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SUNGKAI LEAVES (*PERONEMA CANESCENS*) AMELIORATES THE PLASMA GLUCOSE PROFILE AND THE PANCREATIC HISTOPATHOLOGICAL REGENERATION OF DIABETIC WISTAR RATS

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ABSTRACT

This study aims to test how effective oral infusion of sungkai leaves is on the profile of changes in blood plasma glucose levels and regeneration of pancreatic tissue in diabetic Wistar rats. This research method is a proper experimental design with a randomized pre-post-test design with a control group. The study's results illustrate differences in mice's blood sugar levels before and after the intervention, showing a decrease in blood sugar levels in the K(+), P-1, and P-2 groups, while in the K(+), P-1, and P groups -2. The K(-) group shows a decreasing trend. Apart from that, histopathological examination of the pancreas also showed improvement in each treatment group with a decreasing percentage of pathological events. In conclusion, blood plasma glucose examination showed a significant difference, positively affecting diabetes mellitus, while the rate of pancreatic tissue injury showed a similar form.

Keywords: Sungkai Leaves, Plasma Glucose, Pancreatic Regeneration

INTRODUCTION

Diabetes Mellitus (DM) as a metabolic and glucose homeostasis disorder is triggered by the failure of pancreatic cells to produce sufficient or quality regulatory (hormonal) protein compounds. Diabetic condition causes not only premature death worldwide, but also blindness, heart disease, and kidney failure. The American Diabetes Association (ADA) and the International Diabetes Federation (IDF) estimated that at least 463 million people aged 20-79 years worldwide suffer from DM in 2019 with a prevalence rate of 9.3%. Based on gender, IDF estimated the prevalence of diabetes in 2019, and the following year will reach a value of 9% and above where men have a greater percentage of 0.65% (IDF, 2021; Galicia-Garcia et al., 2020). There are two forms of curative treatment for DM, namely pharmacological and non-pharmacological routes. Some literature studies state that pharmacological therapy for DM has side effects on several organs of the body (heart, kidney, liver, and others) (Hill-Briggs et al., 2021). Problems that arise in chronic DM can lead to complications in several vital organs of the body that increase the risk of death. In addition, diabetes antidiabetic therapy that can last a long time poses a risk of damage and organ failure mentioned above. DM also has comorbid risks of causing non-medical disorders including socio-economic and psychological (Hill-Briggs et al., 2021; Ahriyasna et al., 2023).

As a country that has a diversity of nutritious plant resources, Indonesian plants, and foods also have the potential to create various non-pharmacological ingredients. Some ingredients that have high antioxidant content should be consumed by people with degenerative diseases, especially diabetes, one of which is Sungkai leaf (*Paronema canescens*). This plant is a wild plant, and because of its economic value, people cultivate it as a medicinal plant. Sungkai leaves contain antioxidants such as flavonoids (the largest content), polyphenols, tannin alkaloids, and sposin. Flavonoids are believed to lower blood glucose levels with their ability as antioxidants and have many roles in preventing diabetes and its complications, which are protective roles to ß-cell damage as insulin-producing agents and increase insulin sensitivity. Antioxidants suppress ß-cell apoptosis without altering their proliferation (Ahriyasna & Primal, 2023). As secondary metabolites of polyphenols, flavonoids also provide bioactive effects including anti-viral, anti-inflammatory agent, cardioprotective, antidiabetic, anti-cancer, anti-aging, and antioxidant. Flavonoids as secondary metabolites have antioxidant activity by donating electrons or hydrogen to prevent the formation of free radicals. The antioxidant activity of Sungkai leaves has a total flavonoid content of 1.057±0.002 mg EK/g and has an antioxidant activity with an IC50 value of 44,933 ppm, so it is classified as a very active antioxidant category (Yunarto et al., 2022).

Compounds containing flavonoids and ethanol are expected to help improve diabetes conditions and repair affected tissues such as the pancreas, liver, and kidneys. This improves the tissue repair process in some of the experimental samples used (Naeem et al., 2022; Pratiwi et al., 2022). The number of medicinal plants in Indonesia is estimated at around 1,260 types of plants. One of them is the Sungkai plant (*Paronema canescens*) which is one of the herbal medicines found in Indonesia. According to several research references, empirically Sungkai leaves are used by some people for toothache and fever reduction. In addition, it is also used to treat malaria, colds, fever, and ringworms, used as a bath for women after childbirth, and as a mouthwash. This is because Sungkai leaves contain secondary metabolites such as alkaloids, flavonoids, and tannins (Yunarto et al., 2022; Naeem et al., 2022).

