Original Article

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Effectiveness of Kelakai (*Stenochlaena palustris* (Burm.) Bedd) Extract as an Anti-diabetic through Increased GLUT 4 Expression without Affecting Insulin Levels

Kiki Rizky Ananda, B.Nutr.[1], Muthmainah, M.D., Ph.D.[2],
Nur Hafidha Hikmayani, M.D., M.Clin.Epid., Ph.D.[3]

[1]Master Program of Nutritional Sciences, Graduate School, Universitas Sebelas Maret, Surakarta, Central Java 57126, Indonesia.
[2]Department of Histology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Central Java 57126, Indonesia
[3]Department of Pharmacology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Central Java 57126, Indonesia.

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Abstract:

Objective: Untreated hyperglycemia disrupts insulin secretion and blocks glucose transporter 4 (GLUT 4), consequently affecting the glucose levels of patients with type 2 diabetes mellitus. This study aimed to investigate the effectiveness of kelakai on GLUT 4 expression.

Material and Methods: In this study, a laboratory experiment was performed, with a pre and posttest group design for fasting blood glucose and insulin levels and using posttest only expression GLUT 4 immunohistochemistry methods. A total of 25 rats were divided into 5 groups: nondiabetic, diabetic, diabetic+glimepiride, diabetic+kelakai extract 400 mg/kgBW, and diabetic+kelakai extract 800 mg/kgBW for 21 days.

Results: The 400 and 800 mg/kgBW groups had significant effects (p-value<0.05) on decreasing the fasting glucose levels. A 400 mg/kgBW dose gave a better picture of the fasting glucose levels and reversed the GLUT 4 expression to normal levels. The kelakai extract lowered the fasting blood glucose levels by increasing the GLUT 4 expression in the soleus muscle tissue without affecting the insulin secretion.

Conclusion: Kelakai (*S. palustris* (Burm.f) Bedd) extract significantly reduced the fasting glucose levels by increasing the GLUT 4 expression without affecting the insulin levels at the best dose of 400 mg/kgBB. Thus, it has potential for use in anti-diabetic therapy.

Keywords: diabetes mellitus, GLUT 4, insulin level, kelakai, *Stenochlaena palustris* (Burm.) Bedd

Contact: Kiki Rizky Ananda, B.Nutr.
Master Program of Nutritional Sciences, Graduate School, Universitas Sebelas Maret, Surakarta, Central Java 57126, Indonesia.
E-mail: kiki.rezkyananda13@gmail.com

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Introduction

Diabetes mellitus (DM) is one of the most frequently occurring health problems in the world, with the most common being type 2 diabetes mellitus found in approximately more than 90.0% of the population[1]. Repeated studies have shown that people with diabetes mellitus have a 2 to 4 times higher risk of developing heart disease, hypertension, and dyslipidemia (Jyotsna et al., 2023). The International Diabetes Federation predicts that in 2030 DM cases will reach 643 million, and in 2045 this will increase to 783 million cases[1].

Type 2 diabetes mellitus is a metabolic disease caused by 2 main factors: insufficient insulin secretion by the pancreatic β cells and the inability of insulin-sensitive tissues to respond appropriately to insulin[2]. Insulin is a hormone released by the pancreatic β cells responsible for controlling the balance of blood glucose homeostasis by inserting glucose into cells in various tissues, such as muscles and fat[3]. When damage occurs to the pancreatic β cells, the insulin production decreases, causing a blood glucose level increase[4]. The decreased insulin secretion causes glucose transporter 4 (GLUT 4) transporter activity to decrease[5]. Glucose transporter 4 is a transporter mostly located in the striated muscle that plays a role in facilitating glucose entry into the cells. The reduced GLUT 4 signaling pathway activity impairs the peripheral glucose utilization and affects blood glucose control[6].

Diabetes mellitus treatment is currently symptomatic and should be performed for life; hence, it has the potential to cause various side effects, such as hypoglycemia, diarrhea, bloating, and vomiting[7]. Accordingly, there is a need for alternative treatments that can stabilize blood glucose at normal levels. Plants are currently considered a potential source of bioactive compounds with secondary metabolic content. Treatment with medicinal plants has been considered safer due to their having minimal to no side effects compared with conventional drugs.

