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The Effect of Variant Concentration of Kesambi (*Scheleichera Oleosa*) on Diabetic Animal Models

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Abstract: Diabetes mellitus (DM) is a disease with high prevalence which continues to increase. Existing treatments, either orally or through insulin injection, has varied effectiveness for diabetics. Therefore, an alternative treatment of DM is needed. Kesambi leaves have antioxidant properties, which are thought to have potential as an antidiabetic. Antioxidants are compounds that can reduce the negative impact of free radicals and other oxidants, to inhibit oxidative damage that could worsen the severity of DM. This study aims to determine the antioxidant compound content of kesambi extract and its effects on glucose, insulin, and regeneration of pancreatic beta cells in Wistar rats induced by streptozotocin. Methods: Experimental research with modified pretest and posttest randomized controlled group design, using 60 male Wistar rats divided into 6 thin-layer chromatography (TLC) groups: healthy, sick (streptozocin (STZ) induction), galvus (synthetic drug), and kesambi extract at levels of 100, 200, and 300 mg/Kg BB male Wistar rats. The study showed that kesambi (Schleichera oleosa) extract had antioxidant activity of inhibitory concentration (IC) 50 of 20.29 ppm (very strong), effected the regeneration of pancreatic β cells, improved insulin levels, and decreased blood glucose levels of Wistar rats induced by STZ, dosed optimally at a concentration of 200 mg to improve regeneration of pancreatic β cells, insulin levels and decreased blood glucose levels.

Keywords: diabetes mellitus, insulin, glucose, kesambi (*Schleichera oleosa*), β-pancreas.

不同浓度的凯桑比（油菜花）对糖尿病动物模型的影响

摘要：糖尿病（DM）是一种患病率很高的疾病，并且还在持续增加。现有的治疗方法，无论是口服还是注射胰岛素，对糖尿病患者的疗效各不相同。因此，需要另一种 DM 治疗方法。凯桑比叶具有抗氧化特性，被认为具有抗糖尿病的潜力。抗氧化剂是可以减少自由基和其他氧化剂的负面影响的化合物，以抑制可能加重 DM 严重程度的氧化损伤。本研究旨在确定凯萨比提取物的抗氧化化合物含量及其对葡萄糖、胰岛素和链脲佐菌素诱导的威斯塔大鼠胰腺 β 细胞再生的影响。方法：采用改良前测和后测随机对照设计的实验研究，使用 60 只雄性威斯塔大鼠分为 6 个薄层色谱（薄层色谱）组：健康、生病（链脲佐菌素（STZ）诱导）、惊厥（合成药物）和凯萨比 100、200 和 300 毫克/公斤 BB 雄性威斯塔大鼠的提取物。研究表明，凯斯比（油菜花）提取物具有抑制浓度（我知道了）50 20.29 百万分之一（非常强）的抗氧化活性，影响胰腺β细胞的再生，改善胰岛素水平，降低 STZ 诱导的威斯塔大鼠

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的血糖水平, 最佳剂量为 200 毫克，以改善胰腺 β 细胞的再生、胰岛素水平和降低血糖水平
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关键词：糖尿病、胰岛素、葡萄糖、凯萨比 (油菜花)、β -胰腺。

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder occurring due to disruption in function of the insulin hormone produced by the pancreas [22]. Based on 2018 data from the Indonesian Ministry of Health, Indonesia is ranked fourth of 20 countries which are estimated to have the highest number of DM cases. DM sufferers are prone to develop ROS (reactive oxygen species). The hyperglycemia state in DM triggers glucose autoxidation, resulting in ROS [1]. ROS are free radical compounds that react with other compounds. In the body, ROS tends to react with tissues and damage them. The increase of ROS in the body causes oxidative stress. Furthermore, oxidative stress contributes to islet damage and insulin resistance, which worsens the condition of DM sufferers [1].

ROS reactivity can be inhibited by antioxidants (polyphenols, flavonoids, alkaloids, and tannins) found in plants. Antioxidants can suppress beta cell apoptosis without altering the proliferation of pancreatic beta cells. Antioxidants can bind free radicals, then reduce insulin resistance [2].