Flavonoids are included in the class of phenolic compounds in Sungkai leaves from several research results reveal flavonoids have biological activity as antiviral, antibacterial, anticancer, antioxidant, anti-inflammatory, and hepatoprotective (Naeem et al., 2022; Ahriyasna & Primal, 2023). Flavonoids are substrates for conjugating and hydrolyzing enzymes in the small intestine, liver, and colon, and all of them are conjugated via urinary excretion. Many factors affect the bioavailability of flavonoids in food, such as molecular weight which greatly affects their absorption, glycosylation, and metabolic conversion (Pratiwi et al., 2022). Antioxidants bind to free radicals thereby reducing insulin resistance by lowering Reactive Oxygen Species (ROS). Prevention of ROS by flavonoids by inhibiting the action of xanthine oxidase and nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase enzymes, and metal chelating (Fe^{2+} and Cu^{2+}) prevents redox reactions that generate free radicals (Brata & Wasih, 2021; Wilson et al., 2021; Mutia, 2022).

Hyperglycaemia causes an increase in free radicals in the body, especially ROS, which are the result of cellular metabolism from mitochondria. The formation of ROS that exceeds the antioxidant capacity can cause damage to macromolecules such as fats, proteins, DNA, and pancreatic cells. To reduce damage caused by free radicals or ROS, exogenous antioxidants are needed from the outside through the food consumed, which in the study aims the antidiabetic and anti-inflammatory antioxidants can improve blood glucose levels through cell regeneration mechanisms (Wilson et al., 2021).

Several research show tissue repair after administration of Peronema canescens in some tissues that have experienced injury, degeneration, and even necrosis. Its anti-inflammatory and analgesic properties reduce the previously formalin-induced tissue edema and modulate pain reduction (Ghasemi-Dehnoo et al., 2020; Mutia, 2022). Likewise, the antioxidant content showed an improvement in liver and kidney tissue through an increase in the mean values of ALT and AST in liver tissue, and an increase in plasma urea level, thus triggering the kidneys to excrete urea more quickly (Ghasemi-Dehnoo et al., 2020; Marques et al., 2020). In addition, the enzyme glucosidase is considered a molecular target in diabetes therapy. This enzymatic reaction also stimulates the formation of reactivity of immunological cells, especially phagocytic cells (macrophages) which are also needed in assisting regeneration and tissue protection (Dillasamola et al., 2021; Padhi et al., 2020).

Based on several research results that were cited, the extraction research reveals the antioxidant activity with an IC50 value of 44,933 ppm and found highly reactive activity. Furthermore, another study analyzed *Peronema canescens* on blood pressure and heart rate of experimental animals with hypertension. Its potential test of Sungkai young leaves on health (immune) in mice (*mus musculus*) has shown significant results on the development of immune cells. Recent research from Dillasamola et al., (2021) also used *Peronema canescens* as an immunostimulator that affects the development of macrophage cells both in-vivo and in-vitro but only shows the reactivity of immune cells without identifying the role of these macrophage immune cells in terms of numbers (Wilson et al., 2021; Ghasemi-Dehnoo et al., 2020; Dillasamola et al., 2021; Padhi et al., 2020). We believe so far, there is a few of related research involving Sungkai leaves on the hematological profile through the changes in blood glucose and pancreatic organ regeneration, especially in Indonesia. Based on the function of the bioactive components contained in Sungkai leaves, we were interested in conducting the treatment effect of steeping these leaves on the hematological profile and the pancreas regeneration in diabetes Wistar rats. This research is deemed necessary to be carried out as an effort to reduce the risk and reduce the severity of diabetes mellitus sufferers, as well as the effects of continued drug consumption.

RESEARCH METHOD

This is a true experimental study using a randomized pre-post-test with a control group design. The research was conducted from June 2022 to March 2023 in the Integrated Laboratory of the Faculty of Health Sciences and the Faculty of Pharmacy, Universitas Perintis Indonesia.

Research sample and criterion

The research sample was male white rats of the Wistar strain (*Rattus norvegicus*) aged 2-3 months with 150-250g body weight, the condition of the rats was healthy and had never been used in another experiment. The number of experimental animals per group was determined by the Federer formula (1963). Based on the formula, the sample required a minimum of 6 experimental rats in each group. With the addition of an estimated dropout of 10%, the minimum sample size required for each group is 7 rats. Thus, the total number of experimental animals needed was 28. Where every 28 samples that meet the inclusion criteria that have been set will be given a number, which is then divided into 4 groups.

Moreover, the sample criteria were rats with blood glucose levels >200 mg/dl (after being given Alloxan). Rats were excluded from the study if they were injured or disabled during the study or died during the experiment period. The feed given to the experimental animals was standard (pellets) for all treatment groups until the final stage of the study.

Research ethics and animal acclimatization

The study was conducted after passing the ethical feasibility of involving experimental animals as research subjects by the Health Research Ethics Committee (KEPK) of the Universitas Perintis Indonesia with protocol number 083.2/KEPK.F2/ETIK/2022. It started with the acclimatization of rats for 7 days followed by injection of alloxan for 7 days later on.