Stenochlaena palustris (Burm.f) Bedd, also known as kelakai, is a typical Borneo plant that grows on peat soil. It is often consumed as a vegetable and used as a traditional medicine by the local population, especially the Dayak tribe of Kalimantan. Kelakai contains flavonoids, alkaloids, phenols, tannins, steroids, and terpenoids with antioxidant properties[8] South Kalimantan, EtOH 1:4 w/v. These compounds are reported to have anti-diabetic effects[9-10]. Research has shown that the kelakai extract can inhibit alpha-glucosidase and amylase enzymes.

One study showed that the kelakai frond extract can inhibit alpha-glucosidase enzymes reinforced by the presence of the active component of astragalin compounds[10] followed by silica N60 column chromatography. This study showed that post-harvest treatment significantly protected the AGI activity of S. palustris, while its optimum extract condition was observed with methanol and a smaller particle size (<250 µm. Another study found that the quercetin compound found in the kelakai aerial extract can significantly inhibit alpha glucosidase and amylase enzymes compared to acarbose as a positive control[9]. Another study conducted using the water fraction of kelakai with phenol content demonstrated its ability to bind the alpha-glucoronidase enzyme[11]. Enzyme inhibition can slow down or delay the carbohydrate hydrolysis process in the small intestine, which can consequently control blood glucose levels[9]. However, previous studies were limited to the phytochemical test analysis and in vitro tests; hence, this in vivo study was designed to determine the effect of administering kelakai (*S. palustris*) extract on the fasting blood glucose and insulin levels and GLUT 4 expression.

Material and Methods

Ethical approval

This research was a laboratory experiment, with pre and post-tests with a control design for blood glucose levels, insulin levels and post-test only glucose transporter

GLUT 4 expression, immunohistochemistry methods. Ethical approval of this research comes from the Ethics Committee of the Faculty of Medicine, Universitas Sebelas Maret with registration number 32/UN27.06.11/KEP/EC/2024.

Preparation of extract materials

This research used young and fresh kelakai. To produce simplicia powder, kelakai plants were cleaned, the leaves and stems were taken, cut, and dried under a shade (air-dry), then blended and sieved using a size 60 mesh to make powder. Kelakai powder was macerated using 70% ethanol solvent. The maceration process was repeated 3 times; they became a thick kelakai extract (KE).

Preparation of animal model

A total of 25 Wistar rats were obtained from the Bio Farma laboratory. The maintenance and handling of experimental animals were carried out at the LPPT (Integrated Research and Testing Laboratory) of UGM. Male Wistar rats aged 8 weeks and weighing 200-300 gr were maintained under standard laboratory conditions at a temperature of 22-25 ^{0}C, humidity of 60-70%, and lighting was set with 12 hours bright light and 12 hours dark light. Before modeling, the rats were first adapted for 7 days.

Streptozotocin and nicotinamide induction

A total of 25 Wistar rats were obtained from the Bio Farma laboratory. The maintenance and handling of the experimental animals were performed at LPPT (Integrated Research and Testing Laboratory) at Universitas Gadjah Mada. Male Wistar rats aged 8 weeks and weighing 200–300 g were maintained under standard laboratory conditions at 22–25 °C temperature, 60–70% humidity, and lighting set with 12 hours bright light and 12 hours dark light. Before modeling, the rats first adapted for 7 days.

Experimental design

Twenty-five male Wistar rats were divided into 5 groups as follows: normal control or KN (n=5), negative control of DMT2 or K– (n=5), positive control of DMT2 or KP given glimepiride 0.09 mg/kgBW (n=5), treatment KP1 extract kelakai given 400 mg/kgBW (n=5), and treatment KP2 given extract kelakai 800 mg/kgBW or KP2 (n=5). The rats were given standard feed and drink ad libitum and KE for 21 days. The fasting blood glucose (FBG) and insulin levels were checked before the intervention. After the 21st day of intervention, all animals were sacrificed by anesthesia using xyla and ketamine to reduce pain.

