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The Effectiveness of Nanoemulsion Preparation of Star Anise (Illicium Verum) Extract on Blood Sugar Levels and Pancreas in Male Wistar Rats (Rattus Norvegicus) Induced by Alloxan

Jesslyn Gustin[1],Chrismis Novalinda Ginting[2],Linda Chiuman[3]
[1,2,3]Faculty of Medicine, Universitas Prima Indonesia, Medan, Indonesia

E-mail: Jslyn89@yahoo.com.hk

ABSTRACT
Prolonged hyperglycemia results in the onset of diabetes mellitus... The pancreas is an organ responsible for, among other things, maintaining blood glucose levels at a level that is tolerable by the body. This study aims to test and analyze the effectiveness of a nanoemulsion preparation of star anise (Illicium verum) extract on blood sugar levels and the pancreas in male rats induced by Alloxan. The sample used was 25 rats in 5 groups (positive and negative controls, treatments 1, 2, and 3) where the extract preparation in the treatment was a nanoemulsion of star anise (Illicium verum) extract with concentrations of 5%, 10%, and 20%. The results showed that pancreatic histology showed the highest level of damage in the negative control group, while treatment group 3 (20%) and the positive control showed significant improvement in the structure of the islets of Langerhans. The tissue damage score decreased in treatment group 3, indicating that the star anise nanoemulsion has a protective effect on pancreatic tissue and is able to reduce necrosis and cell degeneration.

Keywords: *Star anise, Nanoemulsion,Pancreas, KGD*

INTRODUCTION
Glucose is the primary fuel for cells. It serves as an energy provider and a precursor for several biosynthetic pathways. Glucose is essential for every cell, and each cell can utilize it according to its specific function. Insulin, secreted by the pancreas, plays a crucial role in glucose homeostasis. Insulin increases glycolysis, glycogenesis, lipogenesis, and protein synthesis, while decreasing gluconeogenesis. When β-cells fail to compensate for this stress, apoptotic cell death occurs, leading to defects in insulin production and secretion. This consequence gradually increases blood glucose levels and leads to hyperglycemia (Giri et al., 2018).
Chronic hyperglycemia mediates irreversible cell damage, known as glucose toxicity. Prolonged hyperglycemia results in diabetes mellitus. Insulin is a hormone essential for the body's utilization and storage of fuels, including saccharides, proteins, and fats. Pancreatic beta cells produce insulin. Individuals with hyperglycemia do not produce enough insulin (Prakash, 2023).
The pancreas functions as an exocrine gland by producing and secreting digestive enzymes, such as amylase, lipase, and trypsin, through acinar cells, which facilitate the breakdown of carbohydrates, fats, and proteins in the digestive tract. Simultaneously, the pancreas functions as an endocrine gland through islet cells of Langerhans, which are responsible for the synthesis and release of vital hormones, including insulin, glucagon, and somatostatin, which are integral to regulating blood glucose levels (Kaprinska & Czauderna, 2022).

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Maintaining normal pancreatic function is crucial. One way to do this is with medicinal plants like star anise. This condition can be addressed by improving lifestyle habits, including increasing antioxidant intake. This includes medicinal plants like star anise (Alias et al., 2024).

Star anise belongs to the Magnoliaceae family and is widely known for its dried fruit, which is a traditional edible and medicinal herb with a long history and extensive use in the pharmaceutical and chemical industries (Shi et al., 2021). Star anise is valued primarily for its warming properties in traditional Chinese medicine, and these properties are believed to improve digestion and alleviate cold and flu symptoms (Zou et al., 2023).

Star anise also has a high essential oil content, which is associated with various medicinal properties, including antimicrobial and antioxidant activity, as well as antiviral, anti-inflammatory, and antitumor properties. Several key components of the essential oil, such as trans-anethole, limonene, α-pinene, and estragole, significantly influence the quality of star anise (Shahrajabian et al., 2019).

