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Original Article

Insulin secretion and repairing pancreatic tissue damage on diabetic mice treated with the extract and active fraction of *Hibiscus surattensis* L. leaves

[Secreción de insulina y reparación del daño del tejido pancreático en ratones diabéticos tratados con el extracto y la fracción activa de hojas de *Hibiscus surattensis* L.]

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Abstract

Context: Diabetes is a chronic health condition with insulin disturbance. *Hibiscus surattensis* L. is a medicinal plant and plays a pivotal role in diabetes.

Aims: To evaluate the effect of *H. surattensis* leaves extract to increase insulin secretion and repair pancreatic tissue damage in streptozotocin (STZ)-induced diabetic mice.

Methods: This research used male mice (Swiss Webster) administrated by STZ 100 mg/kg BW to induce diabetes. Animals were divided into eight groups (n = 6) consisting of normal control, diabetic control, glibenclamide, ethanol extract 50, ethyl acetate fraction, and water fraction with doses of 25 and 50 mg/kg BW, respectively. Treatment was given for 28 days. The evaluation was carried out on blood glucose levels, insulin levels, HOMA-β, and the pancreatic islets by the morphometric analysis were evaluated. All data were analyzed using SPSS 16.0.

Results: Extract and active fraction of *H. surattensis* leaves, especially ethyl acetate fractions (25 and 50 mg/kg BW) significantly ($p<0.05$) reduced fasting blood glucose levels, insulin levels, and the strength of pancreatic β cells (HOMA-β) increased in STZ induced diabetic mice. Histopathological results in pancreatic confirmed the effect of *H. surattensis* to increase the number of islets significantly in Langerhans and pancreatic β-cells compared to the diabetic control group ($p<0.05$).

Conclusions: Extract, and active fraction of *H. surattensis* leaves affect the insulin secretion and repairing the pancreatic tissue damage on diabetic mice, the most optimal therapy on the administration of ethyl acetate fraction.

Keywords: antidiabetic; *Hibiscus surattensis* L.; insulin; pancreas; streptozotocin.

Resumen

Contexto: La diabetes es una enfermedad crónica con alteración de la insulina. *Hibiscus surattensis* L. es una planta medicinal y juega un papel fundamental en la diabetes.

Objetivos: Evaluar el efecto del extracto de hojas de *H. surattensis* para aumentar la secreción de insulina y reparar el daño del tejido pancreático en ratones diabéticos inducidos por estreptozotocina (STZ).

Métodos: Esta investigación utilizó ratones macho (Swiss Webster) administrados por STZ 100 mg/kg BW para inducir diabetes. Los animales se dividieron en ocho grupos (n = 6) que consistían en control normal, control diabético, glibenclamida, extracto de etanol 50, fracción de acetato de etilo y fracción de agua con dosis de 25 y 50 mg/kg de peso corporal, respectivamente. El tratamiento se administró durante 28 días. La evaluación se realizó sobre niveles de glucosa en sangre, niveles de insulina, HOMA-β, y se evaluaron los islotes pancreáticos mediante análisis morfométrico. Todos los datos se analizaron con SPSS 16.0.

Resultados: El extracto y la fracción activa de hojas de *H. surattensis*, especialmente las fracciones de acetato de etilo (25 y 50 mg/kg de peso corporal) redujeron significativamente ($p<0,05$) los niveles de glucosa en sangre en ayunas, los niveles de insulina y la fuerza de las células β pancreáticas (HOMA-β) aumentó en ratones diabéticos inducidos por STZ. Los resultados histopatológicos en páncreas confirmaron el efecto de *H. surattensis* para aumentar significativamente el número de islotes en Langerhans y células β pancreáticas en comparación con el grupo de control diabético ($p<0,05$).

Conclusiones: El extracto y fracción activa de hojas de *H. surattensis* afectan la secreción de insulina y reparan el daño del tejido pancreático en ratones diabéticos, la terapia más óptima sobre la administración de fracción de acetato de etilo.

Palabras Clave: antidiabético; estreptozotocina; *Hibiscus surattensis* L.; insulina; páncreas.

