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The effect of perennial sow-thistle (*Sonchus arvensis*) leaf extract on blood glucose and plasma insulin levels of diabetic mice

FLORENSIA SYNTESA HARYTA[1], SHANTI LISTYAWATI♥, ARTINI PANGASTUTI
Program of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Surakarta 57126, Central Java, Indonesia. Tel./fax.: +62-271-663375, ♥email: shantilistyawati@staff.uns.ac.id

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Abstract. *Haryta FS, Listyawati S, Pangastuti A. 2021. The effect of perennial sow-thistle (*Sonchus arvensis*) leaf extract on blood glucose and plasma insulin levels of diabetic mice. Cell Biol Dev 5: 63-69.* The prevalence of diabetes mellitus (DM) has recently increased, thus encouraging drug exploration, especially from natural compounds. One of the potential medicinal plants to reduce blood glucose levels in people with diabetes mellitus is perennial sow-thistle (*Sonchus arvensis*). This study was conducted to determine the effect of perennial *S. arvensis* leaf extract on mice's blood glucose levels and plasma insulin levels induced by streptozotocin. This study used six treatment groups, namely: normal, positive control (acarbose 0.13 mg/20ggBW), negative control (aquadest), and treatment group of perennial *S. arvensis* leaf extract at doses of 50 mg/kgBW, 100 mg/kgBW, and 150 mg/kgBW. The treatments were done by oral administration daily for 14 days. Measurements of blood glucose levels and postprandial plasma insulin levels were measured at 0, 60, and 120 minutes. The test animals were monitored for 14 days, and fasting blood glucose levels were measured on the 7th and 14th days. Data analysis using one-way ANOVA, and if there is a significant difference, it is continued with Duncan's Range Test (DMRT). The results showed that perennial *S. arvensis* leaf extract could reduce postprandial blood glucose elevation and blood glucose levels in diabetic mice, which were monitored for 14 days. The most effective dose is 150 mg/kgBW. Perennial *S. arvensis* leaf extract increased postprandial plasma insulin levels at 60 minutes; the most effective dose is 50 mg/kgBW.

Keywords: blood glucose, diabetes mellitus, perennial sow-thistle leaves, *Sonchus arvensis*, plasma insulin

INTRODUCTION

Diabetes mellitus (DM), also known as a non-communicable disease, is a systemic disease in Indonesia with a high prevalence and mortality rate. Diabetes mellitus is a disorder in which blood glucose levels are consistently higher (hyperglycemia) than normal due to insulin deficiency or inadequate insulin activity (World Health Organization 2003). Insulin therapy, synthetic antidiabetic medicines such as sulfonylureas, biguanides, glinides (Mohammed et al. 2016), metformin, and thiazolidinedione are used to treat diabetes mellitus in general (Meenatchi et al. 2017). However, these synthetic treatments have limitations, including side effects such as hypoglycemia, anemia, weight gain, and heart failure (Acevedo et al. 2017). Considering the impact caused by synthetic drugs, there is research on herbal medicines derived from nature.

The current paradigm in terms of doing the treatment is back to nature, meaning a return to traditional medicine. Plants are a source of phytochemicals for alternative medicine and antidiabetic functional food. One of the medicinal plants that have the potential to treat diabetes mellitus is the perennial sow-thistle (Latin: *Sonchus arvensis*; Indonesian: *tempuyung*). The results of phytochemical screening on perennial *S. arvensis* leaves showed the presence of flavonoids, phenolics, and steroids (Devi et al. 2019). The flavonoid content in perennial *S. arvensis* leaves is dominated by orientin, quercetin, and kaempferol (Khan 2012). The result of the study of percentage inhibition of infusion of perennial *S. arvensis* leaf samples on the activity of α-amylase and α-glucosidase enzymes showed that these plants have potential as antidiabetics, especially in inhibiting the activity of α-amylase enzymes because they contain active flavonoid compounds (Devi et al. 2019). Inhibition of the α-amylase enzyme can delay and prolong carbohydrate digestion, cause a decrease in the rate of glucose absorption and prevent an increase in postprandial blood glucose levels (Sales et al. 2012). An exploratory ethnopharmacology survey in the community in Dawuan District, Subang Regency, West Java Province found that perennial *S. arvensis* leaves were used as a medicinal plant for diabetes or Diabetes mellitus by the local community (Mulyani et al. 2020). This study examined the activity of perennial *S. arvensis* leaves extract as an antidiabetic using a streptozotocin-induced mouse (*Mus musculus*) animal model.

