Effectiveness of Andaliman Nanoemulsion Extract on Serum Adiponectin Levels in Male Wistar Rats Induced by Streptozotocin

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Abstract. Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterized by insulin resistance and chronic hyperglycemia. Low adiponectin levels are associated with increased T2DM risk. Andaliman, with its antioxidant and anti-inflammatory properties, may influence adiponectin levels and improve insulin sensitivity. This study investigates the effects of andaliman nanoemulsion on adiponectin and blood glucose levels in male Wistar rats. The rats were divided into six groups: normal, negative control, positive control, and three groups treated with andaliman nanoemulsion at doses of 25, 50, and 75 mg/kgBW. Blood glucose was measured before treatment and on days 7, 14, and 21, while adiponectin levels were assessed on day 21 using ELISA. Results showed a significant reduction in blood glucose at the 50 mg/kgBW dose (P¡0.05), but no significant differences in adiponectin levels (P¿0.05). In conclusion, andaliman nanoemulsion shows promise as an antihyperglycemic agent for T2DM management.

Keywords: Adiponectin, Andaliman, Diabetes, Hyperglycemia

1 Introduction

Type 2 diabetes mellitus (T2DM) is one of the most prevalent metabolic diseases worldwide. It is characterized by chronic hyperglycemia due to insulin resistance and impaired insulin secretion. The increasing global burden of T2DM, driven by lifestyle changes and dietary habits, necessitates searching for novel therapeutic agents to manage the condition effectively. Current pharmacological treatments aim to control blood glucose levels but do not completely address underlying metabolic disturbances, such as reduced adiponectin levels and insulin sensitivity. Adiponectin, an adipocyte-derived hormone, regulates glucose levels and fatty acid breakdown. Decreased levels of this hormone are closely linked to the development and progression of T2DM. Hence, increasing adiponectin levels could be a potential target for diabetes management. Traditional herbs and natural products have been explored for potential health benefits, including regulating adiponectin and blood glucose levels.

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Andaliman (Zanthoxylum acanthopodium) is a lesser-known fruit that has garnered attention due to its potent antioxidant and anti-inflammatory properties. Previous studies have suggested that these properties could positively influence insulin sensitivity and adiponectin levels. However, the specific effects of andaliman on T2DM, particularly in its nanoemulsion form, have yet to be extensively studied.

This study aims to explore the effects of andaliman nanoemulsion on adiponectin and blood glucose levels in a T2DM rat model. Using the Self-Nanoemulsifying Drug Delivery System (SNEDDS) to enhance the bioavailability of andaliman, this research investigates whether the nanoemulsion can offer a practical approach to managing hyperglycemia and improving metabolic health in T2DM. The study's findings could contribute to developing new therapeutic strategies for T2DM management.

2 Literature Review

Type 2 diabetes mellitus (T2DM) is the most common metabolic disease. It is mainly characterized by metabolic disorders, such as high blood sugar levels (hyperglycemia), high blood fat levels (hyperlipidemia), and insulin resistance. Diabetes can cause various serious complications, such as coronary heart disease, lower extremity arterial disease, retinal damage (retinopathy), and kidney damage (diabetic nephropathy), which affect the quality of life of many people worldwide [10]. T2DM has become a serious public health problem globally. The latest statistical data analysis shows several new epidemiological characteristics of T2DM. First, diabetes cases continue to increase in developed countries such as the United States and Japan. In addition, it should be noted that T2DM has become an increasingly worrying problem in developing countries. It is estimated that the number of T2DM cases will continue to increase in the next twenty years, with more than 70% of new patients appearing in developing countries, mostly aged 45 to 64 years. Currently, seven of the ten countries with the most significant number of diabetes patients are low- or middle-income countries, including India, China, Russia, Brazil, Pakistan, Indonesia, and Bangladesh, with prevalence rates of 12.1% in India and 9.7% in China, respectively [22]. T2DM is associated with active inflammatory signals and abnormal cytokine production. Chronic low-grade inflammation and increased pro-inflammatory agents are closely related to the development and progression of T2DM. This inflammation also contributes to insulin resistance, impaired beta-cell function, and various other complications of diabetes [4].