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INTRODUCTION

Diabetes mellitus (DM) is a severe metabolic disorder with several etiologies characterized by elevated blood sugar levels (hyperglycemia) accompanied by compromised glucose, lipid, and protein metabolism due to reduced insulin secretion and insulin sensitivity and sometimes correlated with long-term complications in different organs, particularly the skin, liver, kidneys, heart, nerves, and blood vessels (Prihatin et al., 2019). Diabetes is often associated with an increase in the occurrence of global morbidity and mortality. International Diabetes Federation (2019) data showed that approximately 463 million adults (20-79 years) live under diabetes conditions; in 2045, the diabetes incidence is expected to rise to 700 million. Nearly 79% of adults with diabetes live in low and middle-income countries, including Indonesia (Ogurtsova et al., 2017. DM prevalence in Indonesia also continues to increase, from 6.9% in 2013 to 10.9% in 2018 (Ministry of Health, 2019).

Although DM is a chronic disease that has no impact on death directly, this entity can be worse if treatment management is wrong. DM management requires multidisciplinary management, including non-drug therapy (medical nutrition therapy and physical activity) and drug therapy. The management of DM has the goal to reduce DM morbidity and mortality. The strategy to achieve these objectives is keeping plasma glucose levels within the normal range and preventing or minimizing DM complications. On the other hand, the pharmacological treatment of DM without side effects through oral administration of antihyperglycemic drugs remains a problem for the medical community. These drugs are limited by pharmacokinetic properties, secondary failure rates, treatment costs, and side effects. Therefore, the search for new class compounds is critical to overcoming DM problems, ultimately leading to the search for alternative drugs with lower side effects, including utilizing plants (Natarajan and Dhas, 2013).

Several studies have reported that oxidative stress is a crucial factor of vascular injury in patients with T2DM. Hyperglycemia has a relationship with DM and oxidative stress (Bwomnlee, 2001; Folli et al., 2011). Alterations in the antioxidant defense system in diabetes have recently been reported. A natural antioxidant can be used to defend against oxidative stress (Johansen et al., 2005). Notably, it has been reported that almost 90% of the population in developing countries use traditional medicines in primary health care because of their abundance, affordability, safety, and efficacy (World Health Organization, 2002). Ethnobotany information shows that more than 800 plants can be used as traditional medicines to treat diabetes (Patil et al., 2011). Medicinal plants are used as hypoglycemic agents to treat diabetes because they have excellent antioxidant properties and polyphenolic compounds (Kähkönen et al., 1999).

Hibiscus surattensis L. is a plant species from the genus *Hibiscus* (*Malvaceae*), used as a traditional medicine in several countries in Asia and Africa. *Hibiscus surattensis* L., known as 'tamoenju' by the Kaili and Dondo tribes in Central Sulawesi, is one of the traditional medicinal plants used to treat diabetes and anti-hepatitis by local shamans or doctors. The part that was used commonly was the leaves (Fajrin et al., 2015; Triani et al., 2015). *H. surattensis* is categorized as a shrub that abundantly grows in the forest and has not been widely cultivated by local communities. Various ethnobotany and ethnopharmacology studies show that some part of this plant especially leaves, flowers, stems, and bark have multiple properties: anti-inflammatory (Mabona et al., 2013), antimalarial (Yetein et al., 2013), antihypertensive (Gbolade, 2012), wounds, abscesses, gonorrhea (Amri and Kisangau, 2012) and cough (Bassey and Effiong, 2011). The leaves of *H. surattensis* have exhibited a variety of chemical compounds such as alkaloids, steroids, tannins, phenolics, and flavonoids (flavones and flavones) (Raghu, 2015). Several experimental and pharmacological studies have shown the efficacy of *H. surattensis* leaves as antibacterial, antioxidant, anti-inflammatory, and antidiarrheal (Sultana et al., 2018).

Previous research has shown the extract, ethyl acetate fraction, and water fraction of *H. surattensis* leaves had a hypoglycemic effect on the oral glucose tolerance test in normal mice (Yuliet et al., 2018) and as DPP-4 inhibitor (Yuliet et al., 2020). Based on these studies, research on the effects of the extract and active fraction of *H. surattensis* leaves as an antidiabetic is an interesting topic. Further studies should be investigated not only with the glucose tolerance test model but also using animals by inducing streptozotocin (STZ). Moreover, the effect in increasing insulin secretion and repair pancreatic tissue damage in STZ induced diabetic mice should be evaluated.

