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Effectiveness of Chia Seed (Salvia Hispanica) Extract Nano Emulsion Preparation on Alloxan-Induced Blood Sugar and Pancreas of Male Wistar Rats

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ABSTRACT
Type 2 Diabetes Mellitus is one of the most common metabolic disorders worldwide and its development is mainly caused by a combination of two main factors: imperfect insulin secretion by pancreatic β-cells. Serum amylase and lipase levels are used to diagnose pancreatic damage. This study was conducted to test and analyze the effectiveness of Chia seed (Salvia hispanica) extract nanoemulsion preparations on blood sugar and pancreas in male Wistar rats (Rattus norvegicus) induced by Alloxan. The sample of rats used was 25 in 5 groups (positive control, negative control, treatment 1, 2 and 3) where the extract preparation in the treatment was chia seed extract nanoemulsion with concentrations of 5%, 10% and 20%. With the results at a concentration of 20% also providing a decrease in amylase and lipase enzyme levels that were closer to the negative control group, indicating the potential for restoration of pancreatic function.

Keywords: *Chia Seed, Nanoemulsion,Pancreas, Diabetes*

INTRODUCTION
The pancreas is a combined exocrine and endocrine gland that plays a vital role in the human digestive system and nutrient metabolism. The exocrine pancreas secretes digestive enzymes to break down carbohydrates, proteins, and lipids. These enzymes act as biomarkers of pancreatic cell damage. Endocrine function is involved in appetite regulation, glucose homeostasis, and gastrointestinal secretion (Lim et al., 2023). Pancreatic endocrine hormones, particularly insulin, influence exocrine pancreatic function. Insulin has a detrimental effect on exocrine acinar cells. The exocrine acinar cells attached to them contain various enzymes, including amylase and lipase, which aid in the digestion of certain food particles. Amylase is the primary enzyme that breaks down starch into maltose, maltotriose, and dextrins during digestion. Lipase is a digestive enzyme from the pancreas that travels to the intestine, where it helps break down triglycerides into acids and monoglycerides (Ambad et al., 2020).
Serum amylase and lipase levels are used to diagnose pancreatic damage. If the patient experiences severe epigastric pain palpable during the physical examination and the serum amylase level is elevated to 3 times the upper limit of normal or higher, the diagnosis of acute pancreatitis can be made without further imaging. Serum lipase may also be collected, although sequencing the two tests has not been shown to improve diagnostic sensitivity and specificity. Computed tomography (CT) with contrast may also be performed for additional imaging support. Additional laboratory tests such as a complete blood count, basic metabolic panel, alanine aminotransferase (ALT), calcium, albumin, aspartate aminotransferase (AST), and direct and total bilirubin may also be obtained to guide the diagnosis. Magnetic resonance imaging (MRI) and magnetic resonance cholangiopancreatogram (MRCP) can also elucidate pancreatic pathology (Lam et al., 2020).

Nomor ISSN: 3124-1115

Improving metabolic control is the goal of hyperglycemia treatment, which aims to prevent or delay the development of micro- and macrovascular complications in both type 1 and type 2 diabetes. Hyperglycemia promotes the accumulation of free radicals through several metabolic pathways, including decreased antioxidant defenses (Ahangarpour et al., 2018). Therefore, adequate antioxidant intake is necessary, one way of doing this is by utilizing chia seeds.

Chia is considered a complete food because it contains carbohydrates, especially fiber, fatty acids, protein, vitamins, and minerals. Furthermore, chia can be considered a functional food, as it is a source of healthy bioactive compounds, especially antioxidants, with a variety of potential applications.

Based on this background, researchers are interested in testing and analyzing the effectiveness of chia seed extract nanoemulsion preparations on blood sugar and pancreatic function of alloxan-induced male Wistar rats (Rattus norvegicus).

LITERATURE REVIEW

Type 2 Diabetes Mellitus is one of the most common metabolic disorders worldwide and its development is mainly due to a combination of two main factors: imperfect insulin secretion by pancreatic β-cells and the inability of insulin-sensitive tissues to respond to insulin., damage to any of the mechanisms involved can cause a metabolic imbalance that leads to the pathogenesis of type 2 diabetes mellitus (Galacia-Garcia et al., 2020)

The chemical composition of chia seeds has been previously described, highlighting their nutritional value. Their high fiber content, higher than some dried fruits, cereals, and nuts, provides metabolic benefits (Hrnčič et al., 2019).