Adiponectin, which is the most prominent adipokine, is a protein with a molecular weight of 30 kDa that is mainly secreted by fat cells (adipocytes). This protein interacts with various types of cells or tissues/organs through binding to its own receptors and functions to protect the body from obesity, insulin resistance, and inflammation-related diseases [11]. Fibrosis and high-grade local inflammation are the main causes of metabolic disorders caused by insulin resistance. A significant decrease in adiponectin production and secretion often

accompanies these negative effects. Therefore, circulating adiponectin levels may serve as an important indicator of adipose tissue health and reflect the overall metabolic flexibility of the tissue during metabolic perturbations. Healthy adipose tissue tends to secrete more adiponectin, whereas unhealthy adipose tissue, such as that undergoing fibrosis or inflammation, secretes less adiponectin [19].

In laboratory models, streptozotocin (STZ) is one of the most frequently used chemicals to induce diabetes. However, its cytotoxic effects on β cells are highly damaging and mimic the pathology of human type 1 diabetes [15]. Masiello et al. (1998) developed a model of diabetes in mice using STZ and nicotinamide (NA) induction. STZ selectively damages pancreatic beta cells, whereas NA reduces the damage caused by STZ, creating a partial insulin deficiency state that mimics type 2 diabetes. The severity of diabetes induced by the combination of STZ + NA was much milder than that induced by STZ alone; mice experience mild hyperglycemia and do not require exogenous insulin to survive [5].

Andaliman (Zanthoxylum acanthopodium DC) is a native plant from North Sumatra, often referred to as the "Golden Spice of North Sumatra". This plant grows in the Tapanuli area and is used as a spice in traditional Batak Angkola and Batak Mandailing cuisine. Andaliman fruit has a spicy taste and a distinctive aroma in dishes [6]. Andaliman fruit contains bioactive compounds such as alkaloids, flavonoids, and essential oils, which have been shown to have various pharmacological benefits. These benefits include anti-inflammatory, antimicrobial, and antioxidant properties [14]. Phytochemical screening conducted by [17] showed several compounds with anti-diabetic activity, namely Dehydroevodiamine (alkaloid), Leucopelargonidin, and Morin (flavonoid). Based on the description above, researchers hope to continue the research to prove whether the nanoemulsion extract of andaliman (Zanthoxylum acanthopodium DC), whose fruit is often used by the Batak tribe as a cooking spice, has anti-hyperglycemic properties and its effects on the adiponectin hormone, and how the relationship between blood sugar levels and adiponectin hormone levels in male Wistar rats induced with STZ-NA.

3 Methods

3.1 Research Design and Ethical Clearance

The method used in this study is an experimental method with a post-test only control group design. This study has been approved by the Health Research Ethics Committee (KEPK) of Universitas Prima Indonesia with the number: 037 / KEPK / UNPRI / III / 2024.

3.2 Preparation of Andaliman Nanoemulsion

To prepare the nanoemulsion, Methyl Paraben and Propyl Paraben were dissolved in heated and cooled distilled water, then Tween 80 was added to the distilled water and stirred with a magnetic stirrer for 30 minutes at a speed of

5000 rpm (Mass 1). Furthermore, PEG 400 was added to the andaliman extract and stirred with a magnetic stirrer for 20 minutes at a speed of 5000 rpm (Mass 2). After that, Mass 1 and Mass 2 were slowly combined using a dropper, then stirred with a magnetic stirrer for 8 hours at a speed of 5000 rpm, and finally sonicated.

3.3 STZ-NA Induction Procedure and Nanoemulsion

The study involved 30 male Wistar rats aged 6–8 weeks with a body weight of 150–200 grams, which were divided into six groups:

1. Normal group.
2. Negative control group (given STZ 65 mg/kgBW + NA 230 mg/kgBW).
3. Positive control group (given STZ 65 mg/kgBW + NA 230 mg/kgBW + Metformin 9 mg).
4. Treatment group 1 (given STZ 65 mg/kgBW + NA 230 mg/kgBW + nanoemulsion 25 mg/kgBW).
5. Treatment group 2 (given STZ 65 mg/kgBW + NA 230 mg/kgBW + nanoemulsion 50 mg/kgBW).
6. Treatment group 3 (given STZ 65 mg/kgBW + NA 230 mg/kgBW + nanoemulsion 75 mg/kgBW).