MATERIAL AND METHODS

Chemical and reagents

Streptozotocin (STZ) was purchased from Sigma-Aldrich (St. Louis. MO. USA), mouse insulin ELISA kit from Bioassay Technology Laboratory (Shanghai, China), glibenclamide tablets (Daonil 5®, Sanofi-Aventis), One Touch Ultra Easy Glucometer strips (LifeScan. Johnson-Johnson). The reagents and other chemicals were used in analytical grade. The extract, active fraction, and glibenclamide were suspended in 0.5% Na CMC/ sodium CMC as carriers, respectively.

Plant material

H. surattensis leaves were collected from Alindau, Sindue Tobata, Central Sulawesi, Indonesia (0°20'46.2"S 119°47'52.5"E) and authenticated by experts of the herbarium staff in the Department of Biology, School of Life Science and Technology, Bandung Institute of Technology, Bandung, Indonesia (1791/II.CO2.2/PL/2017). Fresh and cleaned plant leaves were dried at room temperature and grounded into the powder using a grinder machine. The powder was stored in airtight containers.

Preparations of ethanol extract and fraction of *H. surattensis* leaves

The powdered samples (3.1 kg) were extracted using 96% ethanol with a ratio of 1:10 (w/v) by the maceration method (5 × 24 hours) and evaporated to dryness under vacuum using a rotary evaporator. The evaporation process was conducted at a temperature of ≤50°C. The percentage yield of the extract was 17.15%. The crude extract was then added with warm distilled water (1:1) to remove chlorophyll and obtain the ethanol-water extract. It was fractionated in a separating funnel using solvents of increasing polarity (n-hexane and ethyl acetate). Extracts, *n*-hexane fraction, and ethyl acetate fraction were filtered and concentrated using a rotary vacuum evaporator at 50°C. The water fraction was dried using a freeze dryer. The percentage yields were 1.27% in *n*-hexane, 12.67% in ethyl acetate, and 33.42% in water. Ethanol extract (EE) and active subfractions (ethyl acetate/EAF and water fraction/WF) were based on previous research stored in airtight containers and placed in the refrigerator at 4°C until further use.

Animals

Forty-eight male Swiss Webster mice (25-30 g) with ages ranging from 8-12 weeks were housed in polypropylene cages with the light-dark cycle of 12 hours in the Animal house of School of Pharmacy, Bandung Institute of Technology, Bandung, Indonesia. All the test animals were acclimatized for seven days and were given standard mice feed and water *ad libitum*. After approval of the protocol by The Ethics Committee for Animal Research of Bandung Institute of Technology, Indonesia (No.07/KEPHP-ITB/11-2017), the experiments were performed.

Experimental protocol

Mice were randomly divided into eight groups of six in each group. The sample size determination followed the WHO regulation by about five animals in an experimental group (World Health Organization, 2000). Doses were selected based on our previous studies (Yuliet et al., 2018). All groups were orally given the tested samples by using oral gavage. Glibenclamide, extract, and active fractions were suspended in 0.5% sodium CMC as the vehicle. Group I was a normal control (healthy mice); Group II was a diabetic control. Group I and II received 0.5% sodium carbox-

ymethylcellulose (prepared in distilled water) as a vehicle at a dose volume of 2.5 mL/200 g BW; Group III diabetic mice received glibenclamide at 0.65 mg/kg BW as a standard antidiabetic drug; Group IV diabetic mice were given *H. surattensis* leaves ethanol extract (50 mg/kg BW); Group V and VI diabetic mice were given ethyl acetate fraction (in doses of 25 and 50 mg/kg BW, respectively); Group VII and VIII diabetic mice were given water fraction (in doses of 25 and 50 mg/kg BW, respectively). All the mice were treated once daily for 28 consecutive days.

Induction of diabetes

Experimental diabetes was induced by a single i.p. injection of 100 mg/kg BW of STZ freshly dissolved in cold 50 mM citrate buffer (pH 4.5). The mice were then kept on 10% sucrose water solution for 48 hours to prevent hypoglycemia. Normal control (non-diabetic mice) received citrate buffer i.p injection. After seven days of STZ injection, blood samples were collected from the tail vein, and fasting blood glucose (FBG) level was determined using a glucometer. Only the mice, which have shown hyperglycemia (FBG level >200 mg/dL) and with signs of polyuria and polydipsia, were considered to be diabetic and were selected for the study (Furman, 2015).