LITERATURE REVIEW

Elevated blood glucose levels have been shown to be a potent activator in the development of cardiovascular disease, cell proliferation and cancer cell development, and mediate inflammation in osteoarthritis (Giri et al., 2018). Diabetes mellitus is a complex chronic condition caused by inherited and/or acquired deficiency of insulin production by the pancreas or by the ineffectiveness of the insulin produced. This disease is defined as a metabolic disorder caused by various factors and characterized by hyperglycemia (elevated blood glucose levels) with disturbances in carbohydrate, fat, and protein metabolism (ADA, 2018). Dysfunction in the islets of Langerhans may play a major role in insulin resistance and the onset of diabetes, where complex interactions between β-cells and other cellular components within the islets are crucial for maintaining homeostasis of the islet microenvironment (Dludla et al., 2023). Intercellular communication within the islets is crucial; its disruption can lead to significant β-cell dysfunction. For example, endothelial cells and macrophages within islets engage in dynamic communication with β-cells, regulating their integrity and function through a finely tuned signaling network (Eguchi & Nagai, 2017).

METHODS

The type of research used in this study is quantitative experimental, employing a true experiment or laboratory experimental design. Experimental research is conducted by controlling all external variables that could influence the experimental activities. This study used a post-test only control group design..

The sample of this study25 Wistar rats were used in each experimental group. The test animals were randomly divided into 5 groups.. The experimental group was divided into positive and negative control groups, treatments 1, 2 and 3. The test animals were acclimatized for 7 days in the laboratory.Department of Pharmacology and Therapeutics, Faculty of Medicine, University of North Sumatra. Research procedures include: acclimatization of test animals, preparation of star anise extract soaking star anise powder using 96% ethanol solvent with a concentration of 2000 ml for 3 days, phytochemical test of star anise extract to see the content of secondary metabolites in the extract, making star anise extract nanoemulsionwith 96% ethanol,Preparation of test animals, administration of treatment, treatment groups 1, 2, and 3 were given extract doses with concentrations of 5%, 10%, and 20%, for the positive control, nothing was given, and the negative control mice were induced by alloxan and given metformin. All test animals were given treatment for 21 days. Then, glucose observations and examination of pancreatic function and histopathology

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of the pancreatic organ were carried out. Then, the data from the research results were tabulated and analyzed with the help of SPSS (Statistics of Package for Social Science).

RESULTS

Phytochemical testing was conducted to identify the secondary metabolite compounds present in star anise extract, which are suspected to have potential as therapeutic agents. Based on the results of the phytochemical tests, it was concluded that star anise extract contains secondary metabolites in the form of flavonoids, saponins, tannins, alkaloids, and triterpenoids.

In this study, the test animals received preconditioning treatment in the form of alloxan induction to induce diabetes mellitus. Alloxan induction was performed on each test animal. The first stage was that the mice were fasted for 18 hours and then induced with alloxan at a dose of 150 mg/kgBW administered via intraperitoneal injection. After 3 days, blood sugar levels were measured. Blood sugar levels were measured by taking 1 mL of blood from the mice through the tail after first cleaning it with alcohol. Then, the blood was dropped onto a glucometer strip and then the strip was inserted into the device to read the results. The successful condition of the induction was indicated by blood sugar levels reaching more than or equal to 200 mg/dl. Blood sugar levels were measured on days 1, 14, and 21. The changes that occurred were observed and the results were as follows:

Table 1 Results of Blood Sugar Level Observations

No	Group	Blood Sugar Level (mg/dL) Mean ± SD		
		Day 1	Day 14	Day 21
1	Negative Control	257± 6.78	253± 6.67	229.6± 7.33
2	Positive Control	258±6.44	187.6± 6.10	101.4±5.85
3	Treatment 1	259.2± 6.37	231.4± 5.72	122.6± 5.22
4	Treatment 2	254.6± 6.94	226.2±6.22	115± 4.94
5	Treatment 3	258±4.47	192 ± 4.58	103.4 ± 6.80