Rats were placed in plastic cages with lids made of ram wire and covered with husks, fed with pellets of 10-15g/day, and drinking water ad libitium. The cage environment was maintained so that it was not humid, the cage temperature was around 25-27°C, and there was an exchange of dark and light every 12 hours. Each group of rats was placed in a separate cage and guarded so that they did not interact with each other, and the health of the rats was monitored every day.

Alloxan and induction in experimental animals

Alloxan as a simple pyrimidine derivative will damage pancreatic beta cells thereby reducing insulin production. Injection of alloxan at a dose of 125 mg/kg of rats' weight via intraperitoneal. Diabetic conditions in rats will be calculated starting 72 hours after alloxan induction. The injection was for once to make the rats have type 2 diabetes mellitus. Blood glucose measurements were analyzed on the 7th day after alloxan induction. Measurement of blood sugar levels when 200 mg/dl means that the rat is indicated to have type 2 diabetes.

In the beginning, Wistar strain rats weighing 150-250gr were divided into 4 groups; The first group of rats as negative control (K-) were only given regular feed, the second group as positive control (K+) were injected with Aloxsan and without having Sungkai ingestion, the third group (P-1) was injected with alloxan and treated with Sungkai leaf steeping as much as 4g/200ml/day for 14 days period. Finally, the fourth group (P-2) rats were injected with alloxan and treated with 8g/200ml/day of Sungkai leaf decoction for 14 days. The infusion of Sungkai leaves was administered orally using a probe for 14 days. The control rats (K-) were only given a standard feed of 15gr/day/rat and were given drinking water ad libitum, just like the other groups.

Sungkai leaf processing and examinations

During the experiment animals were acclimatized, and Sungkai leaves were taken from an Indonesian village called Kampung Gobah Talang Kayu Jao Nagari Sungai Sirah in Silaut Pesisir Selatan Regency of West Sumatra Province. It was taken directly from the trunk as much as 2,5 kg. In making a decoction of Sungkai leaves, the washed leaves (10 leaves) are dried at room temperature of 25-27°C for 3-5 days which should not be exposed to direct sunlight. The dried leaves were blended, and the simplicial leaves were weighed 4 and 8 grams, respectively, to be both boiled with 200 mL of water for 4 minutes and then filtered. The habitual activity of the people of the lunar village manages young leaves of P. canescens as a febrifuge with hands. If it is assumed that the wet weight of a handful of P. canescens leaves is 30 g, the average body weight of an adult is 50 kg and the weight of the mice to be used is ± 8 weeks old on average 30g. This was used as the basis for the dose conversion of the young leaf extract of P. canescens sungkai to adult mice. The steeping dose of Sungkai (Peronema canescens) that will be used in this study is 0.5625 (mouse dose) ~7.0 (mouse to mouse conversion factor), and the final dose was 1.4 g/200 ml/day, and 2.8 g/200 ml/day.

The quality inspection of Sungkai leaves is carried out in several tests, as the first one was the proximate testing, using the 2012 AOAC method. It delivers the moisture, protein, and fat content of these leaves. The second analysis was the antioxidant activity test. Later, a total flavonoid test was conducted by using the AlCl3 calorimetry method with absorbance calculation using UV-VIS spectrophotometry at 428nm wavelength. The calibration curve used standard quercetin in methanol (0.005 mg/ml) with various concentrations of 0g/ml, 5g/ml, 10g/ml, 20g/ml, 40g/ml, and 60g/ml. Sungkai leaf decoction was only for Group III (P-1) for 14 days at a dose of 4g/200 ml/day., and Group IV with an intervention dosage of 8g/200 ml/day. For uniformity, the administration of Sungkai leaves is carried out every day for 14 days from

12.00 to 1.00 p.m. After 14 days, the rats were given an infusion of sungkai4 Sungkai leaves and then the rats' blood sugar levels were checked using a photometer.

Analyzing the blood glucose and pancreatic histopathology

Centrifugation of blood for 15 minutes at a speed of 4000 rpm as much as 10µl sample and read the absorbance at a wavelength of 500nm in a spectrophotometry device. The amount of macrophage cells was measured by counting the number of cells in 5 fields of view microscopic analysis from the peripheral blood smear. The result of the amount of cells will be delivered in average numbers. After the rat's blood was collected, they were dissected with spinal cord dislocation. The pancreas was isolated in a fixative tube which was washed. with NaCl before. The fixation of 36 hours was with 10% NBF. The tissue would be processed to create the histopathological preparation by dehydrating with graded alcohol (60 min) and cleared with xylol twice. pancreas paraffin block will be cut 3mm thick on a microtome device and forwarded to deparaffinating with graded xylol and alcohol concentrate. Stain the tissue preparation with hematoxylin and eosin and dip in graded alcohol (5x) and into xylol I, II, to observe for pancreatic necrosis or regeneration.