Measurement of fasting blood glucose and insulin level

Blood samples were collected from the caudal vein to measure fasting blood glucose level (FBG) on day one before induction day 7 (H-7). After seven days of STZ induction, FBG levels were measured and only those animals showing blood glucose level ≥200 mg/dL were used for the following investigation. The day on which hyperglycemia had been confirmed was designated as day 1 (H1). The fasting blood glucose levels were measured every 7th day during the 28 days of treatment (H7, H14, H21, and H28). FBG levels were measured with a portable glucometer. At the end of the experiment, all mice were euthanized via carbon dioxide inhalation, and their blood samples were collected via cardiac puncture. Blood was collected and centrifuged at 3000 rpm for 15 min to separate blood serum. The clear serum was used to estimation of insulin level. Estimation serum insulin was performed by mouse insulin ELISA kit.

Assessment of homeostasis model assessment

The homeostatic model assessment (HOMA-β%) is a method used to quantify β-cell function (HOMA-β%). HOMA-β% was measured from fasting blood glucose (mg/dL) and fasting serum insulin (mIU/L) concentration by homeostasis model assessment (HOMA) calculated using the HOMA2 calculator software. HOMA2 calculator was downloaded from the University of Oxford: http://www.dtu.ox.ac.uk/ (Diabetes Trial Unit, 2019).

Histopathological study

The pancreas was rapidly removed to avoid its autolysis after a few moments of the sacrifice of the animal. This organ was fixed in 10% buffered formalin. After fixation, tissues were embedded in paraffin. Thick sections were cut at 4-5 µm and stained with Gomori. Histological preparations of Gomori pancreatic staining were observed with a microscope at a magnification of 40 × 10 by a pathologist. The morphometric analysis for the pancreas determined (1) the area of the pancreatic islet (µm^2), (2) the number of islets in each section of the pancreas, (3) the number of pancreatic β-cells. The observation was conducted using an Olympus® CX-21 microscope connected to the Optilab Viewer and Image Raster 3 software.

Statistical analysis

All data were presented as the mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) or nonparametric ANOVA (Kruskal-Wallis test when data do not follow normality or homogeneity of variances) were performed to obtain significant differences between varieties ($p< 0.05$) using Statistical Packages for Social Sciences (SPSS) 16.0. Duncan's or Mann Whitney test (nonparametric) were used to compare the means when differences were statistically significant.

RESULTS

Effect of ethanol extract *H. surattensis* leaves and its fraction on fasting blood glucose level

The mean of the fasting blood glucose level (FBG) of normal mice (H-7) in Fig. 1 at the beginning of the study demonstrated no significant differences. These results indicated that all experimental animal condition before treatment was the same and without hyperglycemic condition. In Fig. 1, blood glucose levels in DM mice after STZ induction had an increase of above 200 mg/dL in all groups (three times increase compared to the normal control group). This condition was classified as hyperglycemia. It was characterized by FBG values above normal and the induction of diabetes. The mean of FBG of diabetic control group mice was higher than the other treatment groups. It indicated the interference with glucose homeostasis, and DM's condition continued over time. This condition might be possible by the influence of STZ on the destruction of pancreatic β-cells and a decrease in insulin secretion.

According to Fig. 1, administration of EE, EAF, and WF showed an excellent impact on the decreasing level of FBG. Giving EE, EAF, and WF reduced significantly of FBG on days 7, 14, 21, and 28 compared to the first day. The decline in FBG on day 7 was not substantially different from diabetic control. The significant reduction was on days 14, 21, and 28 ($p<0.05$). At the end of the study, FBG in treatment decreased significantly compared with the FBG diabetes control group with the hypoglycemic power percentage of glibenclamide (36.66%), EE (43.70%), EAF 25 (45.60%), EAF 50 (52.60%), WF 25 (40.24%), and WF 50 (34.18%).

Effect of *H. surattensis* on insulin level and insulin-related biomarkers

Levels of insulin after 28 days of intervention are presented in Table 1. Insulin levels in the diabetic control group showed the lowest value compared to other treatment groups. The results showed that glibenclamide administration, EE 50, EAF 25, EAF 50, WF 25, and WF 50 from leaves of *H. surattensis* could increase insulin levels by 40.56, 46.15, 30.94, 40.56, 19.93, and 25.17%, respectively. Based on the result, the increased insulin levels showed a non-significant difference in the *H. surattensis* extract groups and fractions with the group given glibenclamide ($p>0.05$). We compared the extract and fraction to determine the optimal activity between extract and fraction. We used 2 doses of ethyl acetate fraction and water fraction at 25 and 50 mg/kg BW to determine whether the increased dose would increase the effect. Glibenclamide was used as a standard to ensure a valid research method.