Examination of FBG and insulin level

FBG was examined using a mouse blood sample, taken through the retroorbital sinus by 0.5ml collected in a sterile microtube. Serum FBG levels were examined 3 times, including 1) Before modeling DM mice, 2) After STZ-Na induction (post-test), 3) After 21 days of intervention (pre-test). Serum FBG levels were examined using Enzymatic Calorimetric-Test method glucose oxidase-peroxidase aminoantypirin. Insulin levels were checked at pre-test and post-test using a standard ELISA kit (Rat Insulin ELISA).

Immunohistochemical examination of GLUT 4 expression in soleus muscle

The examination was performed by immunohisto chemical (IHC) staining using a 2-step Plus Poly-HRP Anti Rabbit IgG Detection System (with DAB solution) protocol method. The soleus muscle taken was fixed with Neutral Formalin Buffer for 24 hours. The tissue was processed into paraffin blocks and sliced using a microtome. The tissue sections were made into slides and incubated with E-IR R215C, E-IR R215A, E-IR R215B, and primary antibodies (Rabbit- IgG). The soleus muscle tissue was observed under a light microscope at 1000x magnification, and each slice was observed in 5 fields of view. GLUT 4 expression appeared brown in the cytoplasm of the skeletal muscle cells. The magnitude of GLUT 4 expression was assessed using the Allred histology score[12,13].

Statistical analysis

The data were processed using Statistical Package for the Social Sciences (SPSS). The pre-posttest data were analyzed using Paired T-test if they were normally distributed, or using Wilcoxon if otherwise. Furthermore, to see the difference in the group average test, Anova was used provided that the data were normally distributed, or would be using Kruskal-Wallis if the data were not normally distributed, then continued with post hoc test. The significant value was p-value<0.05.

Results

Effects of kelakai extract on fasting blood glucose levels

The researchers examined the results of the FBG levels after streptozotocin and nicotinamide (STZ-NA) induction as a marker of the successful modeling of a type 2 diabetes mellitus rat. The average results are depicted in Figure 1, indicating T2DM with a marker of FBG levels >126 mg/dL. Figure 1 shows the fasting blood glucose levels before and after KE administration for 21 days in each group. A significant decrease in the fasting blood glucose levels (p-value<0.05) was observed after KE administration at 400 and 800 mg/kgBW doses. Statistically, the decrease was significantly different from that of the negative group, but not significantly different from the normal and positive controls (glimepiride 0.09 mg/kgBW) in the 400 mg/kgBW dose group during the post-hoc test.

Effects of Kelakai Extract on Insulin Levels

The examination was performed to investigate the increase in the insulin levels produced after KE administration for 21 days of intervention in each group. After examining the statistics, the average insulin levels were found to increase in the 400 mg/kgBW dose group, but were not significant (p-value>0.05). The positive control group (glimepiride 0.09 mg/kgBW) significantly (p-value<0.05) experienced a decrease in insulin levels. The average decrease in the insulin levels also occurred in the 800 mg/kgBW dose group. In addition, the post hoc test produced a p value in the 400 and 800 mg/kgBW dose groups that was significantly (p-value<0.05) different from that in the normal group (Figure 2). This research resulted in the negative control group experiencing an increase in the insulin levels; however, this increase was insignificant (p-value>0.05) because the glucose levels were still in the DM rat category.

Effects of kelakai extract on GLUT 4 expression

In this work, a significant difference (p-value<0.05) was observed in the GLUT 4 expression of the variable group given KE in the soleus muscle (Figure 3). The DM rat experienced a decrease in the GLUT 4 expression in the soleus muscle by 37.2, confirming T2DM, as previously shown in the fasting glucose level study. A significant (p-value<0.05) difference was also observed between the 400 mg/kgBW dose group and the negative control group. In the 800 mg/KgBW dose group, no significant difference from the negative group was found. Interestingly, the GLUT 4 expression level after the 400 mg/kgBW KE administration reversed the GLUT 4 expression to normal levels because no significant difference was found in the GLUT 4 expression in healthy individuals. In addition, a concentration-dependent tendency was observed for the ability of the extract to increase the GLUT 4 expression. In this study, we read the GLUT 4 expression at 400× magnification. Each slice was observed in 5 fields of view, but we displayed the GLUT 4 expression image at 1000× magnification in order to more clearly illustrate that the GLUT 4 expression looks brown in the cytoplasmic cell membrane of the soleus muscle cells.