Previous studies have shown a decreased level of GLUT 2 protein expression (down-regulation) in pancreatic beta cells due to the induction of streptozotocin nicotinamide, which causes toxicity to pancreatic beta cells so they are damaged and reduce insulin secretion [3]. Streptozotocin is selectively toxic to pancreatic beta cells producing insulin [4]. This compound has a glucose-like molecular shape (glucomimetic). When the compound is induced into laboratory mice, the GLUT 2 in pancreatic beta cells will be recognized as glucose. Streptozotocin will be taken to the cytosol [5]. In the cytosol, streptozotocin will undergo a redox reaction that produces ROS. The formation of ROS will cause depolarization of beta cell membranes and an increase in Ca^{2+} so the cytosol will activate various enzymes that cause lipid peroxidation, DNA fragmentation, and protein fragmentation. As a result, pancreatic beta cells become necrotic, so that insulin function and secretion decrease [6].

Treatment of DM uses hypoglycemic drugs such as injectable insulin and oral antidiabetic drugs. The administration of these drugs causes various side effects such as headaches, dizziness, nausea, and anorexia [7]. This has prompted the search for antidiabetic drugs made from natural ingredients. It is hoped that alternative natural ingredients will be more effective and have minimal side effects with the ethnopharmaceutical and ethnobotany approach [8].

Kesambi leaves (*Schleichera oleosa*) contain secondary metabolites in the form of alkaloids, phenolics, tannins, and flavonoids. The highest levels are in phenolics and flavonoids [9]. The antioxidant activity of kesambi leaves is high, with an IC_{50} value of 16.1 ppm [10]. The high content of antioxidants in kesambi (*Schleichera oleosa*) leaves led to the development of kesambi leaf extract (*Schleichera oleosa*) into standardized herbs that have a potential anti-DM effect [11]. Previous studies have not looked at the potential of these leaves by direct indication to test animals (in vivo). The metabolic response of living beings' bodies could be proved. The novelty of this study that it is able to provide an effective antidiabetic solution from the sample so researchers will explore the effect of kesambi leaf extract (Schleichera oleosa) on DM using mice that will be damaged by pancrease beta cells using Streptozotocin.

2. Methods

This study used an experimental method with a modified pretest posttest randomized controlled group design, which was divided into 6 groups, each group consisting of 10 rats. The research was conducted from March to September 2020 at the Makassar Clinical Pharmacy Laboratory, Biopharmaceutical, Faculty of Pharmacy, Hasanuddin University, Makassar Faculty of Mathematics and Natural Sciences Biochemistry Laboratory, Makassar GG Laboratory, and Maros Veterinary Center.

The tools used in this study were Elisa (BIO Rad Mode 650), analytical balance (Startorius), micropipette (Ependorf), a set of minor surgical tools, a routine staining histopathologic examination tool (Haematoxylin Eosin), a maceration kit, a rotary evaporator (Buchi), magnetic stirrer, UV-Vis spectrophotometer (Hewlett Packard), animal scale, centrifuge, syringe, oral needle, binocular microscope, camera and mouse cage.

The materials used in this study were Kesambi leaves taken from Barru Regency, South Sulawesi. White wistar male rats were obtained from the Biopharmaceutical Laboratory of the Faculty of Pharmacy, UNHAS. Other materials were Folin-Ciocalteu reagent, pH 4.5 citrate buffer, distilled water, aqua pro injection (Otsuka), alcohol (E. Merck), buffered formaldehyde, xylol, paraffin, diethyl ether (E. Merck), Na. CMC, STZ ALX-350-010 from

ALEXIS Corporation, animal feed (HG II-B), histopathologic material, Haematoxylin Eosin staining and immunohistochemical staining, Bovin Serum Albumin (BSA), KCl, KH_2PO_4, NaCl, $Na_2HPO_4.H_2O$, NaOH, Tween, $NaNO_3$ (E. Merck), Antibodies, and mouse insulin.

The research sample was obtained from the population by simple random sampling with inclusion criteria (white male rats, strain wistar, age 1.5-2 months, body weight 150-200 grams. During adaptation 14 days before treatment, all rats were not sick; their activity and behavior were normal). Sixty rats were used in the research.

2.1. Kesambi Leaf Extraction

The leaves of Kesambi (*Schleichera oleosa*) were cleaned and dried. A total of 5.90 kg of sample was extracted with n-hexane solvent. Then the phytochemical test was carried out using $FeCl_3$, bromine water, and indophenol. Quantitative analysis of polyphenols was performed using the Folin-Ciocalteu method at a wavelength of 666 nm using a UV-Vis spectrophotometer.