The increasing insulin levels in the group treated by *H. surattensis* showed that the content of active compounds found in *H. surattensis* leaves could trigger insulin secretion. As biomarkers of insulin levels, HOMA-β calculations were conducted. Homeostasis of the assessment β-cell model (HOMA-β) is a mathematical model that reflects pancreatic β-cell function. HOMA-β is one indicator to measure the level of strength of pancreatic β cells that produce insulin (Wysham and Shubrook, 2020). The higher the value % of HOMA-β will alter the strength of β-cells. Table 1 shows that the DM group exhibited HOMA-β (4.82 ± 0.37) lower than the other groups, while the groups treated with glibenclamide, EE 50, EAF 25, EAF 50, WF 25, and WF 50 (14.26 ± 0.79, 23.06 ± 1.84, 19.96 ± 1.05, 25.66 ± 2.96, 14.10 ± 1.46, and 13.66 ± 1.42) significantly can improve the level of strength of pancreatic β-cells in DM mice. The EAF 50 group had the highest HOMA-β value compared to the other treatment groups. The EAF 50 capable of improving the level of pancreatic β-cell strength in DM mice were 5.32 times higher than in diabetic control.

Effect ethanol extract *H. surattensis* leaves and its fraction on histopathological analysis of pancreas

Observation of pancreatic histology was conducted by counting the number of the islet of Langerhans in a pancreatic tissue section, measuring Langerhans area, and counting the number of β-cells. Data on each treatment group are presented in Table 2 and Fig. 2. Based on Table 2, the normal control group showed a difference among

the area of the islet of Langerhans compared to the diabetic control group. This proves that the insulin deficiency test model with STZ was successful. Diabetes mice decreased the number and extent of the islet of Langerhans. This is caused by streptozotocin's mechanism, which induces mice to become diabetic by selectively damaging pancreatic β-cells (pancreatic β-cells degeneration) to cause insulin deficiency (Eleazu et al., 2013). The islet of Langerhans microanatomy described changes in the islet of Langerhans area, number of the islet of Langerhans, and β-cell numbers in each treatment, as presented in Fig. 2. The pancreatic tissue of the diabetic control group had acinar cells, degeneration, and necrosis. The number of β-cells was significantly reduced in the islet of Langerhans compared to the other treatment groups. Severe β-cell damage and insulin secretion were very few resulting in hyperglycemia. The outcome of the morphometric analysis of the islet area, the total number of the islet, and the number of beta cells pancreas showed a significant reduction in the diabetes control group compared to the normal control group by 10.9, 12.6, and 14.2%, respectively.

However, the groups given ethanol extract, ethyl acetate fraction, and water fraction showed an increase in the islet area, the total number of the islet, and the number of β cells indicated by a significant increase compared to the diabetes control group. The morphometric parameters were significantly increased in EE, EAF 25 and 50, WF 25 and 50 with 77.3, 47.8, 43.5, 28.1, and 30.1% exhibited improvement in the area of the islet: 62.5, 24.9, 37.5, 24.9, and 31.3% in the total number of islets, and 29.4, 44.6, 56.4, 62.6, 29.4, and 35.1% in number β cell. Based on pancreatic histological data, it was shown that the group treated by EE and *H. surattensis* leaves fraction had improved pancreatic histology condition. These images showed the tendency to increase the proliferation of pancreatic β-cells. Therefore, it increased the Langerhans island area and regenerated pancreatic β-cells, especially in groups treated by EE and EAF.

