JPPIPA 10(12) (2024)

Jurnal Penelitian Pendidikan IPA

Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

Effectiveness of Andaliman Extract on Blood Sugar Levels of White Mice Induced by STZ HOMA-IR

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Received: September 4, 2024
Revised: November 29, 2024
Accepted: December 12, 2024
Published: December 31, 2024

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DOI: 10.29303/jppipa.v10i12.9163

Abstract: The increasing cases and treatment costs have triggered the search for alternatives, such as Andaliman, which is believed to have antidiabetic and antioxidant activities, induced by Streptozotocin (STZ). Objective: to determine the effectiveness of Andaliman nanoemulsion extract in lowering blood sugar levels, overcoming insulin resistance as measured by HOMA-IR, in white mice induced by Streptozotocin (STZ). The study employed a posttest only controlled group design, using sic groups of mice, each of which had five mice weighing 150–260 grams in STZ induction (65 mg/kg), the positive control group used STZ + Metformin, the treatment group used a dose of Nanoandaliman of 25.50 & 75 mg/KgBB. KGD examination was carried out after 7.14 and 21 days after STZ induction. Results: Based on the lowest KGD measurement on day 21 in the STZ65mg/kgBB + NA50 mg/kgBB treatment group, in the HOMA-IR measurement, the highest value was found in the negative control (STZ 65mg/kgBB) on day 21. This indicates that the decrease in blood glucose levels with STZ65mg/kgBB + NA50 mg/kgBB treatment is better than other treatments on day 21. Conclusion: STZ65mg/kgBB + NA50 mg/kgBB treatment has been proven to be effective in lowering blood sugar levels. It is expected that the results of this study can become an alternative treatment for diabetes in the future.

Keywords: Andaliman extract; Diabetes Mellitus; Nlood sugar; STZ; White Mice

Introduction

Diabetes Mellitus (DM) is one of a disorder metabolism in the form of disease chronic characterized by increased level glucose blood from normal (Ministry of Health of the Republic of Indonesia, 2020). The pathogenesis of DM includes two mechanisms: autoimmune destruction of β pancreatic cell resulting in inadequate insulin production and insulin resistance, which is the main trigger for chronic hyperglycemia (Lovic et al., 2020). Diabetes mellitus become the most cases happening in the world, particularly in the Asian region. Several countries in the world include China, India, the United States, Pakistan, Brazil, Mexico, Indonesia, Germany, Egypt and Bangladesh. Indonesia is in 7th position among 10 countries with the number of sufferers amounting to 10.7 million. The World Health Organization (WHO) stated around 1.5 million mortality happen due to diabetes and more than 80% of deaths occurs in high-income countries low and medium that in 2012 (Tanessa et al., 2023).

Based on the International Diabetes Federation (IDF) report, the number of Type 1 diabetes sufferers in Indonesia reached 41.817 people in 2022. This number places Indonesia in the to rank in ASEAN. The majority Type 1 diabetes sufferers in Indonesia are aged between 20-59 years old, as many as 26.781 people. Then, sufferers under 20 years old as many as 13.311 people and sufferers aged 60 years and over as many as 1.721 people (International Diabetes Federation, 2022).

The prevalence of DM in Indonesia is increasing from 1.5% in 2013 to 2.0% against population aged over 15 years in 2018 (Ministry of health Republic of Indonesia, 2020). The 2018 Basic Health Research/RISKESDAS data explains that the national prevalence of DM is 8.5 % or around 20.4 million Indonesians suffering from DM. Another issue related to the management of diabetes mellitus is the diverse geographical, cultural, and social factors (Soelistijo et al., 2019).

There is an increase amount diabetes mellitus sufferer in every year also the expensive medical costs,

How to Cite:
Dhillon, J. M. K., Chiuman, L., & Rusip, G. (2024). Effectiveness of Andaliman Extract on Blood Sugar Levels of White Mice Induced by STZ HOMA-IR. *Jurnal Penelitian Pendidikan IPA*, *10*(12), 9915–9925. https://doi.org/10.29303/jppipa.v10i12.9163

especially if accompanied by clinical complications (Sukmawati et al., 2018). It makes society want to try traditional treatment, which can be used as an alternative way (Petersmann et al., 2018). Therefore, more and more therapies are developed using traditional medicinal plants to treat diabetes mellitus (Ayu et al., 2014).

Andaliman (Zanthoxylum acanthopodium DC) is a plant specialized in North Sumatra. Andaliman is stated to have an immune system stimulating effect (Adrian et al., 2023; Siregar, 2017). This is considered to be because Andaliman fruit contains active compounds that are believed to make a positive contribution for human health, namely flavonoids, alkaloids, and terpenoids (Ulfa et al., 2020).

Nanoparticles technology have now become a new trend in the drug delivery systems (Begines et al., 2020; Xu et al., 2022; Yusuf et al., 2023). Particles at the nanometre scale have distinctive physical properties compared to several particles at larger sizes, especially in improving the quality of drug compound delivery (Naito et al., 2018; Petersmann et al., 2018). Another advantage of nanoparticle technology is its openness combined with other technologies, thus opening the opportunity to produce more perfect delivery systems (Martien et al., 2012).