2.2. Antioxidant Activity Test

Two ml of sample solution of various concentrations were added with 2 ml of DPPH 0.4 mM and left at room temperature for 30 minutes. Then it was measured on a visible spectrophotometer at a wavelength of 515 nm. The IC50 value is determined by probit between the log data of the concentration and the probit percentage of free radical binding.

2.3. Application to Diabetic Animal Models

Kesambi extract suspension was made: 100 mg/kg mass of rats and 300 mg/kg BW of rats, respectively. Sixty rats that met the inclusion criteria were taken randomly on day 0 and then put into 6 groups. Each group consisted of 10 tails and was kept in separate pens, given standard feed and drink ad libitium.

Furthermore, each group was given the following treatment respectively. All groups consisting of ten rats were fasted first and observed. *Group I* was healthy control rats; *Group II* the rats were induced STZ 60 mg/kg single dose rats as a pain control; *Group III* the rats were induced STZ 60 mg/kg single dose rats by i.p. as a positive comparison. Starting from the seventh day, the rats were given 0.9 mg/200g galvus orally every day for 21 days. In *Groups IV-VI*, the rats were induced STZ 60 mg/kg single dose rats by i.p. Starting from the seventh day 100 mg/kg, 200 mg/kg, and 300 mg/kg of rat kesambi extract were given orally every day for 21 days.

Histopathologic examination of pancreatic β cells, insulin levels and blood glucose levels were performed five times; once before STZ induction, and four times after being induced by STZ, on day 1, 7, 14, 21, and 28. Pancreatic tissue was taken and a blood smear was then performed for histopathologic examination by staining using hematoxylin eosin to quantitatively examine the number of pancreatic β cells. Insulin levels were measured using Enzyme-Linked Immuno Sorbent Assay (ELISA). Blood glucose levels were measured using a glucometer.

2.4. Data Analysis

Data analysis was performed using the ANOVA test to compare the effect of several treatment interventions on blood glucose levels, pancreatic β cell counts, and insulin levels in 6 groups of male Wistar rats.

3. Results and Discussion

The extract was tested by adding a specific reagent for alkaloids, flavonoids, steroids, tannin and saponin. Results of phytochemistry assay are shown in Table 1.

Table 1 Phytochemistry assay

Phytochemistry assay	Result
Steroid	+
Alkaloid	+
Flavanoid	+
Saponin	+
Tanin	+

The results showed that the extract contained a secondary metabolite that has potential as antioxidant agent. The results of this study were consistent with several previous studies [4], [10], [12]. An antioxidant assay was then done to determine antioxidant activity.

3.1. Antioxidant Activity

Testing to determine total phenolic content for antioxidant activity was performed using the Folin-Ciocalteu method. The antioxidant activity was carried out using the DPPH method. The principle of this method is the donation of hydrogen atoms (H^+) from the substance tested to the DPPH radical to become a non-radical compound of dihenyl picryl hydrazine, which is indicated by a color change. The color change that occurs is a color change from purple to yellow; the intensity of the DPPH color change is directly proportional to the antioxidant activity to reduce these free radicals. The IC_{50} results showed antioxidant activity of 20.29 ppm better than its bark stem [13]. From these results, it can be seen that kesambi leaves have very strong antioxidant activity. The IC_{50} value of less than 50 ppm has a very strong antioxidant activity. The active compound contained in the extract is a group of antioxidant compounds.

3.2. Acclimatization Test

Male rats were selected as the test animals because their biological conditions are more stable than those of female rats. Moreover, they are not influenced by the estrous cycle and they have a faster drug metabolism

rate. The rats were acclimatized to the laboratory for 14 days, and they were given pellet feed and drinking water ad libitum.

In the acclimatization process, the rats were weighed to determine the treatment dose to be administered to them. The rats used in the study were healthy, with signs of non-standing hair, white fur, clear eyes, normal behavior, and their body weight increased during the intervention period according to the research method.

Kesambi extract is an ingredient given to the subjects of treatment given in ad libitum. To minimize the variability between the test animals, the test animals had a uniform body weight of 200–300 g and they were between three and four months of age. The selection of rats as test animals was due to their high availability and sensitivity to represent humans in determining blood glucose levels. Meanwhile, rats have metabolic and digestive systems that are similar to those of humans.