MATERIALS AND METHODS

Place and time

This research was carried out at the Central Laboratory of Pharma-Veterinary (PUSVETMA-Laboratorium Pusat Veteriner Farma) Surabaya from November 2020 to January 2021.

Materials

This study used 24 male (*Mus musculus*) mice with an average weight of 25-30 g, 2-3 months old, healthy, and

from the Central Laboratory of Pharma-Veterinary (PUSVETMA), Surabaya, Indonesia. The code of ethics (Ethical clearance) for the use of test animals has been approved by the Health Research Ethics Committee of the Faculty of Medicine, Sebelas Maret University, with the number: 151/UN27.06.6.1/KEPK/EC/2020 on 21 September 2020. The materials used to manufacture the extract are perennial *S. arvensis* leaf simplicia from the Center for Research and Development of Plants and Traditional Medicines (B2P2TOOT), Tawangmangu, Indonesia, aquadest. The chemicals used were distilled water, acarbose, streptozotocin, citrate buffer pH 4.5, and starch soluble.

Experiment design

This study used a completely randomized design (CRD) with six treatments and 4 replications for each treatment.

Procedures

Test animal preparation

Mice were acclimatized for one week while still being fed and watered, so the mice quickly adapted to the environment and prevented stress.

Making diabetic mice

After 7 days of acclimatization, mice fasted for 8 hours and then measured blood glucose levels and body weight. Next, mice were induced by streptozotocin at a dose of 150 mg/kgBW/day for 2 days intraperitoneally (abdominal cavity) in 5 groups of test animals (except the normal group) (Ria et al. 2015). Streptozotocin (STZ) was dissolved in citrate buffer pH 4.5. On the 3rd day after STZ induction, the blood glucose levels in mice were measured using a glucometer and a strip. Measurement of blood glucose levels by taking blood samples in the veins of the eyes of mice using a microhematocrit tube, the blood that comes out is dripped onto a strip. The blood glucose concentration value will appear in mg/dL units on the screen, and when the blood glucose concentration value is above 200 mg/dL then mice are considered to have diabetes mellitus (Malole and Purnomo 1989). After the mice were declared diabetic, the weight of the mice was also measured and marked on the back and tail of the mice with a permanent marker.

Preparation of 5% perennial Sonchus arvensis *leaf extract with the infundation method*

The S. arvensis leaf simplicia weighed as much as 50 grams; 1,000 mL of distilled water was put into the pot in the infusion, while the outer pot of the infusion was filled with water until it hit or immersed the pot in the infusion. Wait for the distilled water to boil (temperature = 90^{o}C); the perennial *S. arvensis* leaf simplicia is put into a deep pot and boiled for 15 minutes, stirring every 5 minutes. The decoction of the perennial *S. arvensis* leaves is filtered with an anti-bacterial filter while still hot; because the filter results do not reach 1,000 mL, sterile distilled water is added through the filter until it is filled with 1,000 mL. The infusion was stored in a refrigerator at 8 ^{o}C.

Preparation of 5% acarbose suspension and 20% starch solution

Acarbose in tablet form was crushed until smooth, weighed as much as 50 mg, dissolved in 10 mL of sterile distilled water, and poured little by little into the mortar. Next, the acarbose solution was put into a flacon bottle. Finally, the starch solution was made by dissolving 8 grams of starch in 40 mL of sterile distilled water and homogenizing it with a hot plate and a magnetic stirrer until dissolved.

Test animal treatment

The test animals were divided into six treatment groups. The treatment for the test animals was given orally. The grouping of test animals is shown in Figure 1.

Measurement of postprandial blood glucose level

The 0th-minute data was utilized to measure postprandial blood glucose levels, which were obtained by drawing blood from the ocular vein. First, the test animals were given 0.5 mL of soluble starch feed orally, then treated, as shown in Figure 1. Then, using blood from the ocular vein, blood glucose levels were assessed again at 60 and 120 minutes.