Insulin receptor sensitivity is known by comparing fasting glucose levels and insulin levels called Homa-IR (Cignarelli et al., 2019; Mendoza et al., 2023). One of the parameters used to assess the level of insulin resistance is Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) (Adriawan et al., 2014; Sukmawati et al., 2024). This study aims to evaluate the effectiveness of Andaliman extract in reducing blood sugar levels and HOMA-IR in white mice exposed to STZ. The results of this study are expected to provide conclusive scientific evidence regarding the potential of Andaliman extract as an anti-diabetic agent capable of overcoming metabolic changes and insulin resistance induced by STZ 65 mg/KgBB in white rats.

Method

Pure experimentation characterizes this kind of research (Dhee, 2020; Prasetyo et al., 2020). This study used a post-test only controlled group design, which took place from December 2023 to March 2024. The research was conducted in a laboratory that has complete equipment and adequate experience. Care and maintenance of test animals were carried out at the Ellio Laboratory. Meanwhile, the formation of Andaliman fruit extract into nanoparticles was done in the Prima Indonesia University Laboratory. The main tools involved standard laboratory equipment, such as micropipettes, tube reaction, measuring cups, and glucometer to measure mice blood sugar levels, blood sampling tools, such as syringe and tube reaction. The materials used are male white mice wistar strain of Andaliman extract, STZ used to induce diabetic conditions. The samples used in this study involved 30 male wistar white mice *(rattus noverticus)* with the age of 2-3 months and weighing 150-260 grams, a healthy and active physique mice, do not have anatomical abnormalities, and never been used as research samples (Maco *et al.,* 2022). The test animals were then divided into six groups of five wistar male white rats each.

Group 1 normal (without treatment), Group 2 negative control (STZ 65mg/kgBB), Group 3 positive control (STZ 65mg/kgBB + Metformin), Group 4 treatment 1 (STZ 65mg/kgBB + Nanoparticles 25mg/kgBB), Group 5 treatment 2 (STZ 65mg/kgBB + Nanoparticles 50mg/kgBB), Group 6 treatment 3 (STZ 65mg/kgBB + Nanoparticles 75mg/kgBB) (Tanessa et al., 2023).

Preparation of Andaliman fruit extract was done by drying 7000 grams of Andaliman fruit through aerating process for three days. Then, Andaliman was mashed until becoming simplisia so that its obtained was as much as 4.088,51 grams. Then, macerated (extracted) using 11 litres of methanol solvent for five days. In five times 24 hours stirred for 10 minutes. After that, the macerated extract was filtered. Then, obtained Andaliman liquid extract as much as 250 ml. After obtaining the Andaliman liquid extract, the extract was then carried out Rotary Vacum Evaporator and in Waterbath until the Andaliman concentrated extract obtained. The concentrated extract of Andaliman obtained was 108-110 grams (Djuang et al., 2022).

Blood glucose measurements of mice were carried out before STZ induction; after its induction, 7 days after STZ induction, 14 days after STZ induction, and 21 days after STZ induction. A glucometer was used to check blood glucose. Examination of blood glucose with a glucometer tool took two minutes with capillary blood being easier to take and less pain, the amount of blood used was small, the supporting examination of blood glucose levels was still debated because whether the difference was caused by the tool and that the glucometer tool results in high results from the spectrophotometer so that there were differences in values that vary with venous blood glucose levels with capillary blood (Ubaedillah, 2019).

The procedure for measuring insulin levels was carried out using an ELISA Kit in accordance with the manufacturer's rules. STZ induction was intended to produce Diabetes Mellitus rats. The dose used was STZ (65 mg/kg) + NA (25, 50 & 75 mg/KgBB) (Ghasemi & Jeddi, 2023). Diabetes Mellitus mice were rats induced with streptozotocin 65 mg/KgBB, where 15 minutes earlier injected NA (25, 50 & 75 mg/KgBB, with the

parameter of increasing fasting blood sugar levels (GDP) taken from heart blood in mice. The normal GDP level of SD rats is 55-135 mg/dl (Najib et al., 2022). Mice were declared DM if the increase in fasting blood sugar was >135 mg/dl after STZ induction, and GDP levels were measured using a glucometer (Najib et al., 2022). Diabetes Treatment Procedure with Streptozotocin (STZ) is that the test animals are fasted first for 10-18 hours. During fasting, test animals were not provided food but with adequate drink. Weighed the body weight of each test animal to determine the dose of Streptozotocin using a scale, marked the tail. The dose of STZ in micd used to cause diabetes was 65 mg/kg and NA (230 mg/kg, BW (Ghasemi & Jeddi, 2023). Mice were injected with a single dose of Streptozotocin (STZ) 65 mg/kg and overnight given 10 percent dextrose to prevent hypoglycaemic and shock in rats (Rajab et al., 2023). After STZ administration, the animals were given access to food and water. Blood sugar checks were performed before and after STZ induction, and animals with glucose levels ≈ 140±8 mg/dL were considered diabetic and selected for further study (Rais et al., 2021).