Data analysis

The data collected is primary data obtained from the results of an examination of blood sugar levels, hematological profiles, and percentage of pancreatic necrosis, and later on, the treatment group to be compared to the control group result. The normality test was carried out with the Saphiro-Wilk test, if the data were normally distributed, it would determine blood sugar levels before and after treatment with the paired t-test. If the data is not normally distributed, then the data will be analyzed using the Wilcoxon non-parametric test. The differences between the four interventions were analyzed through the ANOVA parametric statistical test followed by the LSD and Duncan's Pose Hoc test for normally distributed data. However, if the data is not normally distributed, the Kruskal Wallis non-parametric statistical test will be used which is followed by the Mann-Whitney test.

RESEARCH RESULT

The study included male white rats of the Wistar strain (*Rattus norvegicus*) aged 2-3 months weighing 150-250g which experienced two drops out, which occurred in the second week of the study, the mice that died were positive control mice number seven, and the third week the mice that died were dose-treated mice of P-2 group.

Characteristics of Sungkai Leaves

The results were obtained in the form of a dark green extract. The thick extract obtained was then weighed and the yield was calculated. The yield of the ethanol extract of P. canescens leaves with a fixed weight of 72.88 g is 7.28%, which means that the percentage of the number of substances obtained from the extraction process is 7.28% or there are 7.28 grams of substances obtained from 1000 grams of simplicial. This result is higher than the results obtained in the study of Sungkai leaves from East Kalimantan conducted by Ahmad and Ibrahim (2015), where the extract yield was 3.38%. The next step is to do fractionation. The thick n-butanol fraction obtained was 6.2 g so the. yield of the fraction was 24.8%, which means there were 24.8 parts of the substance from a total of 100 parts. This result is also higher than the previous study conducted on Sungkai leaves from East Kalimantan with a total fraction yield of 3.64%.

Table. 1
Distribution of the Percentage of the Phytochemical Composition
of Sungkai Leaves

Sungkai Phytochemical Composition	%
Proximate test	
Water	2.27
Dusts	0.98
Protein	6.37
Fibers	14.31
Antioxidane test	56.98
Flavonoid test	3.9 mg QE/g

Test results of proximate, antioxidant, and flavonoid tests in Sungkai leaves are presented in the table. The distribution of data in Table 1 shows the content of antioxidant and flavonoid values which is quite high. This condition gives a positive indication of the protective ability of cells and tissues from functional tissue damage in diabetic conditions by decreasing the blood glucose persistently. It also assumed that the high percentage of protein and fibers will lead to the improvement of tissue injury, especially of pancreatic islets.

Rat's Body Weight and Blood Glucose Changes before and after the Sungkai Leaf Intervention

Table. 2
Average rat's Body Weight before and after Research Intervention

Groups	Weight Pre	Weight Post
K-	205.3 g	215.5 g
K+	198.8 g	189.3 g
P1	196.7 g	224.7 g
P2	206.5 g	232.0 g

The paired sample t-test and the Wilcoxon test from Table 2. showed a significant difference in body weight between the groups before and after the intervention of Sungkai leaf decoction in groups K- (P=.001), K+(.002), P-1 (.001), while in group P-2 (.058) there was no difference. If the value of $p \leq .005$ then there is a difference in body weight before and after the intervention, if the value of $p \geq .005$ then there is no difference in body weight before and after the intervention. Data on the difference in body weight of rats before and after the intervention showed an increase in body weight in the K- group (10.17±1.472), P-1 (28.00±9.654), P-2 (14.83±33.090), while in the K+ group (-9.50± 3.782) the occurrence of weight loss because the K+ group was not given intervention (drugs), prolonged urination was characterized by cecum that was always wet, did not have an appetite for food. In addition, the differences that occurred in this study were caused by differences in the metabolic response rate of the rat's body.

To find out whether there was a significant difference in body weight of rats before and after the Sungkai leaf intervention, it was necessary to carry out statistical tests using post hoc tests which can be seen from the following Table 3. Based on this table, in the post hoc test, there is a significant difference in the K+ group against (P-1 and P-2) because the p-value is 0.05. In the group K- against (K+, P-1, and P-2), and P-1 against P-2, there was no difference because of the $p \geq 0.05$. This difference occurred because the K- group was not injected with alloxan so there was no DM and no weight loss occurred. Meanwhile, in groups P1 and P2 that had been injected with alloxan and had DM, there was no weight loss because this group was given the intervention of Sungkai leaves so that they could restrain the metabolic rate through antioxidants found in Sungkai leaves and anti-diabetic.