Measurement of postprandial plasma insulin levels

Measurement of postprandial plasma insulin levels has the same mechanism and blood sample data as the measurement of postprandial blood glucose levels above. Blood samples were tested using an INSULIN ELISA kit mouse and a microplate reader. The blood samples were centrifuged at 3,500 rpm for 10 minutes, and plasma samples containing insulin (antigen) were obtained above other blood components. Once obtained, the plasma insulin levels were measured according to the manual on the INSULIN ELISA kit mouse.

Monitoring blood glucose levels in test animals for 14 days

The experiment of giving perennial *S. arvensis* leaf extract was carried out for 14 days. Monitoring was carried out by measuring the weight of the mice every day to determine the dose of the test material. After the mice were confirmed to have eaten their food intake (CP 511), they were treated according to the group and transferred to a new cage to prevent any remaining feed. Fasting blood glucose levels were measured on day 7 and day 14, and blood was taken from the caudal vein.

Data analysis

Analysis of the data results using one-way ANOVA (One-Way ANOVA) with a 95% confidence level and continued with the Duncan Multiple Range Test (DMRT) test.

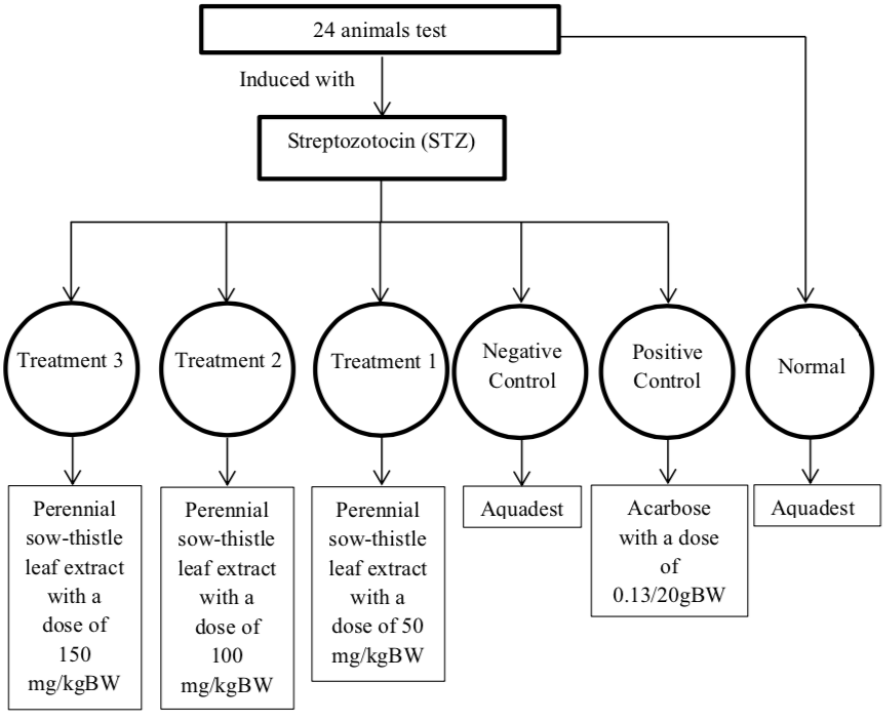

Figure 1. Test animal treatment group

RESULT AND DISCUSSION

Making diabetic mice with streptozotocin

The manufacture of diabetic mice was carried out by inducing streptozotocin at a dose of 150 mg/kgBW/day for 2 days, which can selectively destroy pancreatic β-cells to increase blood glucose levels. Blood glucose levels before and after streptozotocin induction are presented in Table 1.

After the test animals were induced by streptozotocin, blood glucose levels increased in the range of 412 – 539.75 mg/dL. Therefore, the mice were declared to have successfully developed diabetes mellitus (>200 mg/dL) due to the influence of streptozotocin (Malole and Purnomo 1989). The mechanism of action of streptozotocin begins with entry into pancreatic beta cells through the glucose transporter (GLUT-2), which causes DNA damage due to DNA alkylation in the methyl nitrosourea section with the formation of H_2O_2 and inflammatory reactions, resulting in DNA fragmentation. DNA damage activates the enzyme poly (ADP ribose) synthase, an enzyme needed to repair DNA damage. This enzyme requires NAD as its substrate, causing cellular NAD+ and ATP depletion and inhibiting insulin synthesis and secretion. The decrease in ATP synthesis is indicated by dephosphorylation or withdrawal of phosphate groups which produces more substrate for the xanthine oxidase-catalyzed reaction that forms superoxide radicals. The formation of superoxide radicals, resulting in hydrogen peroxide and hydroxyl radicals, is the main cause of pancreatic β-cell damage. In addition, the presence of the N-methyl-N-nitrosourea side chain can release nitric oxide, inhibiting aconitase activity, resulting in mitochondrial dysfunction and causing apoptosis and β-cell necrosis, which eventually causes hyperglycemia (Szkudelski 2001).