On the 14th day, the average blood sugar levels in the negative control group, positive control, treatment group 1, treatment group 2, and treatment group 3 decreased but were not yet included in the normal blood sugar category. On the 21st day, the blood sugar levels of the mice were measured again to see the changes that occurred in all treatment groups. The negative control group got an average result of 229.6 ± 7.33 mg/dl. The positive control group got an average result of 101.4 ± 5.85 mg/dl. Treatment group 1 which was given a 5% concentration of star anise extract nanoemulsion got a result of 122.6 ± 5.22 mg/dl. Treatment group 2 which was given a 10% concentration of star anise extract nanoemulsion got an average result of 115 ± 4.94 mg/dl, treatment group 3 which was given a 20% concentration of star anise extract nanoemulsion had an average value of 103.4 ± 6.80 mg/dL. Then, observations were made on the levels of methylase and lipase, after which histopathological observations of the mouse pancreas and data analysis were carried out.

Reporting Research Results

Observations of changes in amylase levels were carried out after alloxan induction, namely on day 1, day 14, and day 21. The following are the results of observations on amylase levels of test animals during the treatment process:

Table 2 Results of Observation of Amylase Levels After Treatment

No	Group	LevelAmylase(U/L) Mean ± SD		
		Day 1	Day 14	Day 21
1	Negative Control	148.18 ±0.90	145.26± 0.70	139.16 ± 0.83

2	Positive Control	148.09 ± 0.89	126.46 ± 1.14	118.75 ± 0.68
3	Treatment 1	148.57 ± 0.98	136.16 ± 0.70	123.79 ± 0.73
4	Treatment 2	147.99 ± 0.91	133.34 ± 1.07	122.17 ± 0.66
5	Treatment 3	148.04 ± 0.96	128.69 ± 0.55	119.33 ± 0.82

Treatment was given every day at 09.00 am. Observations on amylase levels on the first day showed that the negative control group had an average value of 148.18 ± 0.90 U/L. The positive control group showed an average value of 148.09 ± 0.89U/L. Treatment group 1 had an average of 148.57 ± 0.98 U/L, treatment group 2 was 147.99 ± 0.91 U/L, and treatment group 3 was 148.04 ± 0.96 U/L. These measurement results indicate that alloxan-induced test animals experienced increased amylase levels and were subsequently given treatment according to their respective groups.

On the 14th day, the average amylase levels in the negative and positive control groups, treatment group 1, treatment group 2, and treatment group 3 decreased. On the 21st day, the amylase levels of the mice were measured again to see the changes that occurred in all treatment groups. The negative control group obtained an average result of 139.16 ± 0.83 U/L. The positive control group obtained an average result of 118.75 ± 0.68 U/L. Treatment group 1, which was given a 5% concentration of star anise extract nanoemulsion, obtained a result of 123.79 ± 0.73 U/L. Treatment group 2, which was given a 10% concentration of star anise extract nanoemulsion, obtained an average result of 122.17 ± 0.66 U/L, treatment group 3, which was given a 20% concentration of star anise extract nanoemulsion, had an average value of 119.33 ± 0.82 U/L.

Observations of changes in lipase levels were carried out after alloxan induction, namely on day 1, day 14, and day 21. The following are the results of observations on the lipase levels of test animals during the treatment process:

Table 3 Results of Observation of Lipase Levels After Treatment

No	**LevelLipase(U/L) Mean ± SD**			
	Group	**Day 1**	**Day 14**	**Day 21**
1	Negative Control	153.97 ± 0.82	148.76± 1.00	144.78± 0.79
2	Positive Control	153.97± 0.87	140.35 ±0.63	114.7±0.68
3	Treatment 1	153.43 ± 0.97	145.22± 0.73	137.76±0.74
4	Treatment 2	153.22± 0.95	143.15±0.76	122.48±0.96
5	Treatment 3	153.88±0.93	141.89 ± 0.36	115.20±0.83

Treatment was carried out every day at 09.00 am. Observations on lipase levels on the first day showed that the negative control group had an average of 153.97 ± 0.82 U/L. The positive control group showed an average lipase level of 153.97 ± 0.87 U/L. Treatment group 1 had an average of 153.43 ± 0.97 U/L, treatment group 2 was 153.22 ± 0.95 U/L, and treatment group 3 was 153.88 ± 0.93 U/L. The results of these measurements indicate that the test animals induced by alloxan experienced an increase in lipase levels and were then given treatment according to their respective groups.