Data analysis used the ANOVA test if the data were normally distributed and homogeneous, if the data were not normally distributed and homogeneous, the Kruskal-Wallis test was conducted. If the p value <0.05 was obtained, a further post hoc test was carried out (Priyastama, 2017; Sugiyono, 2020).

Result and Discussion

Andaliman, scientifically known as Zanthoxylum acanthopodium, is an indigenous spice of North Sumatra, Indonesia, renowned for its distinctive taste and suggested therapeutic advantages (Manurung et al., 2021; Setiadi et al., 2022; Tanessa et al., 2023; Tanessa, Praboswara, & Chiuman, 2023). Recent research indicates that andaliman extract may exert a substantial influence on blood glucose levels, rendering it a topic of interest in the realm of diabetes control and metabolic well-being (Yanti et al., 2023). Nanoemulsions extract Andaliman is presented in the figure 1.

Based on Figure 1, it shows the particle size distribution of Andaliman extract nanoemulsion in the particle size range from 0.000 µm to 42,200 µm. At the beginning of the distribution (0.000-0.012 µm), no volume percentage of particles was detected (dQ3(x) % is 0.0%), indicating that the number of particles of that size was very small or zero. The Q3(x) % value remained constant at 0.0%, indicating that the accumulated percentage volume of particles up to that size kept unchanged, while the 100-Q3(x) % value indicated that 100% of the particle volume was above that size. This is consistent in the size range up to 0.096 µm.

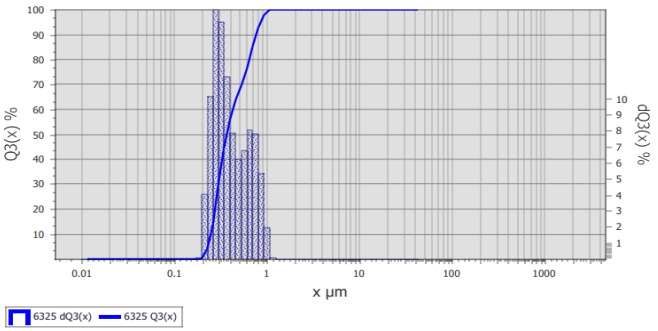

Figure 1. Nanoemulsion of Andaliman Extract (Source: Data processed by researchers, 2024)

Changes began to appear in the 0.195-0.210 µm size range, where dQ3(x) % increased to 4.0% and Q3(x) % to 4.1%, indicating that 4.1% of the total particle volume was below the 0.210 µm size, and 95.9% above it. A significant increase in particle distribution occurred in the 0.225-0.605 µm range, where the cumulative Q3(x)% percentage increased from 14.2% to 84.8%. The largest particle size detected in this data is in the range 36.633-42.200 µm, with all metric values showing 0.0%, signalling that there were no particles of that size in the sample. This data provided a detailed picture of the particle size distribution of the Andaliman extract nanoemulsion, with most particles concentrated in the range of 0.195-0.605 µm, and very few or no particles detected outside of this range.

Providing Andaliman Nanoemulsion Extract to Reduce Blood Sugar Levels

The blood sugar levels before induction and after induction in male wistar white rats (rattus noverticus) can be seen in the table 1. Table 1 shows the average blood glucose levels of male Wistar white mice (Rattus norvegicus) before and after induction. The normal treatment group (K1) showed blood glucose levels of 77 ± 100 mg/dl before induction, which increased to 130 ± 141 mg/dl after induction. In the negative control group (K2) induced with streptozotocin (STZ) and nicotinamide (NA), blood glucose levels increased drastically from 90 ± 92 mg/dl to 267 ± 600 mg/dl after induction. The positive control group (K3), which was also induced with STZ and NA and given metformin, showed an increase in glucose levels from 73 ± 101 mg/dl to 273 ± 600 mg/dl after induction. In the treatment group with doses of STZ and NA and administration of 25 mg/kgBB (K4), blood glucose levels rose from 70 ± 102 mg/dl to 233 ± 600 mg/dl after induction. The group with a dose of 50 mg/kgBB (K5) showed an increase in glucose levels from 73 ± 90 mg/dl to 216 ± 501 mg/dl after induction. The group with a dose of 75 mg/kgBB (K6) showed an increase in glucose levels from 85 ± 100 mg/dl to 237 ± 600 mg/dl after induction. These data indicate that induction with STZ

and NA consistently increases blood glucose levels in mice, with or without metformin administration, and shows variation in response to different doses. From the results of the treatment, the administration of nanoemulsion extract for 21 days could reduce the blood glucose levels of mice. Data on the results of blood glucose measurements in mice during the treatment of 6 treatment groups can be seen in Figure 2.