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 chia seed extract nanoemulsion.withsoaking in 90% ethanol, phytochemical test of chia seed extract to see the content of secondary metabolites in the extract, 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 positive control not given anything, and negative control mice were induced by alloxan and given metformin. All test animals were given treatment for 21 days. Glucose levels were then monitored, followed by pancreatic function and pancreatic histopathology. The data from the study were tabulated and analyzed using SPSS (Statistical Package for Social Science).

RESULTS

Phytochemical testing was conducted to identify the secondary metabolite compounds contained in chia seed (Salvia hispanica) extract nanoemulsions, which are suspected to have potential as natural therapeutic agents. Based on the results of the phytochemical testing, it can be concluded that chia seed (Salvia hispanica) extract contains secondary metabolites in the form of flavonoids, saponins, tannins, alkaloids, and triterpenoids.

Nomor ISSN: 3124-1115

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 involved fasting the mice for 18 hours and then inducing them with alloxan at a dose of 150 mg/kgBW administered via intraperitoneal injection. After three days, blood sugar levels were measured. Successful induction was indicated by blood sugar levels reaching greater than or equal to 200 mg/dl. Blood sugar levels were measured on days 1, 14, and 21.

On the 14th day, the blood sugar levels of the test animals were measured again with the average results of blood sugar levels in the positive control group, treatment group 1, treatment group 2, and treatment group 3 experiencing a decrease but not yet included in the normal blood sugar category. And 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 chia seed extract nanoemulsion at a concentration of 5%, treatment group 2 with a concentration of 10%, and treatment group 3 with a concentration of 20%, experienced a decrease in blood glucose levels to reach normal values, namely below 135 mg / dL. Next, observations were made on serum amylase and lipase to see the function of the pancreas along with its histopathological features.

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 1 Results of Observation of Amylase Levels After Treatment

No	Amylase levels (U/L) Mean ± SD			
	Group	**Day 1**	**Day 14**	**Day 21**
1	Negative Control	110.25±0.90	110.26± 0.91	109.41 ± 1.00
2	Positive Control	151.38 ± 1.12	141.22 ± 0.74	110.26 ± 0.98
3	Treatment 1	153.21 ± 1.25	141.23 ± 1.04	129.58 ± 1.04
4	Treatment 2	152.93 ± 1.60	138.96 ± 0.47	121.31 ± 0.63
5	Treatment 3	153.00 ± 1.27	132.58 ± 1.63	110.06 ± 0.69

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 109.41 ± 1.00 U/L. The positive control group obtained an average result of 110.26 ± 0.98 U/L. Treatment group 1, which was given a 5% concentration of chia seed extract nanoemulsion, obtained a result of 129.58 ± 1.04 U/L. Treatment group 2, which was given a 10% concentration of chia seed extract nanoemulsion, obtained an average result of 121.31 ± 0.63 U/L, treatment group 3, which was given a 20% concentration of chia seed extract nanoemulsion, had an average value of 110.06 ± 0.69 U/L. The results of amylase level measurements on day 21 showed that all treatment groups, including the positive control group given metformin, as well as the treatment groups receiving chia seed 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%, with average values approaching those of the negative control group.

Furthermore, 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:

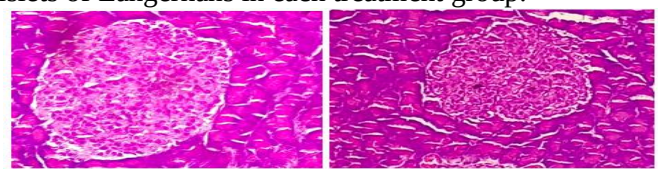
Table 2 Results of Observation of Lipase Levels After Treatment

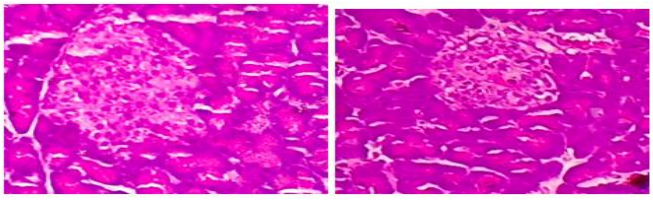
No	Lipase Level (U/L) Mean ± SD			
	Group	**Day 1**	**Day 14**	**Day 21**
1	Negative Control	122.6± 1.01	122.63± 0.99	122.67 ± 1.00
2	Positive Control	163.06 ± 1.00	144.27 ± 0.80	123.13 ± 0.68
3	Treatment 1	164.64 ± 2.44	153.98 ± 1.03	137.30 ± 0.72