Table. 3
Post Hoc LSD Test of Changes in Body Weight before and after Intervention of Decoction of Sungkai Leaves for 14 Days

Groups	Δ Weight (KG)	*P-value*			
		K-	K+	P-1	P-2
K-	10.2±1.47	-	.064	.090	.646
K+	-9.50±3.78		-	.001*	.025*
P-1	28.0±9.65			-	.204
P-2	14.8±33.1				-

Inclusion criteria for blood sugar levels of rats that were sampled were >200mg/dL, to meet these inclusion criteria, the experimental animals were induced with 100 mg/kg body weight of alloxan rats. To determine the blood sugar levels of rats, blood sugar was checked using a photometer (enzymatic/serum). It can be assumed that the Sungkai decoction intervention has a positive effect in reducing the blood glucose level, especially in the group of animals that are given a high dose of extract.

Table. 4
An Average Number of Blood Glucose before and after the Intervention of Sungkai Leaf Decoction

Groups	Pre-blood glucose	Post-blood glucose
K-	87.00 mg/dl	87.83 mg/dl
K+	479.50 mg/dl	439.50 mg/dl
P1	220.50 mg/dl	130.33 mg/dl
P2	210.50 mg/dl	111.33 mg/dl

Based on the analysis Table 4, shows the results of differences in plasma glucose levels before and after the intervention of Sungkai leaves decoction using the paired samples t-test. Data on the difference in blood sugar levels of rats before and after the intervention showed a decrease in blood sugar levels in groups K+(40.00±37.084), P-1 (90.17±24.441), P-2 99.17±14.442). Based on the table above the P value in each group is K + (.046), P-1 (P = .001), P-2 (P = .001) If the p value .005 then there is a significant difference. there is an effect of giving boiled Sungkai leaves on blood sugar levels in Wistar rats induced by diabetes mellitus. If the p-value is .005, there is no difference, which means that there is no effect of giving Sungkai leaves on blood sugar levels. In group K- there was no difference because this group was not injected with alloxan and was not given any intervention.

Table. 5
LSD Post Hoc test Changes in Blood Sugar Levels before and after Intervention of Decoction of Sungkai Leaves for 14 Days

Groups	Δ blood glucose (mg/dl)	*P value*			
		K-	K+	P-1	P-2
K-	-.83±2.93	-	.007*	.000*	.000*
K+	40.0±37.1		-	.001*	.000*
P-1	90.2±24.4			-	.513
P-2	99.2±14.4				-

The post-hoc test in Table 5 shows a significant difference between each group because the p-value is 0.05. In the P-1 group against P-2, there was no difference because the p-value was 0.05. Successful management of DM accompanied by hypo-insulinemia was indicated by an increase in blood sugar levels of rats although statistically it was stated that there was a significant difference in the K- group against (K+, P-1, and P-2), K+ against (P-1 and P-2) but could not distinguish between the P-1 group and the P-2 group.

Pancreatic Tissue Histopathology

Table. 6
Distribution of Macrophage Leukocyte Cell Accumulation and Percentage of Tissue Damage in the Pancreas Organ in Each Research Group

Test Group	Injury percentage	p-value
K-	1.43±1.33	0.00
K+	28.6±3.51	
P-1	19.8±2.01	
P-2	10.1±1.86	

Table 6 explains the injury or damaged tissue of the pancreas organ and shows a decreasing percentage of the pancreas histopathological features of the organ with an indication of percentage damage to the pancreatic islets of Langerhans. Significant improvement was shown in the second dose treatment group with a very significant reduction in damaging areas. The description of differences in tissue damage in the pancreas can be seen in the histopathological figure 1.

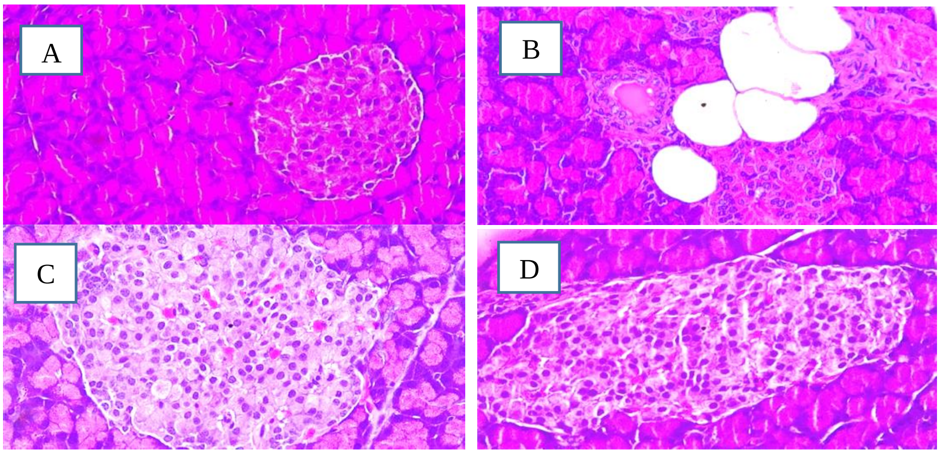

Figure. 2
Percentage of damage to pancreatic islets of Langerhans in rats in the group; A. Negative control, the group of non-diabetic rats, B. Positive control, diabetic rats without Sungkai implementation, C. P1, the implementation of low dose Sungkai decoction (4g/200ml/day), and D. P2, the implementation of high dose Sungkai decoction (8g/200ml/day).