The mechanism of STZ in pancreatic cells can affect glucose causing hyperglycemia as it enters the pancreatic β-cells through the glucose transporter (GLUT-2), thereby causing DNA alkylation. Alkylation or the entry of the methyl group from STZ into the DNA molecule will cause damage to the DNA fragmentation. Subsequently, the DNA damage will trigger the production of synthetic poly (ADP-ribose) enzymes, which are enzymes needed to repair the DNA damage. These enzymes require nicotinamide adenine dinucleotide (NAD) as a substrate so that the NAD^+ content in cells decreases, which causes a decrease in the amount of ATP. Thus, insulin synthesis and secretion are inhibited, thereby causing hyperglycemia [9].

According to the World Health Organization, the normal blood glucose level before meals is approximately 70–130 mg/dL. After meals, the blood glucose level naturally increases; however, it should still be less than 180 mg/dL. Additionally, the blood glucose level during fasting is less than 100 mg/dL. A synthetic drug in the form of galvus was administered to the rats in the third group in order to increase their insulin secretion and ensure a normal blood glucose level. Furthermore, kesambi extract with various doses was administered to the rats in the fourth, fifth, and sixth groups [14].

3.3. ANOVA Test Using SPSS for Windows Software

3.3.1. Glucose Levels

In Table 2, there was a difference in the average blood glucose level with a P value < 0.05. This indicates that the second group experienced an increase in blood glucose level. In this intervention, Ha was accepted and Ho was rejected so that there is induction effect of STZ, the administration of kesambi extract 100, 200, and 300 mg on blood glucose levels.

For the second group induced by STZ and the third group, there was no difference in the average blood glucose levels. This indicates that with the induction of STZ, the blood glucose levels of mice in the third group increased to normal values, indicating that the addition of galvus did not lead to a decrease in the blood glucose levels of the second group.

The results of the average glucose level in the third (with galvus), first, fourth, and fifth groups showed that each group had a P value < 0.05. This shows that the third group produced blood glucose levels that were close to those of normal mice (NaCMC administration) so there is an effect of intervention giving galvus towards normal blood glucose levels. For the sixth group, which was given 300 mg of kesambi extract, there was no difference in the average blood glucose level with a P value > 0.05.

From the results of the average glucose levels of the third, fourth, and fifth groups, Ho was accepted and Ha was rejected, so that the administration of 100, 200, and 300 mg of kesambi extract with NaCMC did not have any effect. Meanwhile, there was a difference in the average blood glucose levels of the sixth group and the first group (given NaCMC) after the ANOVA test. In this case, the effect of the administration of 300 mg of kesambi extract with NaCMC was observed.

Table 2 Post hoc test for glucose levels

Intermediate glucose levels	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig
Group II STZ with several KLP	STZ	NaCMC	81.67200	11.89778	,000
		Galvus	19.17000	11.89778	,118
		100 mg	66.94800	11.89778	,000
		200 mg	97.04000	11.89778	,000
		300 mg	38.76400	11.89778	,005
Group III Galvus with several KLP	Galvus	NaCMC	62.50200	11.80778	,000
		100 mg	47.77800	11.80778	,000
		200 mg	77.87000	11.80778	,000
		300 mg	19.59400	11.80778	,110
Group IV Kesambi extract 100 mg with several group	100 mg	NaCMC	14.50200	11.80778	,224
		200 mg	30.09400	11.80778	,018
		300 mg	-28.18200	11.80778	,025
Group V Kesambi extract 200 mg with several group	200 mg	NaCMC	-15.36800	11.80778	,205
Group VI Kesambi extract 300 mg with several group	300 mg	NaCMC	42.90800	11.80778	,001

3.3.2. Insulin Levels

The results of the average insulin levels showed a difference between the average insulin levels of second group and the first and fifth groups after the ANOVA test, with a P value < 0.05. This indicates that the second group had abnormal insulin levels because STZ acts as a destroyer of the pancreatic β-cell structure, which affects insulin production. However, the addition

of kesambi extract as much as 200 mg causes the production of the hormone insulin at normal levels. In this intervention, Ha was accepted and Ho was rejected so that the effects of STZ induction and the addition of 200 mg of kesambi extract on the insulin levels were observed. In contrast, the third, fourth, and sixth groups had P values > 0.05. This shows that the third group did not have normal insulin levels. In this intervention, the addition of galvus and 100 and 300 mg of kesambi extract had no effect on the normal insulin level, as presented in Table 3.