Table 1. Average Blood Glucose Levels of Mice before and After Induction of Andaliman Nanoemulsion Extract Administration

Group Treatment	Blood glucose levels (mg /dl)	
	Before Induction X ± SD	After Induction X ± SD
K 1 :Normal	77 ± 100	130 ± 141
K2/ Control (-): STZ + NA	90 ± 92	267 ± 600
K3/ Control (+): STZ + NA + Metformin	73 ± 101	273 ± 600
K4: STZ + NA + 25 mg/kgBW	70 ± 102	233 ± 600
K5: STZ + NA + 50 mg/kgBW	73 ± 90	216 ± 501
K6: STZ + NA + 75 mg/kgBW	85 ± 100	237 ± 600

Source: Data processed by researchers, 2024

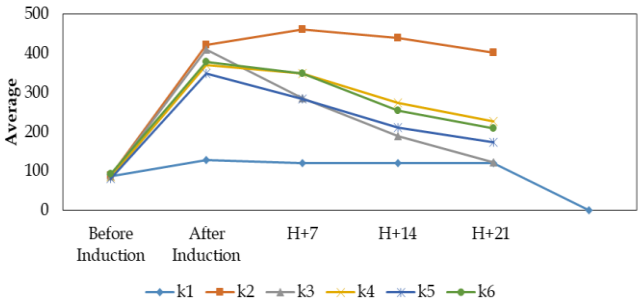

Figure 2. Effectiveness of andaliman nanoemulsion extract blood sugar level reduction (Source: Data processed by researchers, 2024)

Based on Figure 2, it can be seen that there is a significant increase in blood glucose levels in each measurement group after STZ + NA induction. Before induction, blood glucose levels in all groups were within the normal range. After STZ + NA induction, there was a significant increase in blood glucose levels in all treatment groups, with the negative control group (K2) experiencing the greatest increase. On the day 7, the blood glucose level in the K2 group increased to 460.2 mg/dL, while the positive control group (K3) given Metformin experienced a decrease in blood glucose levels to 286.8 mg/dL.

On days 14 and 21, the group provided Andaliman nanoemulsion showed a better reduction in blood glucose levels with an increase in dose producing a more significant effect. On day 21, blood glucose levels in group K5 (50 mg/kgBB) dropped to 173.2 mg/dL lower than groups K4 (25 mg/kgBB) and K6 (75 mg/kgBB) but remained higher than the positive control group (K3).

When compared to the normal group (K1), all treatment groups showed an increase in blood glucose levels after induction. However, group K5: STZ + NA + 50 mg/kgBB is a group treated with Andaliman nanoemulsion showed a better reduction in blood glucose levels.

Influence of Andaliman Nanoemulsion Extract Administration on the Level of Insulin Resistance

The effect of the administration of andaliman nanoemulsion extract on the level of insulin resistance, evaluated through changes in the value of insulin resistance in white mice subjected to STZ induction can be seen in the figure 3.

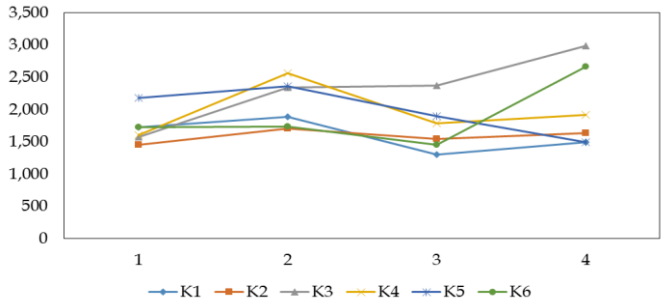

Figure 3. 1elisa kit (Source: Data processed by researchers, 2024)

Based on Figure 3, it can be seen that the measurement of insulin levels using the Elisa Kit shows a clear difference between the study groups. The normal group (K1) had relatively stable insulin levels, with values ranging from 1.307 to 1.892. The negative control group (K2) administered STZ + NA showed lower insulin levels, with values ranging from 1.457 to 1.712, indicating a significant hyperglycaemic effect.

The positive control group (K3) given STZ 65mg/kgBB + Metformin showed higher insulin levels than the negative control group, with values ranging from 1.574 to 2.994, indicating the effectiveness of Andaliman nanoemulsion in increasing insulin levels. In the group given Andaliman nanoemulsion at a dose of 25 mg/kgBB (K4), insulin levels were in the range of 1.609 to 1.920. The dose of 50 mg/kgBB (K5) showed higher insulin levels, ranging from 1.495 to 2.368, while the dose of 75 mg/kgBB (K6) showed the highest insulin levels with a range of 1.459 to 2.671.

Overall, the data indicated that the administration of Andaliman nanoemulsion and Metformin can increase insulin levels, with higher doses showing greater effects. The positive control group and the group

with the higher dose of Andaliman nanoemulsion showed a significant increase in insulin levels compared to the negative control group.

Influence of Andaliman Nanoemulsion Extract Administration on HOMA-IR

The influence giving Andaliman nanoemulsion extract on the level of HOMA-IR can be seen in the figure 4.