The test animals were given an acclimatization period of 7 days to adapt to the new environment. During this acclimatization period, the test animals were fed and watered three times a day. The room temperature was maintained at 18-19 degrees Celsius, with air humidity between 30-70%. In addition, the test animals were also kept away from noise sources during this acclimatization process [16]. The test animals were fasted overnight before being given an intraperitoneal injection of NA (230 mg/kg body weight, in saline), 15 minutes before the intraperitoneal injection of STZ (65 mg/kg body weight), which was dissolved in 0.1 M cold citrate buffer (pH 4.5) just before use. After administration of NA-STZ, the animals were given free access to eat and drink ad libitum (Rais et al., 2021). The treatment group was given nanoemulsion orally according to the required dose.

3.4 Blood Glucose Level Measurement

Blood glucose levels were measured using a glucometer through the tail of the rat. Fasting blood glucose was evaluated after 2 days, and animals with glucose levels of around 140±8 mg/dl were considered diabetic and selected for further research. Blood glucose level measurements were performed every 7 days, namely on days 7, 14, and 21 [15], [9].

3.5 Blood Sampling

On day 21, the mice were anesthetized using ketamine at a dose of 80 mg/kgBW, and then 2-3 ml of blood was taken through the intracardial blood vessels. The blood taken was stored in a vacutainer tube [3]. The American Veterinary Medical Association (AVMA) recognizes barbiturates, such as pentobarbital, and dissociative agents like ketamine as acceptable methods for achieving a humane death when administered independently. In contrast, various alternative methods may be employed in laboratory environments; however, these approaches necessitate specific conditions to ensure a humane outcome. This is due to their increased likelihood of operator error or safety risks, insufficient documentation in scientific literature, or the need for an additional method to confirm death. Examples of such methods include halogenated anesthetics (e.g., halothane, isoflurane, or sevoflurane), carbon dioxide, cervical dislocation, and decapitation. The latter two techniques should only be utilized when anesthetics would compromise the study's objectives. Furthermore, the AVMA delineates a range of unacceptable methods, which encompasses neuromuscular blocking agents, potassium chloride (when not preceded by anesthesia), urethane, and diethyl ether, the latter of which is both irritating and flammable, thereby causing distress to the animal and posing hazards to the human operator [21].

3.6 Serum Adiponectin Level Measurement

Serum Adiponectin levels were measured on day 21 using the Sandwich ELISA kit method from Bioassay Technology Laboratory with Cat. No. E0758Ra, according to the manufacturer's instructions.

3.7 Statistical Analysis

This study analyzed data using SPSS with several statistical tests. To test the difference in average blood sugar levels between groups, the ANOVA test was used with a significance level of 0.05. If the ANOVA assumptions were not met, the Kruskal-Wallis test was used as a non-parametric alternative. In addition, Pearson's correlation test was used to assess the relationship between blood sugar levels on day 21 and serum adiponectin concentrations, while Spearman's test was used if the data did not meet the assumption of normality.

4 Results

In this study, it was found that there was no significant difference in blood sugar levels before induction with a p value ¿ 0.05. In blood sugar levels after induction, day 7, day 14, day 21 showed a significant difference, this is indicated by a p value ¡ 0.05 using Kruskal-Wallis analysis. There was an increase in the average blood sugar level in the Negative Control Group compared to the Normal Group and there was a decrease in the average blood sugar level in the Positive Control

Group and all Groups given Nanoemulsion compared to the Negative Control Group. Data on blood sugar level variables for each group can be seen in Table 1.

Table 1. Blood Sugar Levels of Each Group

Treatment Group	Blood Sugar Level, mg/dl				
	Before Induction	After Induction	Day 7	Day 14	Day 21
Normal	87,6 (77-100)	129,4 (112-141)	120 (102-130)	121,6 (115-128)	120,8 (108-131)
Negative Control	89,4 (86-92)	421,4 (267-600)	460,2 (300-600)	438,8 (271-600)	402,2 (237-500)
Positive Control	87,2 (73-101)	409,4 (259-600)	286,8 (170-417)	190,4 (132-275)	121,8 (100-155)
Nanoemulsion 25 mg/kgBW	85,2 (70-102)	371 (233-600)	348,4 (280-472)	274 (207-350)	226,2 (175-277)
Nanoemulsion 50 mg/kgBW	81,4 (73-90)	349 (216-501)	283,4 (209-432)	211 (177-274)	173,2 (147-210)
Nanoemulsion 75 mg/kgBW	93 (85-100)	378,2 (237-600)	349,6 (255-503)	255 (203-310)	208,4 (169-287)
P Value	0,521	0,022	0,004	0,001	0,000

In this study, there was no significant difference in serum Adiponectin levels between treatment groups as seen from the p value ¿ 0.05 (0.820) in the Kruskal - Wallis test. Data on serum adiponectin levels can be seen in Table 2.