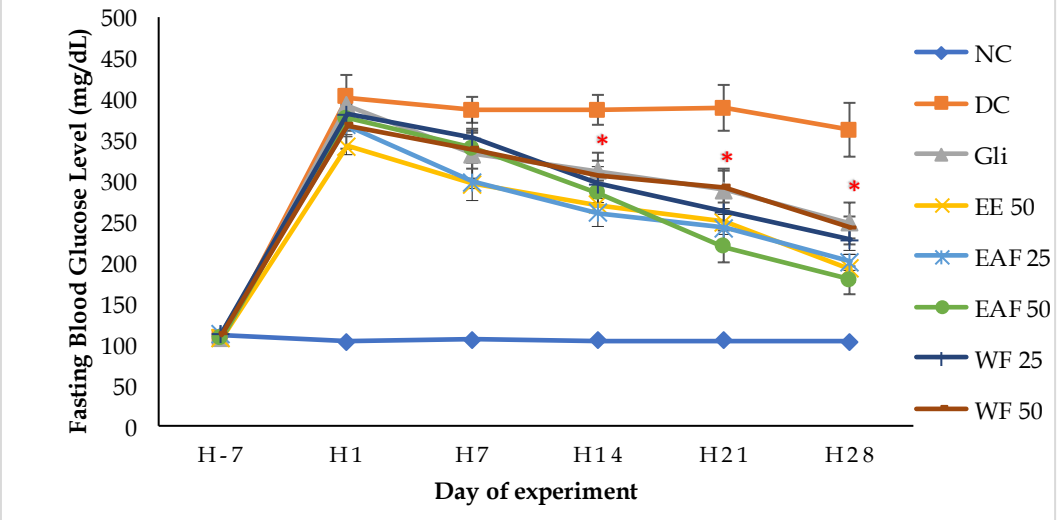

Figure 1. Average of fasting blood glucose of treated mice.

H-7: day 1 first level of blood glucose (STZ administration with dose 100 mg/kg BW, i.p); H1: day 7 STZ induction; H7, H14, H21, and H28 the 7th-28th day treatment time with normal control (NC), diabetic control (DC), glibenclamide with dose 0.65mg/kg BW (Gli); ethanol extract (EE), ethyl acetate fraction (EAF) and water fraction (WF). *p<0.05 significant differences with respect to diabetic control.

Table 1. Effect ethanol extract *H. surattensis* leaves and its fraction on insulin levels and HOMA-β.

Group	Insulin (mIU/L)	Increase of insulin secretion (%)	HOMA-β (%)
Normal control	9.16 ± 0.87^{b}	60.14	80.32 ± 4.65^{d}
Diabetic control	5.72 ± 0.48^{a}	-	4.82 ± 0.37^{a}
DM + glibenclamide	8.04 ± 0.56^{ab}	40.56	14.26 ± 0.79^{b}
DM + EE 50	8.36 ± 0.89^{ab}	46.15	23.06 ± 1.84^{c}
DM + EAF 25	7.49 ± 0.52^{ab}	30.94	19.96 ± 1.05^{bc}
DM + EAF 50	8.04 ± 1.22^{ab}	40.56	25.66 ± 2.96^{c}
DM + WF 25	6.86 ± 0.88^{ab}	19.93	14.10 ± 1.46^{b}
DM + WF 50	7.16 ± 0.95^{ab}	25.17	13.66 ± 1.42^{b}

Values are given as mean ± SEM (n = 6) followed by different lowercase superscript letters that are significantly different at $p<0.05$ according to the Duncan test. DM: Diabetes mellitus; EE: ethanol extract; EAF: ethyl acetate fraction; WF: water fraction.

Table 2. Effect ethanol extract *H. surattensis* leaves and its fraction on the area of the islet, the total number of islet, and number β cells of the pancreas.

Group	Area of islets (μm²) × 1000	Total number of islet	Number β-cells
Normal control	5.056 ± 0.323^{c}	5.33 ± 0.88^{c}	70.33 ± 2.33^{e}
Diabetic control	0.549 ± 0.113^{a}	0.67 ± 0.33^{a}	10.00 ± 0.58^{a}
DM + glibenclamide	1.690± 0.238^{ab}	2.00 ± 1.00^{ab}	20.67 ± 1.45^{b}
DM + EE 50	3.908 ± 0.348^{c}	3.33 ± 0.67^{bc}	31.33 ± 2.85^{c}
DM + EAF 25	2.418 ± 0.712^{b}	1.33 ± 0.33^{ab}	39.67 ± 4.06^{d}
DM + EAF 50	2.201 ± 0.259^{b}	2.00 ± 0.00^{ab}	44.00 ± 1.15^{d}
DM + WF 25	1.418 ± 0.279^{ab}	1.33 ± 0.33^{ab}	20.67 ± 3.18^{b}
DM + WF 50	1.521 ± 0.499^{ab}	1.67 ± 1.20^{ab}	24.67 ± 0.67^{bc}

Values are given as mean ± SEM (n=3) followed by different lowercase superscript letters that are significantly different at $p<0.05$ according to the Duncan test. DM: Diabetes mellitus; EE: ethanol extract; EAF: ethyl acetate fraction; WF: water fraction.