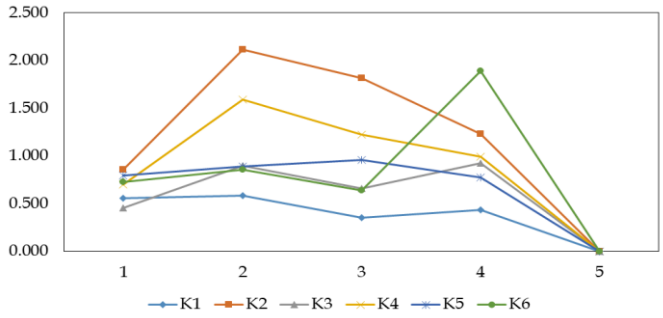

Figure 4. HOMA-IR (Source: Data processed by researchers, 2024)

Figure 4, it can be seen that the effect of Andaliman nanoemulsion extract on the level of HOMA-IR shows that the lowest HOMA-IR value is obtained in the positive control group (STZ 65mg/kgBB + Metformin). These results indicate that the combination of STZ and metformin is effective in reducing insulin resistance, as reflected by lower HOMA-IR values compared to other groups. Metformin, as a widely known antidiabetic drug, plays an iportant role in improving insulin sensitivity and reducing glucose production by the liver, which seems to dominate the effect of insulin resistance in this group. The significant reduction in HOMA-IR values in this group indicated that metformin provided stronger protection against STZ-induced insulin resistance compared to the other treatments, including Andaliman nanoemulsion extract.

Meanwhile, the highest HOMA-IR value was obtained in the negative control (STZ 65mg/kgBB). These results indicate that treatment with STZ produces the highest level of insulin resistance compared to other groups. STZ is a known agent that can damage pancreatic beta cells and induce type 1 diabetes mellitus, resulting in decreased insulin production and increased insulin resistance. The high HOMA-IR values in the negative control group indicated that STZ caused significant impairment in insulin function and sensitivity, which was not addressed or compensated by any intervention in this group.

Data Analysis

The results of statistical analysis of incision wound healing research data using the SPSS (Statistical Package for the Social Sciences) application to determine the influence of the effectiveness of Andaliman nanoemulsion extract in reducing blood sugar levels, overcoming insulin resistance measured through Streptozotocin (STZ) induced HOMA-IR. Group 1 was normal (without treatment), Group 2 negative control (STZ 65mg/kgBB), Group 3 positive control (STZ 65mg/kgBB + Metformin), Group 4 treatment 1 (STZ 65mg/kgBB + Nanoparticles 25mg/kgBB), Group 5 treatment 2 (STZ 65mg/kgBB + Nanoparticles 50mg/kgBB), Group 6 treatment 3 (STZ 65mg/kgBB + Nanoparticles 75mg/kgBB). Statistical testing of the research hypothesis was done using the One Way Anova test. The table below presents the results of the data normality test.

Table 2. Results of Blood Sugar Level Normality Test

Group		Shapiro Wilk		
		Statistics	df	Sig.
Before	K1: Normal	.958	5	.792
	K2/Control (-): STZ + NA	.957	5	.787
	K3/Control (+): STZ + NA + Metformin	.998	5	.999
	K4: STZ + NA + 25 mg/kgBW	.969	5	.871
	K5: STZ + NA + 50 mg/kgBW	.909	5	.463
	K6: STZ + NA + 75 mg/kgBW	.890	5	.357
After	K1: Normal	.931	5	.601
	K2/Control (-): STZ + NA	.941	5	.670
	K3/Control (+): STZ + NA + Metformin	.919	5	.522
	K4: STZ + NA + 25 mg/kgBW	.915	5	.499
	K5: STZ + NA + 50 mg/kgBW	.928	5	.586
	K6: STZ + NA + 75 mg/kgBW	.930	5	.595
Day 7	K1: Normal	.866	5	.251
	K2/Control (-): STZ + NA	.843	5	.173
	K3/Control (+): STZ + NA + Metformin	.879	5	.303
	K4: STZ + NA + 25 mg/kgBW	.852	5	.199
	K5: STZ + NA + 50 mg/kgBW	.856	5	.213
	K6: STZ + NA + 75 mg/kgBW	.911	5	.475
Day 14	K1: Normal	.977	5	.919
	K2/Control (-): STZ + NA	.850	5	.195
	K3/Control (+): STZ + NA + Metformin	.930	5	.595
	K4: STZ + NA + 25 mg/kgBW	.989	5	.977
	K5: STZ + NA + 50 mg/kgBW	.873	5	.277
	K6: STZ + NA + 75 mg/kgBW	.894	5	.378
Day21	K1: Normal	.952	5	.750
	K2/ Control (-): STZ + NA	.811	5	.098
	K3/ Control (+): STZ + NA + Metformin	.923	5	.550
	K4: STZ + NA + 25 mg/kgBW	.985	5	.960
	K5: STZ + NA + 50 mg/kgBW	.756	5	.034
	K6: STZ + NA + 75 mg/kgBW	.845	5	.178

*. This is a lower bound of the true significance.
a. Lilliefors Significance Correction
Source: SPSS Output Results, 2024

Based on the results of the Shapiro wilk test, blood sugar levels in all treatment groups showed on day 21 there was a p value <0.05, which means that the blood glucose level data on day 21 was not normally distributed, so the Kruskal wallis test was continued. Homogeneity test results can be seen in the table below:

Table 3. Test of 1Sugar Level Homogeneity

		Levene Statistics	df1	df2	Sig.
Before	Based on Mean	2,022	5	24	.112
	Based on Median	1,600	5	24	.198
	Based on Median and with adjusted df	1,600	5	16,377	.215
	Based on trimmed mean	2,014	5	24	.113
After	Based on Mean	1,935	5	24	.126
	Based on Median	1,437	5	24	.247
	Based on Median and with adjusted df	1,437	5	18,203	.258
	Based on trimmed mean	1,883	5	24	.135
Day 7	Based on Mean	3,575	5	24	.015
	Based on Median	1,508	5	24	.225
	Based on Median and with adjusted df	1,508	5	18,390	.236
	Based on trimmed mean	3,428	5	24	.018
Day 14	Based on Mean	7,965	5	24	.000
	Based on Median	5,608	5	24	.001
	Based on Median and with adjusted df	5,608	5	10,363	.009
	Based on trimmed mean	7,999	5	24	.000
Day 21	Based on Mean	13,214	5	24	.000
	Based on Median	1,935	5	24	.126
	Based on Median and with adjusted df	1,935	5	6,193	.219
	Based on trimmed mean	11,893	5	24	.000

Source: SPSS Output Results, 2024

Based on the results of homogeneity of blood sugar levels in all treatment groups, it shows that on day 7, day 14 and day 21 have a significant value <0.05, which means that the blood glucose level data on day 7, day 14 and day 21 are not homogeneous because the value (p <0.05).

Based on Table 4, it can be seen that the results of the Kruskal-Wallis test of blood sugar levels in each group on day 7, day 14, and day 14. On day 7, the Kruskal-Wallis test results of blood sugar levels obtained a significant value of 0.004 <0.05 means that the ρ value is greater than α = 0.05, so the alternative hypothesis (Ha) is rejected. So, it can be concluded that there is a significant difference between each treatment group on HOMA IR blood sugar levels of white mice induced by STZ on day 7. On day 14, the results of the Kruskal Wallis test on blood sugar levels obtained a significant value of 0.001 <0.05 means that the ρ value is greater than α = 0.05, so the alternative hypothesis (Ha) is rejected. It can be concluded that there is a real difference between each treatment group on the HOMA IR blood sugar levels of white mice induced by STZ on day 14. Day 21, the results of the Kruskal Wallis test on blood sugar levels obtained a significant value of 0.000 <0.05 means that the ρ value is greater than α = 0.05, so the alternative hypothesis (Ha) is rejected. It can be concluded that there is a real difference between each treatment group on HOMA IR blood sugar levels of white mice induced by STZ day 21.

Table 4. Kruskal Wallis Test Results on Blood Sugar Levels

Test Statistics[a,b]	Day7	Day 14	Day21
Kruskal-Wallis H	17,126	21,215	23,981
df	5	5	5
Asymp . Sig.	.004	.001	.000
a. Kruskal Wallis Test			
b. Grouping Variable: Group			

Source: SPSS Output Results, 2024

Mann Whitney Test is a non- parametric test used to determine whether there is a difference in the average of two samples.

Table 5. Results of 2-Whitney U Test of Blood Sugar Levels

Test Statistics[a]	Day 7	Day 14	Day 21
Mann-Whitney U	.000	.000	.000
Wilcoxon W	15,000	15,000	15,000
Z	-2,611	-2,611	-2,619
Asymp . Sig. (2-tailed)	.009	.009	.009
Exact Sig. [2*(1-tailed Sig.)]	.008 [b]	.008 [b]	.008 [b]
a. Grouping Variable: Group; b. Not corrected for ties.			

Source: SPSS Output Results

Based on the results of the Man Whitney U test of blood sugar levels, it can be concluded that there is a significant comparison between each treatment group on the blood sugar levels of STZ-induced white mice on day 7, day 14, and day 21. This is because the significant value <0.05. Anova test was conducted with 95% confidence level.

Table 6. ANOVA Test Results for Blood Sugar Levels

		Sum of Squares	df	Mean Square	F	Sig.
Before	Between Groups	381,100	5	76,220	.961	.461
	Within Groups	1903,200	24	79,300		
	Total	2284,300	29			
After	Between Groups	291197,467	5	58239,493	3,622	.014
	Within Groups	385874,400	24	16078,100		
	Total	677071,867	29			

Source: SPSS Output Results, 2024

Based on the data in table 6 above, it reveals that the blood sugar level before STZ induction obtained a significant value of 0.461 so that (P>0.05) this value indicates there is no significant difference between each treatment group on blood sugar levels HOMA IR white mice before STZ Induction.

While blood sugar levels after STZ induction obtained a significant value of 0, ,014 so that (P <0.05) the value indicates that there is a real difference between each treatment group on blood sugar levels HOMA IR white mice after STZ Induction. The statistical test was continued using the LSD post hoc test because the data were normally distributed and homogeneous (Prabowo et al., 2021). The results of the LSD post hoc test can be seen in the table 7.