DISCUSSION

Rat Body Weight Before and After Sungkai Leaf Decoction Intervention

The paired sample t-test and Wilcoxon test showed a significant difference in body weight between the groups before and after the intervention of Sungkai leaf decoction in groups

K- (P=.001), K+(.002), P-1 (. 001), P-2 (.058). The body weight of rats before and after the intervention showed an increase in body weight in the K- group (10.17±1.472), P-1 (28.00±9.654), P-2 (14.83±33.090), while in the K+ group (-9.50± 3.782) the occurrence of weight loss due to not being given intervention (drugs), causing damage to every insulin receptor cell in the rat body, for the consequences, there is no food storage in the body caused by oxidative stress. In addition, oxidative stress can also be caused by fatty acids found in adipocyte cells. Where if fatty acids are not in the form of triacylglycerol compounds will cause damage to blood vessels and oxidative stress occurs (Abidov et al., 2022).

This research finding is similar to Xian et al., (2020) that there is an increase in body weight when blood glucose increases, this is because insulin, as known as a glucose absorption receptor through a special membrane, is shown a sensitive condition that results in an increasing trend in blood glucose due to delayed glucose uptake, resulting in weight gain in rats. This is also supported by Eluehike's & Onoagbe’s (2018) research result which states that there was a significant increase in body weight in animals given extracts containing antioxidants (tannins) compared to the control group. This is because the mice experienced fat loss from adipose tissue and amino acid catabolism in muscle tissue. The degradation of structural proteins caused the mice to feel hungry quickly and their food intake increased (Xian et al., 2020; Gobinath et al., 2022). We assumed this correlation suitable to the explanation that the lack of insulin capacity, the glucose as the main energy source for cells, has an inadequate process to become metabolic energy. They were detained in blood streams and tend to be manifested in the adipose tissue, which increases the body weight of the organism. In diabetics, the pancreas fails to convert glucose into energy, resulting in insulin resistance. Because the body needs energy, the alternative is to break down fat into energy. If the energy is not sufficient, then muscle protein is broken down, so that over time you lose weight. Thus, diabetic rats are accompanied by weight loss because their pancreas is damaged.

The body weight of the rats in the treatment group increased in weight because there was an improvement process by being given a decoction of Sungkai leaves. The decoction of Sungkai leaves is given so that there is time for recovery/improvement of blood sugar levels in Wistar rats because the flavonoids contained in Sungkai leaves can mutilate the repair of pancreatic beta cell tissue damaged by alloxan-induced and can reduce blood sugar levels by stimulating pancreatic beta cells to produce insulin and improve impaired pancreatic beta cell function. Based on the post-hoc test, there was a significant difference in the K+ group against (P-1 and P-2). In the group K- against (K+, P-1 and P-2), and P-1 against P-2, there was no difference at all. This difference occurred in groups P1 and P2 that had been injected with alloxan and had DM, there was no weight loss because this group was given the intervention of Sungkai leaves so that they could restrain the metabolic rate through antioxidants found in Sungkai leaves and anti-DM. The successful management of DM accompanied by hypo-insulinemia was indicated by an increase in blood sugar levels of rats although statistically it was stated that there was a significant difference in the K- group against (P-1 and P-2).

Differences in body weight between groups may occur due to differences in the initial weight of the mice before the intervention in each group. The state of weight gain can be caused by excessive intake of nutrients continuously, resulting in excessive fat storage. Fatty acid deposits in the form of chemical compounds in the form of triacylglycerol contained in adipocyte cells can protect the body from the toxic effects of fatty acids. Free fatty acids can circulate in the blood vessels throughout the body and cause oxidative stress which we know as lipo-toxicity. Likewise, the weight loss of rats that occurred in the K^+ treatment group may have occurred because the rats experienced oxidative stress caused by DM but were not given any intervention to cope with the DM.

Blood sugar levels of rats before and after administration of Sungkai leaf intervention.

Based on the table analysis, shows the results of differences in blood sugar levels before and after the intervention of decoction of sungkai leaves using the t-test. Data on the difference in blood sugar levels of rats before and after the intervention showed a decrease in blood sugar levels in the K+, P-1, and P-2 groups while in the K- indicated a decrease. Based on the p-value, it indicates a significant difference which means that there is an effect of giving sungkai leaf decoction on blood sugar levels in Wistar rats induced by diabetes mellitus, and it shows that Sungkai leaves have an antidiabetic effect or act as an antihyperglycemic agent.