The results of the average insulin levels between KLP III (given galvus) and KLP I (given NaCMC) after the ANOVA test showed a difference in the average insulin levels with a P value < 0.05. This shows that the third group had normal insulin production, and the effect of galvus intervention on normal insulin levels was observed. Meanwhile, in the fourth, fifth, and sixth groups with P values > 0.05, Ho was accepted and Ha was rejected. There was no effect of the intervention between galvus and kesambi extracts of 100, 200, and 300 mg. The same thing was also found in the first, fourth, fifth, and sixth groups, as shown in Table 3.

Table 3 Post hoc test for insulin amount

Insulin Level	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig
Group II STZ with several Group	STZ	NaCMC	42.27200	11.33492	,001
		Galvus	6.57000	11.33592	,568
		100 mg	19.25000	11.33592	,102
		200 mg	35.95000	11.33592	,004
		300 mg	20.77000	11.33592	,079
Group III Galvus with several Group	Galvus	NaCMC	35.70200	11.33592	,006
		100 mg	12.68000	11.33592	,274
		200 mg	29.38000	11.33592	,016
		300 mg	14.20000	11.33592	,222
KLP IV Kesambi extract 100 mg with several group	100 mg	NaCMC	14.50200	11.33592	,053
		200 mg	30.09400	11.33592	,15
		300 mg	-28.18200	11.33592	,89
KLP V Kesambi extract 200 mg with group NaCMC	200 mg	NaCMC	-15.36800	11.33592	,58
KLP VIKesambi extract 300 mg with group NaCMC	300 mg	NaCMC	42.90800	11.33592	,076

3.3.3. Number of β-Pancreatic Cells

The mean number of pancreatic beta cells in the first to the sixth group with all treatments after the ANOVA test showed no difference in the average number of pancreatic beta cells with a P-value > 0.05. In this intervention, Ho is accepted, Ha is rejected so that there is no effect of the intervention between each treatment. This can be seen in Table 4.

Table 4 Mann-Whitney test for number of β-pancreatic cells

		Group	N	Mean Rank	Sum of Ranks	Asymp. Sig (2-tailed)
Number of β-Pancreatic Cells on group I with NaCMC	KLP II	NaCMC	5	8.00	40.00	.009
		STZ	5	3.00	15.00	
	KLP III	NaCMC	5	8.00	40.00	.009
		Galvus	5	3.00	15.00	
	KLP IV	NaCMC	5	8.00	40.00	.009
		Kesambi extract 100 mg	5	3.00	15.00	
	KLP V	NaCMC	5	7.40	37.00	.047
		Kesambi extract 200 mg	5	3.60	18.00	
	KLP VI	NaCMC	5	8.00	40.00	.009
		Kesambi extract 300 mg	5	3.00	15.00	
Number of β-Pancreatic Cells on group II with STZ	KLP III	STZ	5	4.80	24.00	.465
		Galvus	5	6.20	31.00	
	KLP IV	STZ	5	5.00	25.00	.802
		Kesambi extract 100 mg	5	6.00	30.00	
	KLP V	STZ	5	3.80	19.00	.076
		Kesambi extract 200 mg	5	7.20	36.00	
	KLP VI	STZ	5	4.00	20.00	.117
		Kesambi extract 300 mg	5	7.00	35.00	
Number of β-Pancreatic Cells on group III with Galvus	KLP V	Galvus	5	5.60	18.00	.047
		Kesambi extract 200 mg	5	7.40	37.00	
	KLP VI	Galvus	5	3.80	19.00	.076
		Kesambi extract 300 mg	5	7.20	36.00	
Number of of β-Pancreatic Cells on group III IV 100 mg with Kesambi extract	KLP V	Kesambi extract 100 mg	5	3.80	19.00	.076
		Kesambi extract 200 mg	5	7.20	36.00	
	KLP VI	Kesambiextract 100 mg	5	3.80	19.00	.076
		Kesambiextract 300 mg	5	7.20	36.00	
Number of β-Pancreatic cell on group IV 200 mg with Kesambi extract	KLP VI	Kesambiextract 200 mg	5	7.20	36.00	.076
		Kesambiextract 300 mg	5	3.80	19.00	

3.4. In-Vivo Kesambi Extract Activity in Wistar Rats on Blood Glucose Levels

There is a positive correlation between the treatment dose and the average blood glucose level of the rats given the extract of kesambi. This explained that the mean blood glucose levels continued to decrease with the administration of kesambi extract. For determining the effect of kesambi extract on decreasing blood glucose levels and which groups had significant differences, an analysis was performed using an unpaired t-test.