Table 7. 3LSD Post Hoc Test of Blood Sugar Levels

Group	Group Comparison	Significance
K1: Normal	K2/Control (-): STZ + NA	.001 *
	K3/Control (+): STZ + NA + Metformin	.002 *
	K4: STZ + NA + 25 mg/kgBW	.006 *
	K5: STZ + NA + 50 mg/kgBW	.011 *
	K6: STZ + NA + 75 mg/kgBW	.005 *
K2/Control (-): STZ + NA	K1: Normal	.001 *
	K3/Control (+): STZ + NA + Metformin	.882
	K4: STZ + NA + 25 mg/kgBW	.536
	K5: STZ + NA + 50 mg/kgBW	.376
	K6: STZ + NA + 75 mg/kgBW	.595
K3/Control (+): STZ + NA + Metformin	K1: Normal	.002 *
	K2/Control (-): STZ + NA	.882
	K4: STZ + NA + 25 mg/kgBW	.636
	K5: STZ + NA + 50 mg/kgBW	.459
	K6: STZ + NA + 75 mg/kgBW	.701
K4: STZ + NA + 25 mg/kgBW	K1:Normal	.006 *
	K2/Control (-): STZ + NA	.536
	K3/Control (+): STZ + NA + Metformin	.636
	K5: STZ + NA + 50 mg/kgBW	.786
	K6: STZ + NA + 75 mg/kgBW	.929
K5: STZ + NA + 50 mg/kgBW	K1: Normal	.011 *
	K2/Control (-): STZ + NA	.376
	K3/Control (+): STZ + NA + Metformin	.459
	K4: STZ + NA + 25 mg/kgBW	.786
	K6: STZ + NA + 75 mg/kgBW	.719
K6: STZ + NA + 75 mg/kgBW	K1: Normal	.005 *
	K2/Control (-): STZ + NA	.595
	K3/Control (+): STZ + NA + Metformin	.701
	K4: STZ + NA + 25 mg/kgBW	.929
	K5: STZ + NA + 50 mg/kgBW	.719

Information: (*) Significant; ( ) Not significant

In group 1 (without treatment), it can be seen that there is a significant comparison with group 2 negative control (STZ 65mg/kgBB), group 3 positive control (STZ 65mg/kgBB + Metformin), group 4 treatment 1 (STZ 65mg/kgBB + Nanoparticles 25mg/kgBB), group 5 treatment 2 (STZ 65mg/kgBB + Nanoparticles 50mg/kgBB), and group 6 treatment 3 (STZ 65mg/kgBB + Nanoparticles 75mg/kgBB) because the significant value <0.05. In group 2 negative control (STZ 65mg/kgBB), it can be seen that there is a significant comparison with group 1 (without treatment). This is because the significant value <0.05.

In group 3 positive control (STZ 65mg/kgBB + Metformin), there is a significant comparison with group 1 (without treatment) because the significant value <0.05. In group 4 treatment 1 (STZ 65mg/kgBB + Nanoparticles 25mg/kgBB), it can be seen that there is a significant comparison with group 1 (without treatment) because the significant value <0.05. In group 5 treatment 2 (STZ 65mg/ kgBB + Nanoparticles 50mg/ kgBB), it can be seen that there is a significant comparison with group 1 (without treatment) because the significant value <0.05. In group 6 treatment 3 (STZ 65mg/ kgBB + Nanoparticles 75mg/ kgBB), there is a significant comparison with group 1 (without treatment) because the significant value <0.05.

Discussion

The particle size distribution of the Andaliman extract nanoemulsion revealed that at the beginning of

the size range (0.000-0.012 µm), no particle volume was detected, with all particles being above this size. Significant changes began to be seen at 0.195-0.210 µm, where 4.1% of the particle volume was below this size. Most particles are concentrated in the 0.225-0.605 µm range, with the cumulative percentage increasing from 14.2% to 84.8%. The largest particle size detected was in the range 36.633-42.200 µm. However, no particles were detected in this size. Overall, the data showed that the majority of Andaliman extract nanoemulsion particles focused on the size range of 0.195-0.605 µm, with very few particles outside this range.

The results of this study showed that the administration of Andaliman nanoemulsion extract with a nanoparticle dose of 50 mg/kgBB proved to be more effective in reducing blood sugar levels compared to doses of 22 mg/kgBB and 75 mg/kgBB in white mice induced with STZ 65 mg/kgBB. This suggests that a dose of 50 mg/kgBB provides a more optimal therapeutic effect in reducing hyperglycemia in the mice model, possibly because this dose achieves the right balance between effectiveness and potential side effects, whereas lower or higher doses may not provide equally effective results. A dose of 50 mg/kgBB may be optimal in terms of absorption, metabolism, and biological activity of the extract, resulting in a more significant reduction in blood sugar levels.

Experimental animals that have been induced by Streptozotocin were provided Nanoparticles with a size between 1 and 100 nanometers. Where the function of nanoparticles was to deliver drugs in small particle sizes to allocate faster dissolution in the blood, targeted drug delivery systems in a certain way, permeation of drugs across epithelial and endothelial barriers, to deliver drugs at the site of action, and combined therapy of two different modalities or drugs (Tanessa et al., 2023). Nanoemulsion became one of the forms of drugs that could go directly to specific areas quickly with preparations in nano form. In this study, the administration of Andaliman Extract Nanoemulsion at a dose of 50mg/kgBB was more effective than the dose of 25 mg/kgBB and 75mg/kgBB was effective in reducing blood sugar levels.