Pindan et al., (2021) finding stated that the positive Sungkai leaves contain flavonoids, alkaloids, steroids, triterpenoids, phenolics and sponins. Other research findings she cited also mentioned that the phytochemical test results on the ethanolic extract of Sungkai leaves were positive for flavonoid, alkaloid, phenolic, steroid, sponin and tannin group compounds. Flavonoids are known to have antioxidant activity related to antidiabetic activity and are believed to be able to protect the body against damage caused by reactive oxygen species, to inhibit the occurrence of degenerative diseases such as DM (Nguyen et al., 2020). Regarding the healing mechanism of diabetes, flavonoids are thought to play a significant role in increasing antioxidant enzyme activity and being able to regenerate damaged pancreatic cells so that insulin deficiency can be overcome. Flavonoids contained in plants are thought to also improve insulin receptor sensitivity. Thus, the presence of flavonoids has a beneficial effect on DM (Azeem et al., 2023). Flavonoids can lower blood glucose levels with their ability as antioxidants. Flavonoids are protective against damage to cells as insulin producers and can restore insulin receptor sensitivity on cells and even increase insulin sensitivity. Antioxidants can suppress cell apoptosis without altering pancreatic cell proliferation. Antioxidants can bind to free radicals which have been proven in several studies, so that they can reduce insulin resistance (Unuofin et al., 2020; Azeem et al., 2023).

Based on observations for 21 days starting from acclimatization to giving treatment, the implementation showed that the group of DM rats given flavonoids (flavonoids) experienced a decrease in blood glucose level. Based on the results of statistical tests showed that there was a significant difference ($p<0.05$) between the DM group that was treated (P-1 was 4 g/200 ml/day, and P-2 was 8 g/200 ml/day), and those who were not given treatment (K+). This indicates that P-1 and P-2 (treated with Sungkai leaves/flavonoids) can prevent the continuation of the DM condition which is characterized by a gradual decrease in plasma glucose level for 2 weeks, until it reaches normal conditions. There was no difference between the P-1 and P-2 groups because the low-dose intervention was able to improve the condition of diabetes mellitus (lowering blood sugar levels), in principle, if the low dose was able to improve the DM condition, there was no need to use a high dose.

Flavonoids are known to have antioxidant activity related to antidiabetic activity and is believed to be able to protect the body against damage caused by reactive oxygen species, to inhibit the occurrence of degenerative diseases such as DM (Nguyen et al., 2020; Cheng et al., 2023). Regarding the healing mechanism of diabetes, flavonoids are thought to play a significant role in increasing antioxidant enzyme activity and being able to regenerate damaged pancreatic cells so that insulin deficiency can be overcome. Flavonoids contained in plants are thought to also improve insulin receptor sensitivity. Thus, the presence of flavonoids has a beneficial effect on DM. Flavonoids can lower blood glucose levels with their ability as antioxidants. Flavonoids are protective against damage to cells as insulin producers and can restore insulin receptor sensitivity on cells and even increase insulin sensitivity. Antioxidants can suppress cell apoptosis without altering pancreatic cell proliferation. Antioxidants can bind to free radicals which have been proven in several studies so that they can reduce insulin resistance (Unuofin et al., 2020; Gusti et al., 2021).

Pancreatic Histopathology of Rats Treated with Sungkai Leaves

This study showed an increased percentage of pancreatic islets of Langerhans damage. This is an indication that an inflammatory response has occurred from the injury site. This is an indication of the improvement of pancreatic Langerhans tissue due to the high antioxidant value of Sungkai leaves and the high amount of flavonoid composition (Abidov et al., 2022). Flavonoids, a group of natural substances with variable phenolic structures, are well known for their beneficial effects on health, and efforts are being made to isolate ingredients. Flavonoids are now considered an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal, and cosmetic applications. Having attributed for anti-oxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties, it also coupled with their capacity to modulate key cellular enzyme function. Some research discovery indicates this reduces cardiovascular mortality rates, prevents CHD, and is a potential drug in preventing chronic diseases and future research directions. They are also known to be potent inhibitors for several enzymes, such as xanthine oxidase (XO), cyclo-oxygenase (COX), lipoxygenase, and phosphoinositide 3-kinase (Mutha et al., 2021).