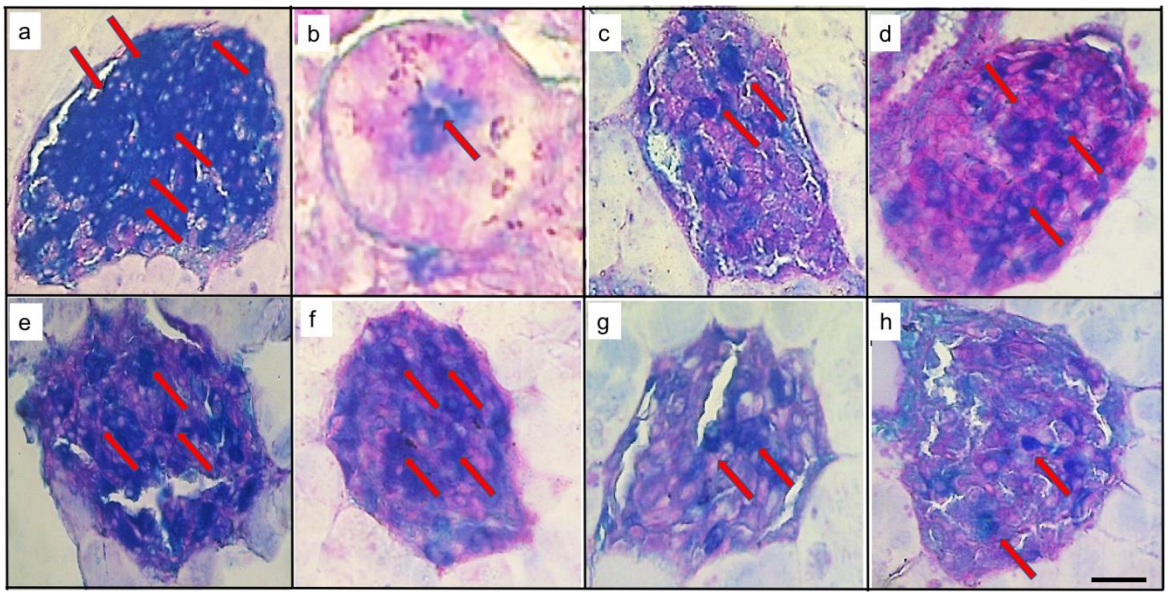

Figure 2. Histological alteration of pancreatic islets in mice after 28 days of the treatment (Gomori staining 400×).

(A) Normal control; **(B)** Diabetic control; **(C)** DM + glibenclamide; **(D)** DM + EE 50; **(E)** DM + EAF 25; **(F)** DM + EAF 50; **(G)** DM + WF 25; **(H)** DM + WF 50 (Scale bar 50 µm; ⟸: β cell). DM: Diabetes mellitus; EE: ethanol extract; EAF: ethyl acetate fraction; WF: water fraction.

DISCUSSION

In this study, STZ induction was used to create experimental diabetes in mice. STZ works by forming highly reactive free radicals that can damage cell membranes, proteins, deoxyribonucleic acid (DNA), and disrupt insulin production (Wilson et al., 1988). When the DNA is damaged, it activates poly ADP-ribosylate and eventually, free radicals are forming. Furthermore, this condition damaging pancreatic β-cells. Specifically, the STZ binds to GLUT-2, which facilitates the entry of STZ into the cytoplasm of pancreatic β-cells, increasing mitochondrial depolarization; as a result, the inclusion of Ca^{2+} ions followed by excessive energy use resulting in a lack of energy in the cell. This mechanism triggers insulin production to be disrupted, resulting in insulin deficiency, which leads to all the glucose, not whole processed, resulting in increased glucose levels in the body (Szkudelski, 2001). Increased glucose levels induce other metabolic disorders and produce free radicals. In diabetic conditions, β-cell dysfunction occurs against insulin secretion stimuli by increasing blood glucose levels. Elevated blood glucose (hyperglycemia) and free fatty acids stimulate the formation of reactive oxygen species (ROS), reactive nitrogen species (RNS), and oxidative stress (Banerjee and Vats, 2014). This can interfere with pancreatic β-cells and insulin resistance, so that it can worsen the condition of diabetes.