On the 14th day, the average lipase levels in the negative control group, positive control, treatment group 1, treatment group 2, and treatment group 3 decreased. On the 21st day, the lipase levels of the mice were measured again to see the changes that occurred in all treatment groups. The negative control group got an average result of 144.78 ± 0.79 U/L. The positive control group got an average result of 114.7 ± 0.68 U/L. Treatment group 1 which was given a 5% concentration of star anise extract nanoemulsion got a result of 137.76 ± 0.74 U/L. Treatment group 2 which was given a 10% concentration of star anise extract nanoemulsion got an average result of 122.48 ± 0.96 U/L, treatment group 3 which was given a 20% concentration of star anise extract nanoemulsion had an average value of 115.20 ± 0.83 U/L.

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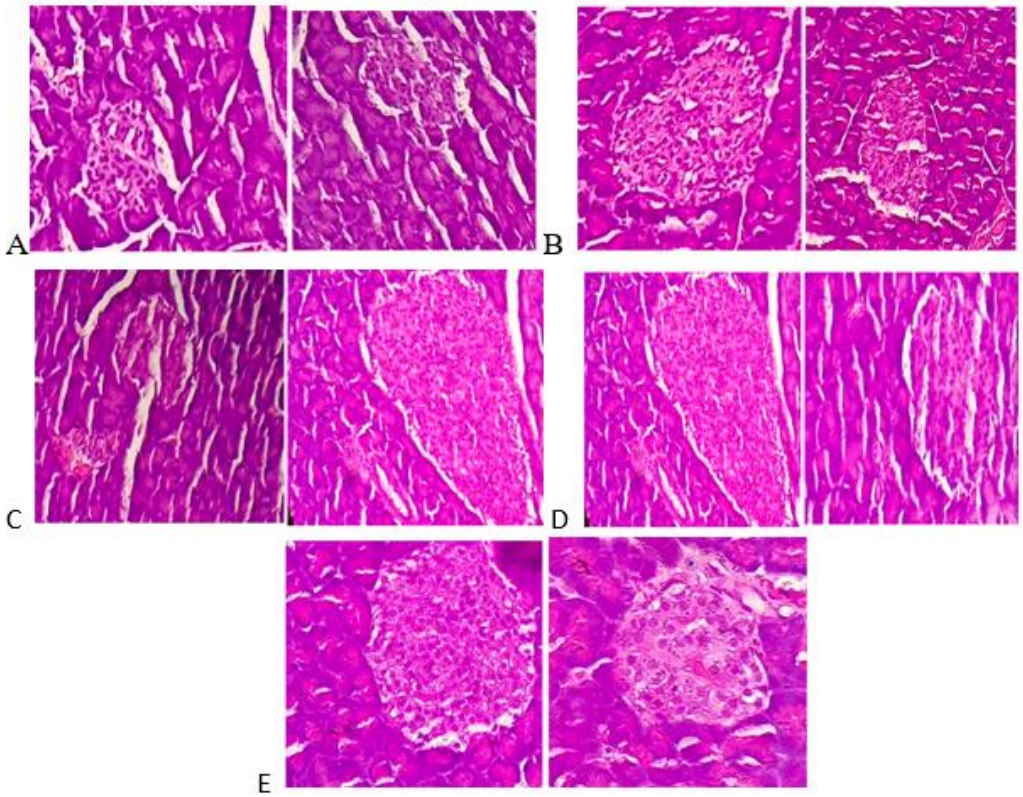
Histopathological examination of the pancreas was then performed. Histopathological examination of the pancreas was performed by assessing the structure of the islets of Langerhans in each treatment group. The results are as follows:



Description: A= negative control, B= positive control, C= treatment 1, D= treatment 2, E= treatment 3

Figure 1. Histopathology of Rat Pancreas

Based on the scoring results, the negative control group received a score of 0. This indicates that the histological structure of the pancreas remains normal, characterized by well-defined islets of Langerhans boundaries, good cell count and morphology, and the absence of necrotic cells. The negative control group served as the reference group for defining the other groups.