The effect of perennial *Sonchus arvensis* leaf extract on postprandial blood glucose levels surges

Based on table 2, there was a spike in blood glucose levels from minute 0 to minute 60. The positive control group had the lowest spike in blood glucose levels because it used acarbose which has a mechanism of action by inhibiting the pancreatic α-amylase enzyme, which works to competitively hydrolyze polysaccharides in the lumen of the small intestine, so there is no spike in blood glucose levels that are too high. The DMRT (Duncan's Multiple Range Test) analysis showed no significant difference between the perennial *S. arvensis* leaf extract group and the positive control group (acarbose). That proves that perennial *S. arvensis* leaf extract has a mechanism of action like acarbose, which can inhibit the α-amylase enzyme in breaking down amylose starch into glucose.

Table 1. Average blood glucose levels of mice before and after streptozotocin induction

Average blood glucose level (mg/dL)	
Before streptozotocin induction	**After streptozotocin induction**
139.50	501.75
120.50	412.00
138.50	539.75
117.50	481.00
138.75	514.50

The α-amylase enzyme has the mechanism of action of initial hydrolysis of the α-(1,4) glycosidic bonds in starch into shorter oligosaccharides with lower molecular weights, such as glucose and maltose. Inhibition of the action of the α-amylase enzyme on the absorption of starch after meals (postprandial) can interfere with or slow down the breakdown of starch, reducing the availability of glucose and maltose and affect the glucose-insulin system, which slows absorption and reduces blood glucose concentrations so spikes can be more controlled. The starch solution is a substrate that can bind to the gap between the carboxyl ends of the A and B domains of the α-amylase enzyme (Souza and Magalhaes 2010). The α-amylase inhibitor can work by imitating the transition position of the pyranosidic unit from the substrate, so it is suspected that the inhibition mechanism is in the form of competitive inhibition (Kim et al., 2008). The flavonoid content in S. arvensis leaves is dominated by orientin, quercetin, and kaempferol (Khan 2012). Tadera et al. (2006) revealed that one of the flavonoid compounds that have the potential to inhibit the α-amylase enzyme is quercetin. The inhibition of the activity of the α-amylase enzyme is related to the hydroxyl group possessed by quercetin. (Yuang et al. 2014). Several studies state that flavonoid compounds also have the inhibitory power of carbohydrate hydrolyzing enzymes. Quercetagetin compounds can inhibit the action of α-amylase enzymes, hydrogen bonds formed between the carboxyl group of the Asp197 side chain on the active site of the α-amylase enzyme and the hydroxyl groups on ring B of quercetagetin can bind covalently, forming a stable bond between quercetagetin and the α-amylase enzyme which results in substrate (starch solution) can no longer bind to the active site of the enzyme so that the product cannot be formed (Piparo et al. 2008). Non-existing products cause glucose absorption in the blood to be controlled so that spikes in blood glucose levels after eating in people with diabetes mellitus can be prevented. The results follow the research by Devi et al. (2019), which states that perennial *S. arvensis* leaves can inhibit the activity of the α-amylase enzyme because it contains phytochemical compounds, particularly flavonoid active compounds.