Table 2. Serum Adiponectin Levels in Each Group

Treatment Group	Adiponectin Serum (mg/l)			P Value
	Med	Min	Max	
Normal	13,1	10,7	16,4	0,820
Negative Control	13,8	11,5	15,8	
Positive Control	14,1	13,1	15,4	
Nanoemulsion 25 mg/kgBW	12,8	10,9	14,4	
Nanoemulsion 50 mg/kgBW	12,7	11,4	13,6	
Nanoemulsion 75 mg/kgBW	13,1	11,6	15,4	

From the data output in Table 3, a correlation coefficient figure of -0.114 was obtained, which means that the negative correlation between KGD on day 21 and Adiponectin is very weak. The Sig. (2-tailed) value is 0.549, because the Sig value¿ 0.05, the relationship between KGD on day 21 and Adiponectin is not significant.

Table 3. Spearman's rho Correlation Test between Blood Glucose Levels on Day 21 and Adiponectin

	Blood Glucose Level day 21	Adiponectin Levels
Correlation Coefficient	1000	-0,114
Sig. (2-tailed)	.	0,549
N	30	30

5 Discussion

The STZ-NA rat model in type 2 diabetes was created based on the protection provided by NA against the β-cell toxic effects of STZ [7]. Based on research [20], blood glucose (BG) measurements after STZ-NA induction showed an increase in blood sugar levels because STZ releases Nitric Oxide, which contributes to the release of free radicals and increases cell damage activity, thereby disrupting insulin production by β cells in the pancreas of Langerhans. The results of phytochemical tests conducted by [1] on the active components of andaliman extract using ethanol solvents showed that this andaliman fruit extract contains almost all secondary metabolites, except steroids/triterpenoids. Secondary metabolites in andaliman extract that have a role as antidiabetics include alkaloids, flavonoids, and saponins. Alkaloids are effective against hyperglycemia by

increasing glucose consumption and glycogen synthesis. Flavonoids target various molecules involved in regulating several pathways, such as increasing β cell proliferation, stimulating insulin secretion, reducing apoptosis, and controlling hyperglycemia by regulating glucose metabolism in the liver. Saponins work by inhibiting the activity of the enzyme α-glucosidase in the intestine, which is responsible for converting carbohydrates into glucose [12], [2], [13].

Some drugs or natural substances are susceptible to degradation due to low pH, enzyme activity, or evaporation, which can affect their bioavailability as herbal medicines. Therefore, the use of nanoemulsions with the Self Nanoemulsifying Drug Delivery System (SNEDDS) can be an effective alternative to protect drugs from these adverse conditions [18]. In this study, administration of Andaliman Extract Nanoemulsion with doses of 25 mg/kgBW, 50 mg/kgBW, and 75 mg/kgBW was proven to be effective in lowering blood sugar levels. The data showed that the decrease in blood sugar levels had a P value ¡ 0.05, while for adiponectin, the P value > 0.05. This shows that Andaliman Extract Nanoemulsion has an effect on lowering blood sugar levels, but has no effect on adiponectin levels.

[8] argued that plasma adiponectin levels were negatively correlated with the development of insulin resistance and type 2 diabetes mellitus. This is in line with the results of this study, which showed an insignificant negative correlation ($P > 0.05$) between blood sugar levels and serum adiponectin levels.

6 Conclusion

In this study, nanoemulsion of andaliman fruit extract (Zanthoxylum Acanthopodium) at a dose of 50 mg/kgBW was effective in reducing blood sugar levels in Wistar rats induced by STZ-NA, but did not show effectiveness on adiponectin levels.

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The findings of this study should be viewed in light of several limitations. Among them are differences in body weight characteristics and baseline blood sugar levels between groups before the experiment which impacted the results. Also the use of chemical agents such as streptozotocin and nicotinamide to induce type 2 diabetes mellitus which is not as perfect as natural induction. However, due to time constraints, chemical agents ultimately became our best option in this study.

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