Based on the measurement of insulin restitution, the positive control group (K3), which received STZ 65 mg/kgBB + Metformin was higher than the other groups. This suggests that although Metformin is a commonly used diabetes drug and effective in lowering blood sugar levels, the combination of STZ and Metformin may not fully overcome insulin resistance caused by STZ. The high insulin resistance in this group may indicate that treatment with Metformin has not been optimal in modulating insulin metabolism in STZ-induced mice, and there are other factors that influence its effectiveness in reducing insulin resistance. Further analysis was required to understand the mechanism behind the increased insulin resistance in this group and to evaluate whether different doses or combinations of treatment may provide better results.

In addition, the highest HOMA-IR was obtained in the negative control group which was only given STZ 65 mg/kgBB. This indicates that without intervention, STZ 65 mg/kgBB significantly caused increased insulin resistance in white mice. This condition reflects the direct impact of STZ as a diabetes inductor, resulting in impaired glucose metabolism and poorer insulin sensitivity. The increased HOMA-IR value in this group indicates that STZ causes more severe disturbances in insulin function.

Administration of STZ to experimental animals can significantly increase blood glucose levels. After STZ induction, it increased in all groups. This situation is classified as hyperglycemia characterized by glucose levels above normal, which results in decreased insulin secretion resulting in DM (Nurhidajah & Nurrahman, 2016). STZ, or Streptozotocin, is a compound known to produce insulin, thus disrupting glucose regulation in the body. When STZ is administered, it induces diabetes by causing a decrease in insulin production, which results in the body's inability to control blood glucose levels effectively. As a result, blood glucose levels increased due to the lack of insulin that could regulate glucose uptake into the body's cells. This increase in blood glucose levels was the main indicator of metabolic disorders caused by STZ and was used as a parameter to evaluate the efficacy of therapeutic interventions in diabetes models (Pratama et al., 2023).

Based on the results of the Man Whitney U test, there is a significant difference between each treatment group on HOMA IR blood sugar levels of white mice induced by STZ on day 7, day 14, and day 21, In addition, the LSD post hoc test shows that there is a significant difference between each treatment group on HOMA IR blood sugar levels of white mice induced by STZ after STZ induction but there is no significant difference before STZ induction. These results indicate that the treatments provided to the groups have significantly different effects on blood sugar levels and HOMA-IR in STZ-induced white mice. This significant difference can be caused by the effect of the treatment given to each group.

The findings of this study are consistent with previous research documenting the efficacy of Andaliman fruit (Zanthoxylum acanthopodium) extract in enhancing the histological condition of peripheral nerves in Wistar rats (Rattus norvegicus) induced by alloxane (Simanullang et al., 2022). Research findings investigating the impact of Andaliman (Zanthoxylum Acanthopodium DC) extract nanoemulsion on lipid profiles in male Wistar rats induced by streptozotocin

(STZ) indicate that a 25 mg/KgBW dose of Andaliman fruit extract nanoemulsion is the most effective in enhancing Triglycerides and HDL levels (Tanessa et al., 2023). Diabetes Mellitus is a persistent metabolic condition marked by elevated blood glucose levels and disrupted metabolism of carbohydrates, lipids, and proteins, caused by inadequate efficacy of insulin. The findings of this study can serve as a basis for ongoing experimental investigation on the efficacy of Andaliman extract in regulating blood glucose levels.

Conclusion

The administration of Andaliman nanoemulsion extract with a nanoparticle dose of 50 mg/kgBB proved to be more effective in reducing blood sugar levels compared to doses of 22 mg/kgBB and 75 mg/kgBB in white mice induced with STZ 65 mg/kgBB. The positive control group (K3), which received STZ 65 mg/kgBB+Metformin, showed higher insulin levels. In contrast, the highest HOMA-IR value was obtained in the negative control group which was only given STZ 65 mg/kgBB. Based on the results of the Man Whitney U test, there is a significant difference between each treatment group on HOMA IR blood sugar levels of white mice induced by STZ on day 7, day 14, and day 21. Although promising, the efficacy of Andaliman extract in regulating blood sugar levels necessitates additional research, namely through extensive human clinical studies. Although current data indicates some advantages, further study is required to comprehensively comprehend its effectiveness, ideal dosage, and long-term consequences.

Acknowledgments
The authors would like to thanks to University for give occasion for this research.

Author Contributions
Investigation, J.K, L.C and G.R; formal analysis, J.K, L.C and G.R and G.R; resources, J.K, L.C and G.R; data curation , J.K, L.C and G.R: writing—original draft preparation, J.K, L.C and G.R; writing—review and editing, , J.K, L.C and G.R: visualization, J.K, L.C and G.R; supervision, , J.K, L.C and G.R; project administration, A.P, S.C.S.A and A.S.A; funding acquisition, , J.K, L.C and G.R. All authors have read and agreed to the published version of the manuscript.

Funding
The author personally funds the collection, analysis, interpretation of data, and the writing of the manuscript for this project.

Conflicts of Interest
There are no conflicts of interest among any of the authors.

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