The positive control group exhibited mild impairment. The islets of Langerhans remained fairly well-defined, but showed a slight decrease in cell number and mild degeneration. No necrosis was observed, but these changes indicate initial diabetes-induced damage that has not yet significantly improved.

In treatment group 1 (given a 5% concentration of star anise extract nanoemulsion), the islets of Langerhans in this group showed blurred boundaries, with a significant decrease in cell number. Cell degeneration and some changes in cell shape were also observed, although necrosis was not widespread. This indicates that the 5% concentration is not optimal enough to structurally repair pancreatic tissue damage.

In treatment group 2 (10% concentration), the islets of Langerhans still showed less distinct boundaries, decreased cell count, and changes in cell shape. The presence of degeneration indicates that structural improvement has not reached a significant level.

Meanwhile, treatment group 3 (with a 20% concentration) showed more significant improvements. The islets of Langerhans were clearly defined, the cell count decreased slightly, and degeneration was minimal, with no necrosis observed. This score was comparable to the positive control, indicating that a high dose of star anise nanoemulsion has the potential to provide a protective effect against pancreatic tissue damage. Here are the scores:

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Table 4 Pancreatic Histopathology Score

Group	Mean Score ± SD	Interpretation
Negative Control	3.2 ± 0.44	Shows unclear boundaries, necrosis begins to appear, and many cells have abnormal shapes.
Positive Control	1.2 ± 0.44	Blurred boundaries, decreased cell count, cell degeneration, and some changes in cell shape
Treatment 1	2.6 ± 0.54	The boundaries are unclear, necrosis begins to appear, and many cells are abnormal in shape.
Treatment 2	2.2± 0.44	The boundaries are unclear, necrosis begins to appear, and many cells are abnormal in shape.
Treatment 3	1.6±0.89	Blurred boundaries, decreased cell count, cell degeneration, and some changes in cell shape

The results of the normality test using the Shapiro-Wilk method obtained significance values of 0.812 for the negative control group, 0.982 for the positive control, 0.919 for treatment group 1, 0.760 for treatment group 2, and 0.525 for treatment group 3. A significance value (p) exceeding 0.05 indicates that the data is normally distributed. Therefore, all groups in this study, both control and treatment, have normal data distribution.

The results of the homogeneity test using the Levene test can be seen in the table above. The significance value is 0.689. The significance value obtained is greater than 0.05, so it can be concluded that the negative control group, positive control, treatment 1, treatment 2, and treatment 3 come from populations that have the same variance, or are homogeneous. The results of the One-Way ANOVA test in the table above show that the resulting significance value is 0.000 or <0.05. Based on these data, it can be concluded that there is a significant difference between the control group and the treatment group.

DISCUSSION

This study aims to evaluate the effectiveness of star anise (Illicium verum) extract nanoemulsion preparation on blood glucose levels and histopathological features of the pancreas in male white rats (Rattus norvegicus) of the Wistar strain induced with alloxan. The study subjects consisted of male Wistar rats with a body weight ranging from 160 to 200 grams and aged 2 to 3 months. A total of 25 rats were used and randomly divided into five treatment groups to ensure a homogeneous distribution and avoid research bias. The five groups include: (1) a negative control group induced by alloxan and given distilled water; (2) a positive control group induced by alloxan and given metformin as a comparison therapy; (3) treatment group 1 induced by alloxan and given star anise extract nanoemulsion with a concentration of 5%; (4) treatment group 2 given a concentration of 10%; and (5) treatment group 3 given a concentration of 20%. This study was designed to observe the effect of various concentrations of nanoemulsion preparations on biochemical parameters

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and histological structure of the pancreas, in order to evaluate the therapeutic potential of star anise extract as an antihyperglycemic agent.

The research procedure began with alloxan induction in the test animals. The first stage was that the mice were fasted for 18 hours and then induced with alloxan at a dose of 150 mg/kgBW with a volume of 1 mL/300gBW administered intramuscularly in the abdomen of the mice. After 3 days, blood sugar levels were measured. The administration of alloxan aims to make the mice diabetic. Blood sugar levels were measured using a glucometer. Blood sugar levels were measured by taking 1 mL of the mice's blood through the tail after first cleaning it with alcohol. Then the blood was dropped onto a glucometer strip and then the strip was inserted into the device to read the results.