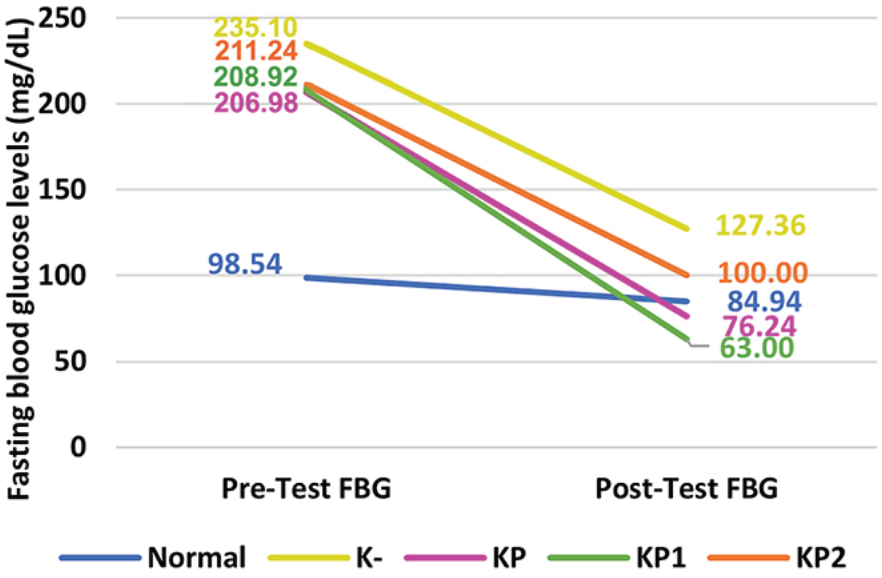

Figure 1 Changes in fasting blood glucose (FBG) levels after treatment. Normal control, negative control (K–) of DMT2, positive control (K+) of DMT2 given glimepiride 0.09 mg/kgBW, treatment (KP1) extract kelakai given 400 mg/kgBW, and treatment (KP2) given extract kelakai 800 mg/kgBW

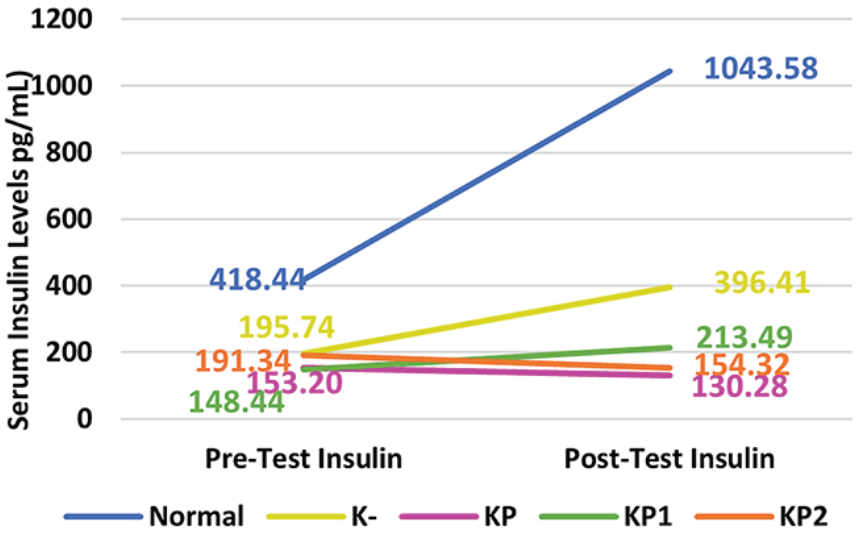

Figure 2 Changes in insulin levels after treatment. Normal control, negative control (K–) of DMT2, positive control (K+) of DMT2 given glimepiride 0.09 mg/kgBW, treatment (KP1) extract kelakai given 400 mg/kgBW, and treatment (KP2) given extract kelakai 800 mg/kgBW or KP2