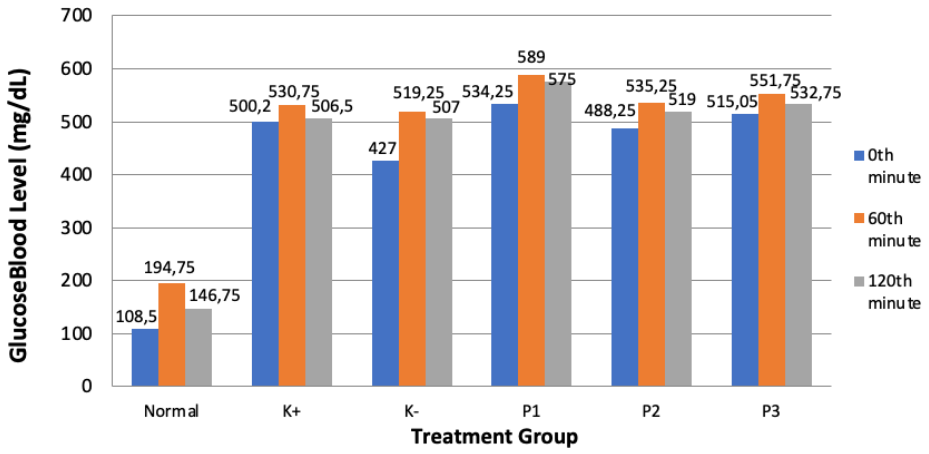

Figure 2 Measurement of postprandial blood glucose levels from 0-120 minutes in mice given starch solution and treatment after the 0th minute. Note: K+ (Positive Control, acarbose), K- (Negative Control, aquadest), P1 (Treatment 1, *S. arvensis* leaf extract with a dose of 50 mg/kgBW), P2 (Treatment 2, *S. arvensis* leaf extract with a dose of 100 mg/kgBW), P3 (Treatment 3, *S. arvensis* leaf extract with a dose of 150 mg/kgBW)

Table 2. Postprandial blood glucose levels at 0-120th minutes

Treatment group	Average difference in blood glucose levels (mg/dl)	
	Minutes 0–60	Minutes 60-120
Normal	86.25[b]	48.00[b]
K + (Acarbose)	30.50[a]	24.25[a]
K – (Aquadest)	92.25[b]	12.25[a]
P1 (*S. arvensis* Leaf extract with a dose of 50 mg/kgBW)	54.75[a]	14.00[a]
P2 (*S. arvensis* Leaf extract with a dose of 100 mg/kgBW)	47.00[a]	16.25[a]
P3 (*S. arvensis* Leaf extract with a dose of 150 mg/kgBW)	36.25[a]	19.00[a]

Note: K + (Positive Control), K – (Negative Control). In the same column, superscripts with different letters indicate significant differences between treatment groups.

The effect of administering perennial *Sonchus arvensis* leaf extract on postprandial plasma insulin levels

Based on the DMRT (Duncan's Multiple Range Test) analysis results, the difference in plasma insulin levels from 0-60 minutes in the normal group significantly differed from the other treatment groups. That is because the normal group is the group with the condition of the test animals in a healthy condition (not having diabetes), so they can raise the highest plasma insulin levels. The positive control and treatment groups of perennial *S. arvensis* leaf extract were not significantly different, but the group differed significantly from the negative control groups. That indicates that the positive control group and the perennial *S. arvensis* leaf extract treatment group had the same ability to increase plasma insulin levels. Still, they were not as effective as the normal group. The negative control group was significantly different from the other treatment groups, indicating that distilled water could not increase plasma insulin levels like other treatment groups. The data analysis results of the difference in plasma insulin levels from 60-120 minutes showed that the negative control significantly differed from the normal treatment group, the positive control group, and the perennial *S. arvensis* leaf extract treatment. That is because the negative control blood glucose level from 60-120 minutes decreased the least compared to the other treatment groups, so plasma insulin in the negative control was still trying to restore blood glucose levels to normal (0th minute).