The mean blood glucose levels of Wistar rats in the second group were significantly different from the first and fifth groups, with p-values < 0.05. They were not significantly different between the second, third, and fourth groups, with p-values > 0.05. This shows that the administration of kesambi extract at a concentration of 200 mg affects reducing blood glucose levels in rats induced by STZ (Ho is rejected, and Ha is accepted).

In the second group, after being induced with STZ starting on days 7, 14, 21, and 28, there was an increase in blood glucose levels. In the third group, after being given galvus synthetic drug on days 7, 14, 21, and 28, the sugar levels began to decrease compared to the second group, but glucose levels remained high. In the fourth, fifth, and sixth groups, each rat was given

kesambi extract with a concentration of 100, 200, and 300 mg/kg of rats on days 7, 14, 21, and 28. There was a decrease in blood glucose levels. In the fifth group, there was a significant decrease in blood glucose levels towards normal glucose levels close to glucose levels of the first group. At a concentration of 200 mg, the extract worked optimally to reduce blood glucose levels in Wistar rats that had experienced hyperglycemic due to STZ induction. This can be because kesambi leaf extract and galvus have suppressed the increase in blood glucose levels by activating pancreatic β cells for insulin production (Fig. 1).

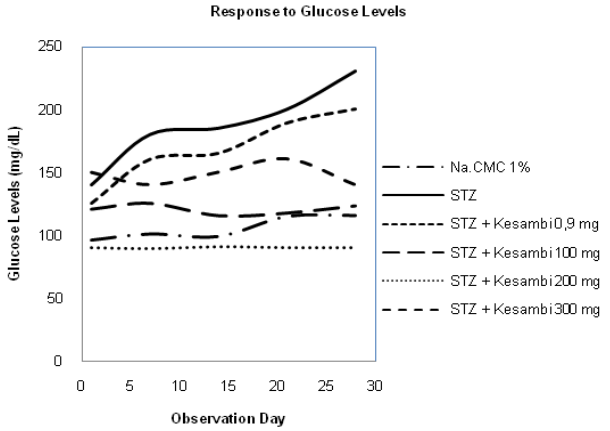

Fig. 1 Response to glucose levels

Insulin has four effects that can lower blood glucose levels and increase carbohydrate storage. Among others, insulin makes it easier for glucose to enter most cells. Glucose molecules do not easily penetrate cell membranes in the absence of insulin. Thus, most tissues rely heavily on insulin to absorb glucose from the blood and use it. Insulin increases the facilitated diffusion (mediated by carriers) of glucose into glucose-dependent cells through the transporter recruitment phenomenon [15].

Antioxidants are thought to reduce blood glucose levels by inhibiting glucose absorption from the gastrointestinal lumen [10], increasing glucose utilization in peripheral tissues, thus acting directly on pancreatic β cells by triggering the activation of the cAMP signal cascade (cyclic Adenosine Monophosphate) in strengthening insulin secretion which is sensitized by glucose.

Antioxidants protect pancreatic β cells from the toxic effects of free radicals produced under chronic hyperglycemia conditions. The administration of antioxidants can increase the mass of pancreatic β cells and maintain the insulin content in them. In cells with insulin receptors (muscle cells, adipose cells, and liver cells), the binding of free radicals will increase insulin signaling in intracellular GLUT4 translocation to the cell membrane; then, they will be able to take glucose from the blood [6]. In general, reducing oxidative stress can reduce insulin resistance and inhibit β-pancreatic cell damage [13].

Antioxidants can prevent complications or progression of diabetes mellitus by cleaning excessive free radicals, breaking the chain of free radical reactions [16], binding metal ions (chelating), and blocking polyol pathways by inhibiting the enzyme aldolase reductase [16]. Antioxidants also have an inhibitory effect on the alpha glucosidase enzyme through hydroxylation and substitution in the β ring [17]. The principle of this inhibition is similar to the acarbose which has been used as a drug for the treatment of diabetes mellitus. This is done by producing a procrastination in the hydrolysis of carbohydrates and disaccharides, absorption of glucose, and inhibiting the metabolism of sucrose to glucose and fructose [18].

The antihyperglycemic effect is thought to be the effect of the secondary metabolite content of the antioxidant extract of kesambi. Antioxidants have a hypoglycemic effect with several mechanisms: inhibiting the glucose absorption, increasing glucose tolerance, stimulating insulin release or acting like insulin, increasing glucose uptake by peripheral tissues and regulating enzymes that play a role in carbohydrate metabolism [19].

