in cells exerts its effects through cAMP formation, causing cell depolarization and an increase in cytosolic calcium concentration (Ca2+), ultimately resulting in increased insulin secretion.65 While the release of insulin by GLP-1R activation is known to be highly dependent on cAMP formation, there is also a role for intracellular Ca2+ and the activation of mitogen-activated protein kinase protein kinase such as ERK1/2.66 Moreover, for many relevant therapeutic effects of GLP-1R activation, including appetite modulation, the underlying GLP-1R- mediated signals are not fully understood. However, physiological responses are known to be a combination of several signaling pathways. Recently, several small-molecule compounds have been identified that can activate GLP-1R. According to a study by Koole et al., quercetin can modulate the activation of ligand-specified GLP-1R.67 Quercetin selectively modulates intracellular Ca2+ signals and activates the cAMP and ERK 1/2 pathways. In separate experiments, trials on streptozotocin-induced diabetic mice showed significantly lower plasma glucose levels, urine output, and urinary glucose content when these animals were treated with an appropriate amount of quercetin compared to diabetic mice control. This suggests that quercetin may have potential in the management of type II diabetes, an effect possibly mediated in part through GLP- 1R modulation.68

The study results indicate that GLP-1 levels and GLP-1R expression increase, while RAGE expression decreases in diabetic mice treated with HSL compared to diabetic mice without HSL administration. It can be concluded that HSL provides protective effects on the heart under diabetic conditions by increasing GLP-1, GLP-1R, and inhibiting RAGE expression.

Based on the study findings, HSL can induce an increase in GLP-1 levels and GLP-1R expression, as well as inhibit RAGE expression, which is not significantly different from the effects of quercetin administration. HSL and quercetin have been shown to provide protective effects on the diabetic heart through the GLP-1 pathway. GLP-1 can block AGE-RAGE activation, thereby reducing inflammatory effects and oxidative stress conditions.

CONCLUSION

The results showed that the expression of GLP-1 and GLP-1R was higher and the expression of RAGE was lower in diabetic mice given HSL compared to diabetic mice without HSL. It can be concluded that HSL provides a potential protective effect on the heart in diabetic conditions through increasing GLP-1, GLP-1R and inhibiting RAGE expression.

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