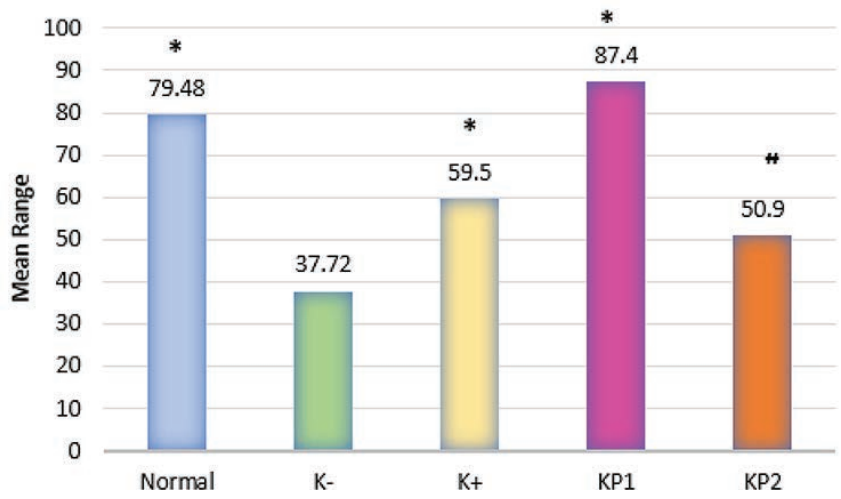

Figure 3 Average Allred Score of GLUT Expression 4. Normal control, negative control (K–) of DMT2, positive control (K+) of DMT2 given glimepiride 0.09 mg/kgBW, treatment (KP1) extract kelakai given 400 mg/kgBW, and treatment (KP2) given extract kelakai 800 mg/kgBW or KP2. After DM rats were intervened with kelakai extract for 21 days (post-test), *=There is a significant difference to K–. #=There was no significant difference to K– during the post-hoc test

Discussion

Hyperglycemia is a condition experienced by patients with diabetes mellitus. If left untreated, it can further damage the pancreatic β cells, making diabetes more progressive and ultimately causing microvascular and macrovascular complications[14]. In cases of T2DM, hyperglycemia occurs because of abnormalities in insulin secretion and resistance or both[2]. In this work, the STZ–NA-induced T2DM model rats increased their fasting blood glucose levels, reduced their insulin secretion, and showed a weakened GLUT 4 expression compared with the normal control groups. The STZ–NA administration caused damage to the pancreatic β cells and further reduced their capability to produce insulin, resulting in a decreased GLUT 4 expression. The mouse model did not undergo KE treatment and maintained hyperglycemia throughout the experiment, indicating a successful induction of the T2DM mouse model. The KE administration significantly lowered the fasting blood glucose levels by increasing the GLUT 4 expression without affecting the insulin levels.

This research resulted in a decrease in the fasting glucose levels in the untreated T2DM model rats, but the decrease was not greater than that of the intervention group. The decrease in the glucose levels was still under the category of the T2DM rat 126 mg/dL, in line with previous studies, which also modeled a T2DM rat using anti-diabetic agents with the same STZ–NA dose and fasting glucose levels of >126 mg/dL[15]. Other studies also found ≥126 mg/dL[16]. In contrast, the negative group experienced a higher average increase in insulin levels, but the IHC results of the GLUT 4 expression in the soleus muscle tissue appeared to have a lower percentage compared to the intervention and positive control groups. This was in line with research, that after the STZ–NA induction, the GLUT-4 expression decreased[17]. Other studies produced similar results, that is, STZ–NA induction affected the GLUT 4 action; hence, the