3.5. The Activity of Kesambi Extract in Vivo in Wistar Strain Rats on Insulin Levels

The relationship between the treatment dose and the average insulin levels in the blood of rats will be demonstrated after giving them kesambi extract. This explains that the mean insulin levels decreased with the administration of kesambi extract. To determine the effect of kesambi extract on decreasing insulin levels and which groups had significant differences, an analysis was performed using the unpaired t test. The results of the mean blood glucose levels of Wistar rats at the second group were significantly different from the first and fifth group with p values <0.05. However, there was no significant difference between the second, third, and fourth, with p values > 0.05. This shows that giving the kesambi extract at a concentration of 200 mg has an effect on decreasing the blood insulin levels in STZ-induced rats (Ho is rejected and Ha is accepted).

At the second group, after being induced with STZ starting on days 7, 14, 21, and 28, there was an increase in the insulin levels. In the third group, after being given the synthetic drug galvus on days 7, 14, 21 and 28, the insulin levels started to decrease as compared to the second group, but the levels remained high. In the fourth, fifth, and sixth groups, the extract was given kesambi with a concentration of 100, 200 and 300 mg/kg of rats on days 7, 14, 21, and 28, in which there was a decrease in blood insulin levels. In the fifth group, there was a significant decrease in blood insulin toward normal levels approaching the first group's insulin levels. At a concentration of 200 mg, kesambi extract worked optimally to reduce blood insulin levels

in Wistar-strain rats that had experienced hyperglycemia due to STZ induction [20].

Insulin levels increased after STZ induction. This is due to the destruction of pancreatic β cells. In pancreatic β cells, the STZ reaction will cause the oxidation of the peptide glutathione in the cells and form dialuric acid, which can produce free radicals in the form of hydrogen peroxide (H2O2), superoxide anion (O2), and nitric oxide (NO), thus damaging the DNA chain of pancreatic β cells. Damage to the DNA of pancreatic β cells causes necrosis and results in problematic insulin production and secretion [10].

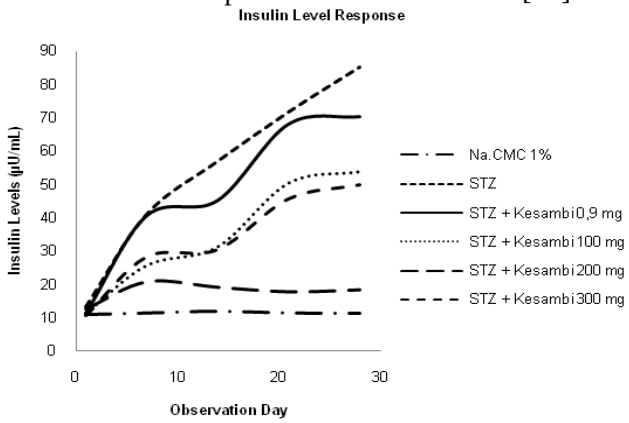

Fig. 2 Insulin level response

Treatment with kesambi extract is thought to play a role in restoring impaired glucose homeostasis by lowering blood glucose levels and tends to increase plasma insulin levels. The active compound extract of kesambi that plays a role in blood glucose homeostasis is unknown, but it is suspected that the acetone-water-soluble compound is an unknown antioxidant group. The active compounds of this antioxidant class act as antioxidants that can prevent and reduce free radicals by reacting directly to these free radicals [21].

3.6. In Vivo Activity of Kesambi Extract in Wistar-Strain Rats on the Number of Pancreatic β Cells

The relationship between treatment dose and the number of pancreatic β cells in rats given the extract of kesambi shows that the number of pancreatic β cells affects the administration of kesambi extract. To determine the effect of kesambi extract on the number of pancreatic β cells and which groups had significant differences, an analysis was performed using an unpaired t-test.

The results of the mean number of pancreatic β cells in Wistar strain mice at the second group were significantly different from the first and fifth groups, with p values <0.05, but between groups 2, 3, and 4 the numbers were not significantly different, with p values >0.05. This shows that giving kesambi extract at a concentration of 200 mg affects the number of pancreatic β cells in mice induced with STZ. (Ho is rejected and Ha is accepted.)