The administration of streptozotocin is known to cause pancreatic β-cell necrosis, so insulin synthesis and secretion are disrupted. Increased blood glucose levels can stimulate pancreatic cells to secrete insulin. Insulin secretion is biphasic, consisting of phase 1 and phase 2. Phase 1 occurs after stimulation of pancreatic β-cells and lasts briefly (about 10 minutes) and is followed by a continuous phase 2. Phase 2 insulin secretion lasts relatively longer; how high the peak can be determined by how much blood glucose levels are at the end of phase 1 (Jensen et al., 2008). Insulin secretion begins with the entry of glucose through the glucose transporter 2 (GLUT-2) to enter the pancreatic β-cells. Glucose undergoes a process of glycolysis and phosphorylation in the cell, increasing ATP production. The ATP molecules formed to inhibit the ATP-sensitive K+ channel, resulting in the depolarization of the plasma membrane, then the opening of a voltage-gated Ca^{2+} channel. This situation allows the entry of Ca^{2+} ions, causing an increase in intracellular Ca^{2+} ions, which function to activate insulin secretion. Perennial *S. arvensis* leaves can increase insulin secretion at 60 minutes, presumably because the leaves are dominated by flavonoids, especially quercetin and kaempferol, which can increase the induction of insulin secretion by glucose in functioning pancreatic cells (Gupta et al. 2012). Kaempferol can increase ATP production, causing the closure of ATP-sensitive K^+ channels and depolarization of cell membranes (Zhang and Liu 2011). That is supported by research conducted by Bermont et al. (2020), which provides evidence that kaempferol can increase secretion in a pancreatic-cell model by increasing mitochondrial Ca^{2+}. Kittl et al. (2016) reported that there was a 50% inhibition of ATP-sensitive K^+ channels in pancreatic β-cells due to quercetin administration. This effect increases Ca^{2+} levels, which trigger insulin for insulin exocytosis (Bardy et al. 2013).

Correlation of postprandial plasma insulin levels to postprandial blood glucose levels

Based on the results above, the R-value is 0.303, close to 0 and away from 1, which indicates no strong or weak correlation between plasma insulin levels and blood glucose levels. The R^2 value is 9.2%, which indicates that postprandial plasma insulin levels influence 9.2% of postprandial blood glucose levels and other factors influence the remaining 90.8%. Another factor affecting postprandial blood glucose levels in this study is the inhibitory effect of α-amylase enzymes carried out by acarbose (positive control) and flavonoid compounds in the treatment of perennial *S. arvensis* leaf extract to reduce postprandial spikes in blood glucose levels. The regression coefficient value is negative, indicating that the correlation between plasma insulin levels and blood glucose levels goes the opposite. If plasma insulin levels increase, blood glucose levels decrease.

The effect of administering perennial *Sonchus arvensis* leaf extract for 14 days on blood glucose levels

Observation of perennial *S. arvensis* leaf extract for 14 days, counted from the first day of administration of perennial *S. arvensis* leaf extract. This observation was conducted to observe whether perennial *S. arvensis* leaf extract could effectively reduce blood glucose levels for 14 days in diabetic mice. Mice were fed with type CP 511 feed made from corn, soybeans, bran, and wheat. The results of measuring blood glucose levels on the 7th and 14th days are presented in table 4.

The one-way ANOVA test $P<0.05$ showed a significant difference between the treatment groups on the 7th and 14th days. All groups except the negative control group experienced a significant decrease in blood glucose levels because distilled water could not repair pancreatic β-cells that had necrosis due to streptozotocin administration. The positive control group using acarbose on day 14 can reduce blood glucose levels until the mice are no longer in the diabetes category (> 200 mg/dL) (Malole and Purnomo 1989).

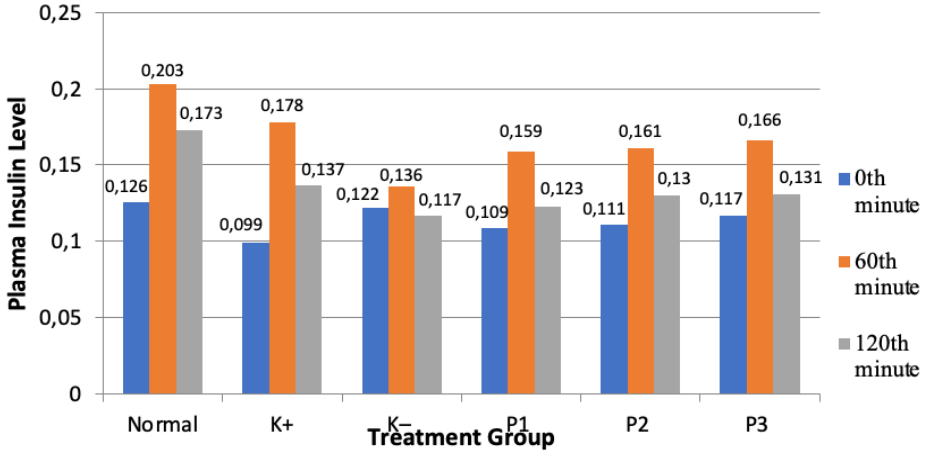

Figure 3. Postprandial plasma insulin levels from 0-120 minutes in mice given starch solution and treatment after 0-minute measurement. Note: K+ (Positive Control, acarbose), K- (Negative Control, aquadest), P1 (Treatment 1, *S. arvensis* leaf extract with a dose of 50 mg/kgBW), P2 (Treatment 2, *S. arvensis* leaf extract with a dose of 100 mg/kgBW), P3 (Treatment 3, *S. arvensis* leaf extract with a dose of 150 mg/kgBW)