The administration of extracts and active fractions (ethyl acetate fraction and water fraction) of *H. surattensis* leaves can significantly reduce FBG better than glibenclamide as a standard antidiabetic drug (based on the results in Fig. 1). One of the effects is due to the stimulation of β-cells of the pancreas to secrete insulin. This can be seen in the measurement of insulin levels, where there was an increase of insulin levels in the group given the extract and the active fraction of the leaves of *H. surattensis*, which was significant compared to the diabetic control (Table 1). Insulin deficiency in STZ-induced mice is caused by pancreatic β-cell damage. Insulin deficiency can be caused by decreased ability of insulin in peripheral tissues (insulin resistance) and β-cells dysfunction, which results in the pancreas not being able to produce

enough insulin to compensate for insulin resistance (Cerf, 2013).

The ability to regenerate pancreatic β-cells in diabetic mice given extracts and active fractions of leaves *H. surattensis* causes an increase in insulin levels and a decrease in FBG. This can be seen in the value of HOMA-β diabetic control, which differed significantly compared to the other treatment groups (Table 1). The improvement of pancreatic tissue can consequently increase insulin secretion so that glucose blood can be absorbed into cells and changed into energy or stored as glycogen. This is also in line with the correlation analysis between blood glucose levels and insulin, which negatively correlates to an increase in plasma insulin levels that will reduce blood glucose levels (Röder et al., 2016).

Pancreatic histology in the diabetic control group showed irregular endocrine cell composition, changes in morphological structure, empty islet of Langerhans due to degeneration of pancreatic β-cells, reduced the islet of Langerhans, and reduction of β-cell counts. However, in the group given extracts and active fractions of *H. surattensis* leaves, there was a significant increase in the number of pancreatic β-cells compared to diabetic control (Table 2). These improvements included endocrine cells on Langerhans' islet, which began to regenerate towards normal forms, although some endocrine cells degenerated. Removal or reduction of free radicals (antioxidant effects) in β-cells may reduce β-cell damage and improve insulin function (Newsholme et al., 2019). Blood glucose levels in diabetic mice can be reduced (hypoglycemic effect).

Our study results indicate the ability to increase insulin secretion due to repair or regeneration of the pancreatic tissue, especially pancreatic beta cells after being given the extract and active fraction of *H. surattensis* leaves. This effect is also related to antioxidant compounds, mainly phenolic and flavonoids found in *H. surattensis*.

Phenolic compounds and flavonoids are known to have antidiabetic and antioxidant effects (Vinayagam and Xu, 2015; Eid and Haddad, 2017; Sarian et al., 2017). Our previous study found that *H. surattensis* leaves, especially ethyl acetate fraction, contained high phenolic and flavonoid compounds and had an antioxidant activity based on *in vitro* experiment. LCMS/MS analysis demonstrated the presence of major compounds such as quercetin, kaempferol, morin, and trifolin (Yuliet et al., 2020).

CONCLUSIONS

The optimal dose of ethanol extract and active fraction from *H. surattensis* leaves was 50 mg/kg BW to increase insulin secretion and repair the pancreatic tissue damage on diabetic mice. Improvement in the histological picture of the pancreatic tissue mostly because of the potential to regenerating pancreatic beta cells. Therefore, it could increase insulin secretion and increase glucose metabolism in STZ-induced diabetic mice. Further studies are warranted to reveal responsible compounds and establish clinical uses as a nutraceutical supplement for diabetes.

CONFLICT OF INTEREST

The authors declare no conflicts of interests.

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AUTHOR CONTRIBUTION:

Contribution	Yuliet	Sukandar EY	Atik N	Adnyana IK
Concepts or ideas	x	x		x
Design	x	x	x	
Definition of intellectual content	x			
Literature search	x	x		
Experimental studies	x		x	
Data acquisition	x		x	x
Data analysis				x
Statistical analysis		x		x
Manuscript preparation	x		x	
Manuscript editing	x	x		
Manuscript review	x	x	x	x

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