STZ causes damage to pancreatic β cells. One of the STZ mechanisms causes hyperglycemia related to the formation of free radicals, including NO, O2, and H2O2, which can cause cell DNA fragmentation due to STZ cytotoxics. STZ has been shown to increase reactive oxygen species (ROS) that contribute to DNA fragmentation and trigger changes in cells. STZ selectively accumulates in pancreatic β cells through the low-affinity GLUT2 glucose transporter on the plasma membrane. The entry of the methyl (alkylation) group from STZ into the DNA molecule will cause damage to the DNA fragment. The DNA damage will activate cellular poly adenosine diphosphate (ADP) - nicotinamide adenine dinucleotide (NAD +), further reducing adenosine triphosphate (ATP) [10]. This increase in ATP dephosphorylation will provide a substrate for xanthine oxidase and will speed up the reaction to give the superoxide anion as the end product. Activation of hydrogen peroxide and other hydroxyl radicals will result from the formation of this superoxide anion.

The infected control group (the second group) showed the occurrence of necrosis and degeneration as indicated by the decreased number of pancreatic β cells. This is because the second group did not receive an extract of kesambi leaves (*Schleichera oleosa*). Whereas in the third, fourth, fifth, and sixth group, the number of pancreatic β cells increased on days 7, 14, 21 and 28. It is suspected that the increased number of pancreatic β cells occurred due to the administration of extracts of kesambi (*Schleichera oleosa*), which contains antioxidants. In the fifth group, there was a significant increase in value, with numbers approaching those of the first group, namely the healthy group (Fig. 3).

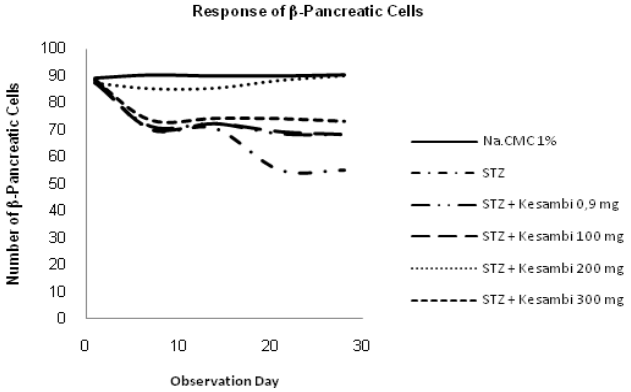

Fig. 3 Response of pancreatic β cells

Langerhans Island is formed from ductal precursor cells and can reduce oxidative stress and help restore the function of pancreatic β cells. Antioxidants help regenerate pancreatic β cells, reduce oxidative stress, and show the incidence of diabetes through reducing fat levels and increasing levels of Superoxide Dismutase (SOD), catalase and glutathione peroxide. Antioxidants can play a role in capturing free radicals or function as natural antioxidants [10]. This antioxidant activity allows antioxidants to capture or

neutralize free radicals (such as ROS or RNS) associated with phenolic OH groups so that they can improve the state of damaged tissue; in other words, the inflammatory process can be inhibited [10].

Antioxidants that play a role in reducing blood glucose levels will increase pancreatic beta cell count, insulin secretion and sensitivity levels, and the production of alpha-glucose inhibitors. The intended antioxidant compounds that play a role in lowering blood glucose levels are flavonoid polyphenols compounds, saponins, and tannins, using reactive oxygen species (ROS). Flavonoids can capture free radicals directly through the donation of hydrogen atoms that are on the OH group and conjugate the double bond. Free radicals are made inactive. Polyphenols act as antioxidants because they have hydroxyl groups that can donate hydrogen atoms to free radical compounds and stabilize reactive oxygen compounds (ROS), and have hydroxyl ketone groups that can act as metal chelles [10].

Tannins and saponins function as inhibitors of the activity of the alpha-glucosidase enzyme, which is an enzyme in digestion that converts carbohydrates into glucose, before glucose absorption is reduced [23].

4. Conclusion

A kesambi extract (*Schleichera oleosa*) has a very strong antioxidant activity. The administration of a kesambi extract (*Schleichera oleosa*) affected the regeneration of pancreatic cells, improved insulin levels, and decreased blood glucose levels of STZ-induced Wistar rats. There is an effect of administering a graded dose of a kesambi extract (Schleichera oleosa) to pancreatic cell regeneration, i.e., increasing insulin levels and lowering blood glucose levels of STZ-induced Wistar rats; it works optimally on kesambi leaf extract with a concentration of 200 mg. Further investigations should be carried out on kesambi extracts; therefore, an isolation process is needed. By knowing the content of pure compounds, it is possible to know the interactions as antidiabetic drugs.

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