There is strong evidence indicating that natural phytochemicals, as found in Sungkai leaves, can modulate the synthesis and release of different mediators of inflammation. Plant extracts that are rich in flavonoids, mainly flavanols and pro-anthocyanidins, have a high tendency to be anti-inflammatory, antibiotic, and antioxidant agents. Inflammation, as a physiological response to innate immunity, intends to protect the organism against microorganisms and noxious agents. In this response, macrophages are central cells that become activated and produce pro-inflammatory mediators in response to several stressors or inflammatory stimuli, including pancreatic damage in diabetic organisms31. We believe that the presence of macrophage cells in the injury tissue site, in pancreatic organs specifically, stimulates the anti-inflammatory compounds to improve tissue damage repair. This means that macrophages should migrate intensively to the injury site through the proximate bloodstreams.

This phenomenon is in line with the decreasing percentage of pancreatic Langerhans damage in the two treatment groups. Tissue repair in the pancreas in the group given high concentrations of Sungkai leaves (P-2) showed a significant effect where the tissue picture almost had morphology in the negative control group (without any treatment). The high content of antioxidants and flavonoids in Sungkai leaves is believed to be an indication of tissue repair in the pancreas because it has the function of regenerating cells that experience inflammation and injury, such as cells that have accumulated membranes. The other research interpretation also gives the same view that there is an improvement in the destructive effect of Langerhans pancreas in diabetic rats through an increase in C-peptide in experimental animal groups that were intervened with herbal ingredients with high antioxidant and anti-atherogenic content (Helmi et al., 2023).

Organs and tissues are continuously threatened by the damage caused by free radicals and reactive oxygen species, which are produced during normal oxygen metabolism or are induced by exogenous injury. The mechanisms and the sequence of events by which free radicals interfere with cellular functions generally seem to be lipid peroxidation, which destroys cell membranes. This cellular damage could change the osmotic pressure and homeostasis, leading to cell swelling and eventually necrosis condition. Free radicals can attract various inflammatory mediators, contributing to a tissue inflammatory response and destruction. According to Arora et al., (2021) research result, rich flavonoid plants such *Tephrosia purpurea* (*T. purpurea*) have been generated as a traditional medicine intervening in diabetes mellitus. Furthermore, its effectiveness in regenerating the pancreas damage in diabetes has not been studied deeply. Related to the present study was undertaken to evaluate pancreatic β-cells regeneration, antioxidant and anti-hyperlipidemia potentials in plants flavonoid composed have a significant antidiabetic, pancreatic regeneration, and anti-hyperlipidemia effect. The histopathological analysis of the flavonoid effect revealed that it improved the β-cell granulation of islets and prevented the β-cells damage, which was further confirmed by morphometric analysis (Arora et al., 2021).

Related to what we found in the histopathological analysis of pancreatic preparation, these flavonoid compounds, as an antioxidant activity, in Sungkai leaves have a positive effect on improving the pancreatic tissue generation towards induced diabetes mellitus. We also believe that the tendency of tissue recovery in the pancreas will directly stimulate the secretion of insulin in the bloodstream, which indicates the positive recovery of the organ. It was approved from the histopathological appearance of the damage percentage of the pancreas in each group. In the histopathological features in this study, the K+ groups show some of the acini cells and Langerhans islet lesions. It also featured the shrinkage of Langerhans islets with injury or even degeneration and necrosis of cell components where its nucleus appeared densely basophilic and karyolysis condition. In the intervention groups examined (P-1 and P-2), there was a degeneration of the β cell in the Langerhans islet noticed. It has the reduction of injury sites and acini cells tent to be normal morphology. Once again, this histological feature has a positive progress on pancreatic regeneration and insulin production. From recent studies, the various diversity of flavonoid structures makes it difficult to establish common effects in the pancreas. However, some research data have revealed the direct effects of flavonoids on insulin secretion, as well as on prevention of beta-cell apoptosis, and they could even act via modulation of proliferation. The mechanisms of action involve mainly their antioxidant properties, but other pathways might also take place (Zhou et al., 2023).

CONCLUSION

The average blood sugar level of Wistar rats before intervention with Sungkai leaf was approved in the intervention groups. It reveals that there is a difference in average blood sugar levels before and after intervention with Sungkai leaf decoction, and even in the rat’s body weight changes. Related to the diabetic condition, it shows the degeneration of the pancreatic tissue with some injury and necrotic sites identified. Eventually, the Sungkai leaves intervention has improved the regeneration of the pancreas organ. In two intervention groups, it reveals the Langerhans islet and acini tissue refinements. It means that Sungkai leaf compounds have a positive effect on improving pancreas damage with the regeneration of pancreas breakage and stimulating anti-inflammatory compounds.

SUGGESTIONS

It is recommended for further researchers to be able to carry out other phytochemical tests in the leaves which have positive effects as antioxidant activity of the fractions and be able to isolate other chemical compounds. Furthermore, it is recommended to continue this study to identify the side effects and the ingredients accumulation in excretion organs. Lastly, there is a consideration for clinical trials in humans so that they can be used in the health sector to produce human doses in diabetic therapy.

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