Table 3. Postprandial plasma insulin levels at 0-120th minutes

Treatment group	Average difference in blood glucose levels (mg/dl)	
	minutes 0–60	minutes 60-120
Normal	0.076c	0.029b
K + (Acarbose)	0.055b	0.041b
K – (Aquadest)	0.036a	0.018a
P1 (*S. arvensis* Leaf extract with a dose of 50 mg/kgBW)	0.050b	0.036b
P2 (*S. arvensis* Leaf extract with a dose of 100 mg/kgBW)	0.049b	0.031b
P3 (*S. arvensis* Leaf extract with a dose of 150 mg/kgBW)	0.048b	0.035b

Note: K + (Positive Control), K – (Negative Control). In the same column, superscripts with different letters indicate significant differences between treatment groups.

Table 4. Average blood glucose levels on the 7th and 14th days in mice that were treated for 14 days

Treatment group	Average blood glucose level (mg/dL)	
	Day-7	Day-14
Normal	117.00a	105.75a
K + (Acarbose)	299.75b	185.50b
K – (Aquadest)	543.25f	581.25f
P1 (*S. arvensis* Leaf extract with a dose of 50 mg/kgBW)	501.25e	413.75e
P2 (*S. arvensis* Leaf extract with a dose of 100 mg/kgBW)	427.00d	346.00d
P3 (*S. arvensis* Leaf extract with a dose of 150 mg/kgBW)	334.25c	231.75c

Note: K + (Positive Control), K – (Negative Control). In the same column, superscripts with different letters indicate significant differences between treatment groups.

The administration of acarbose in diabetic patients has improved pancreatic-cell function (Tyagita et al. 2021). Increased blood glucose levels increase insulin secretion to improve the function of the remaining pancreatic β-cells (Chen et al. 2014). A significant decrease was also found in the perennial *S. arvensis* leaf extract group at doses of 50 mg/kgBW, 100 mg/kgBW, and 150 mg/kgBW. This decrease was thought to be due to the dominant flavonoid group in the S. arvensis leaf extract. Flavonoids are protective against the damage experienced by pancreatic β-cells and can increase insulin sensitivity. In addition, flavonoids have properties as antioxidants. Antioxidants can reduce Reactive Oxygen Species (ROS); during the formation of ROS, oxygen and electrons will bind to free electrons that come out due to leakage of the electron chain, and the reaction will produce ROS in the mitochondria (Annisa et al. 2014). Free radicals originate from the mechanism of destruction by streptozotocin. Antioxidants in flavonoids can donate hydrogen atoms so that flavonoids are oxidized and bind to free radicals, making free radicals become more stable compounds (Ajie 2015). It was also revealed by Sujono et al. 2014 that as antioxidants, flavonoids work by inhibiting free radicals through redox reactions that can reduce hydrogen donors

and reactive oxygen. One of the compounds from the flavonoid group is quercetin, which can stimulate progenitor cells in the urinary tract pancreas to differentiate to form new islets of Langerhans cells in diabetic rats (Riffai et al. 2012). Research conducted by Khan (2012) states that perennial *S. arvensis* leaves can be used as an effective and safe source of antioxidants as well as ethnomedical that can be developed into drugs because of the presence of flavonoid compounds in the form of kaempferol, myricetin, and quercetin. Based on the results in table 4, the most effective group of perennial *S. arvensis* leaf extract in lowering blood glucose levels is the perennial *S. arvensis* leaf extract at 150 mg/kgBW dose.

This study concludes that perennial *S. arvensis* leaf extract can reduce spikes in postprandial and blood glucose levels for 14 days. The most effective dose of perennial *S. arvensis* leaf extract to reduce blood glucose levels is a dose of 150 mg/kgBW. In addition, perennial *S. arvensis* leaf extract can increase plasma insulin levels at 60 minutes, with an effective dose of 50 mg/kgBW.

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