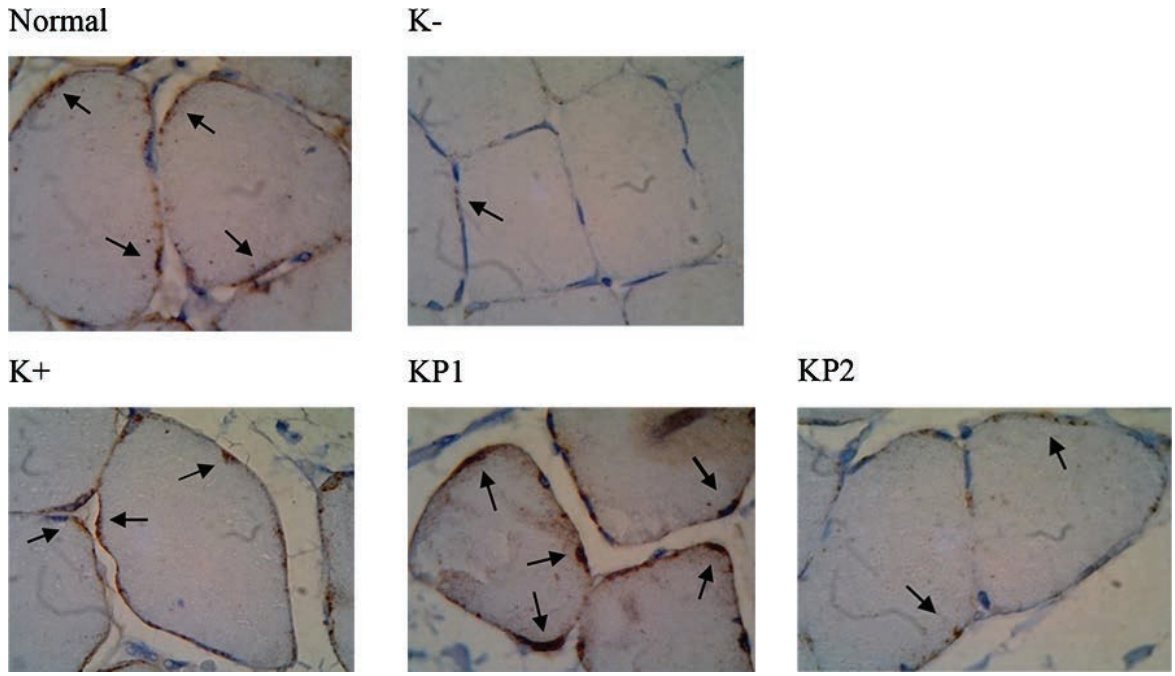

Figure 4 The immunohistochemical staining of GLUT 4 expression in soleus muscle cells was conducted at 1,000x magnification, with brown spots visible in each treatment group. The immunohistochemistry results were calculated using the Allred score method and viewed based on the minimum range. The results for the normal (non-diabetic) group were 79.48, for the diabetic (K-) group 37.72, for the glimepiride 0.09 mg/kgBW (K+) group 59.50, for the KP1 (dose of 400 mg/kgBW) group 87.40 and for the KP2 (dose of 800 mg/kgBW) group 50.9.

negative control mouse modeling without any intervention still maintained hyperglycemia during the experiment[18].

A disruption in the absorption of the blood glucose levels was observed in the type 2 diabetes mellitus model after the STZ–NA induction. Streptozotocin caused the methylation of the pancreatic β cell DNA, such that the DNA was fragmented and resulted in further overstimulation of the PARP enzyme resulting in NAD+ depletion that ultimately caused the ATP depletion[19]. The ATP depletion resulted in a decrease in the sodium pump (Na+ pump), which caused sodium accumulation in the cell. If this condition continues, the cell will rupture and eventually die[20]. To prevent the pancreatic β cells as a whole from dying, NA is given to inhibit the PARP enzyme, such that both the NAD+ and ATP depletions are reduced, limiting the number of pancreatic β cells that die, resulting in the modeling of diabetic rats that can produce insulin[19].

This study showed that increasing the dose of the kelakai extract from 400 (KP1) to 800 mg/kgBB (KP2) is not always directly proportional to the decrease in the GDP levels, where after 21 days of kelakai extract administration, the GDP levels in KP2 (100 mg/dL) were still higher than in KP1 (63 mg/dL). The relationship between dose and effect can be explained based on pharmacodynamic interactions. The relationship is a "U"-shaped dose–response curve, wherein the effect first decreased and then increased as the dose increased[21]. The study results are in line with those in the previous research, where the moringa leaf extract dose of 400 mg/kgBB resulted in a lower reduction in the GDP levels compared to the 600 mg/kgBB dose[22].