The results of blood glucose level measurements on the 21st day showed that all treatment groups, including the positive control group that received metformin, treatment group 1 that was given a 5% concentration of star anise extract nanoemulsion, treatment group 2 with a 10% concentration, and treatment group 3 with a 20% concentration, experienced a decrease in blood glucose levels to reach normal values, namely below 135 mg/dL. Meanwhile, the negative control group that was only given distilled water had blood sugar levels still greater than 200 mg/dL. The most significant decrease occurred in the positive control group and treatment group 3.

Further measurements were performed on amylase levels. The results of amylase measurements on day 21 showed that all treatment groups, including the positive control group given metformin, as well as the treatment groups receiving star anise extract nanoemulsion at concentrations of 5%, 10%, and 20%, experienced a decrease in amylase levels. The most pronounced decrease was recorded in the positive control group and group 3 with a concentration of 20%. The negative control group, which was only given distilled water, had the highest amylase levels among all treatment groups, especially the positive control group. This decrease in amylase levels indicates the recovery of pancreatic function.

Lipase levels were measured on day 21, indicating that all treatment groups, including the positive control group given metformin and the treatment groups receiving star anise extract nanoemulsion at concentrations of 5%, 10%, and 20%, experienced a decrease in lipase levels. The most significant decrease was observed in the positive control group and group 3 with a concentration of 20%. The group with a concentration of 5% showed the smallest decrease in lipase levels compared to the other groups, which was considered high.

Observations continued on pancreatic tissue. Based on histological observations of the pancreas, the negative control group showed the highest level of tissue damage (score 3.2 ± 0.44), characterized by indistinct islet boundaries, severe degeneration, and necrosis. The positive control group given metformin experienced less severe tissue damage (1.2 ± 0.44), indicating better preserved cell structure.

Treatment groups 1 (5%) and 2 (10%) experienced moderate tissue damage, with scores of 2.6 ± 0.54 and 2.2 ± 0.44, respectively, characterized by unclear cell boundaries, abnormal cell shapes, and the appearance of necrosis. Meanwhile, treatment group 3 (20%) showed better tissue repair (1.6 ± 0.89) compared to the previous two treatments, with mild degeneration and no severe necrosis. Thus, it can be concluded that administration of star anise extract nanoemulsion at a concentration of 20% has protective potential for pancreatic tissue, which is close to the effectiveness of metformin as an antidiabetic therapy.

Improving pancreatic function cannot be separated from the content contained in star anise extract nanoemulsion.The improvement in pancreatic function observed in the treatment group, particularly when given a nanoemulsion of star anise extract with a concentration of 20%, cannot be separated from the bioactive content of the extract.

CONCLUSION

1. Star anise extract (Illicium verum) contains various secondary metabolite compounds such as flavonoids, saponins, tannins, alkaloids, and triterpenoids.

2. Administration of star anise extract nanoemulsion at concentrations of 5%, 10%, and 20% significantly reduced blood glucose levels in alloxan-induced mice. The most significant reduction occurred in treatment group 3 (20%), whose effectiveness was equivalent to metformin ($p > 0.05$). This indicates that star anise has potential as an antihyperglycemic agent through protective mechanisms against pancreatic β cells and increased insulin sensitivity.
3. There was a significant decrease in amylase levels in treatment group 3 (20%) and the positive control group, compared to the negative control group and other treatments ($p < 0.05$). The decrease in amylase levels reflects an improvement in exocrine pancreatic function that was previously stressed by alloxan-induced hyperglycemia.
4. Pancreatic histology showed the highest level of damage in the negative control group, while treatment group 3 (20%) and the positive control showed significant improvement in the structure of the islets of Langerhans. Tissue damage scores decreased in treatment group 3, indicating that star anise nanoemulsion has a protective effect on pancreatic tissue and is able to reduce necrosis and cell degeneration.

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