Interesting things were found in this study, although the groups given the kelakai extract intervention (KP1 and KP2) did not show any change relevant to the increasing insulin levels similar to the positive control group (KP) given glimepiride. The resulting GLUT 4 expression was stronger than the negative control group, as seen from the IHC score calculation. In addition, the administration of the 400 mg/kgBB dose of kelakai extract (KP1) resulted in a stronger GLUT 4 expression when compared to the 800 mg/kgBB dose group (KP2) and the positive group given glimepiride. These results are in line with the GDP levels where the 400 mg/kgBB dose of the KP1 group produced lower GDP levels after the kelakai extract intervention.

In this study, the kelakai extract caused a decrease in the GDP levels, not by increasing the insulin levels but by increasing the GLUT 4 expression better than the glimepiride. The stronger GLUT 4 expression in the DM rats given the kelakai extract compared to those that were not given the intervention could be caused by the presence of antioxidant compounds in the kelakai plant. Previous research found that kelakai extract has an antioxidant activity classified as strong to very strong. In that study[8] South Kalimantan, EtOH 1:4 w/v, the kelakai extract produced IC50 of 47.2+0.11 µg/mL. It will cause oxidative stress in untreated DM rats, which will ultimately accelerate disease complications with the presence of antioxidant compounds that can inhibit the production of free radicals and their messy reactions by inhibiting the production of inflammatory mediators and repairing damaged molecules[23].

Based on the phytochemical test of the antioxidant groups in the kelakai leaf extract, namely, flavonoids, alkaloids, steroids, tannins and phenolics[8-24] South Kalimantan, EtOH 1:4 w/v, this study is similar to the study that produced a kelakai leaf extract containing flavonoids, saponins, tannins, alkaloids, and steroids[25]. Based on research[9], 11.74+10 mg CE/g alkaloids, 23,450.14 mg QE/g flavonoids, and 193.97 + 0.11 mg/GAE phenols can be found in the kelakai extract. The presence of these bioactive substances is known to stimulate the IRS, PI3K, and Akt pathways, accelerating the phosphorylation of the insulin signaling pathway, thereby increasing the GLUT 4 translocation to the muscle cytoplasmic membrane, which ultimately increases blood glucose level absorption[26-30] 2-diacyl-sn-glycerol (DAG)

The study results are in line with those from other research[31], which found serpentine, which is an alkaloid component that can increase the GLUT 4 translocation without affecting insulin levels. Another study conducted[32] found phenol compounds that can reduce the blood glucose levels through an increased GLUT 4 expression that is even better than anti-diabetic drugs without insulin reliance. In addition, the research conducted[33] resulted in extracts with flavonoid content that can increase the GLUT 4 expression in the rat skeletal muscles.

Flavonoid compounds are believed to provide health benefits, one of which is their ability to act as an anti-diabetic agent[34]. Flavonoids play a role in increasing the GLUT 4 expression in the striated muscle[35]. In addition, alkaloid compounds play a role in increasing the GLUT 4 translocation, increasing the Ca^{2+} signaling, and activating AMPK and PPAR-Y to control T2DM[36]. Phenolic compounds are believed to have anti-diabetic effects that work by increasing the GLUT 4 expression in the muscle tissue through a PI3K–Akt pathway-dependent mechanism[37] . This study demonstrated that KE administration to T2DM rats increases the GLUT 4 expression in the soleus muscle and decreases the fasting blood glucose levels without affecting the insulin levels.

Conclusion

Kelakai (*S. palustris* (Burm.f) Bedd) extract significantly reduced the fasting glucose levels by increasing the GLUT 4 expression without affecting the insulin levels at the best dose of 400 mg/kgBB. Thus, it has potential for use in anti-diabetic therapy.

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Conflict of interest

The authors have no conflicts of interest to declare.

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