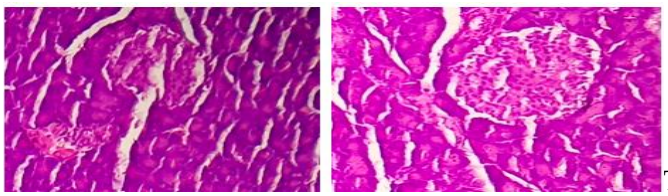
Nomor ISSN: 3124-1115

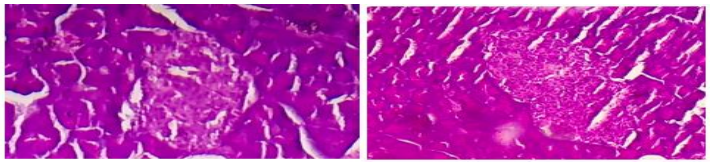
4	Treatment 2	163.42 ± 0.90	143.68 ± 0.95	130.72 ± 0.88
5	Treatment 3	163.83 ± 1.15	140.89 ± 0.57	122.60 ± 1.06

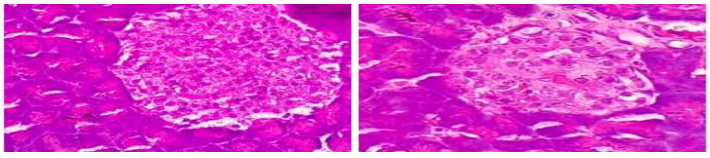
On the 21st day, the lipase levels of the mice were measured again to see the changes that occurred in all treatment groups. The negative control group obtained an average result of 122.67 ± 1.00 U/L. The positive control group obtained an average result of 123.13 ± 0.68 U/L. Treatment group 1 which was given a 5% concentration of chia seed extract nanoemulsion obtained a result of 137.30 ± 0.72 U/L. Treatment group 2 which was given a 10% concentration of chia seed extract nanoemulsion obtained an average result of 130.72 ± 0.88 U/L, treatment group 3 which was given a 20% concentration of chia seed extract nanoemulsion had an average value of 122.60 ± 1.06 U/L. Lipase levels were measured on day 21, showing that all treatment groups, including the positive control group given metformin and the treatment groups receiving chia seed 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%, with average values approaching those of the negative control group.
Then, histopathological observations of the mouse pancreas were conducted. Histopathological observations of the pancreas were conducted by assessing the structure of the islets of Langerhans in each treatment group.


Negative Control Group


Positive Control Group


Treatment Group 1


Treatment Group 2


Treatment Group 3

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 islet 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 still had fairly clear boundaries, but a slight decrease in cell number and mild degeneration were observed. No necrosis was found, but these changes indicate initial damage due to diabetes induction

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that has not been significantly repaired. In treatment group 1 (given chia seed extract nanoemulsion at a concentration of 5%), the islets of Langerhans in this group showed blurring of boundaries, with a significant decrease in cell number. Cell degeneration and some changes in cell shape were also found, although necrosis had not yet spread. This indicates that the 5% concentration is not optimal enough to repair structural pancreatic tissue damage. Treatment group 2 (10% concentration) showed that the islets of Langerhans still showed less defined boundaries, a decrease in cell number, and changes in cell shape. The presence of degeneration indicates that structural repair has not yet reached a significant level. Treatment group 3 (with a 20% concentration) showed more significant improvements. The islet boundaries were quite clear, the cell number decreased slightly, and degeneration was minimal, with no necrosis observed. These scores were equivalent to the positive control, indicating that a high dose of chia seed nanoemulsion has the potential to provide a protective effect against pancreatic tissue damage.

Then, in the analysis results, for the normality test using the Shapiro Wilk test. With the results of the normality test for amialse and lipase. The significance value (p) exceeding 0.05 indicates that the data is normally distributed. The results of the homogeneity test for amialse conducted using the Levene Test were 0.719 and lipase 0.804. The significance value obtained was greater than 0.05, so it can be concluded that the negative control group, positive control, treatment 1, treatment 2, and treatment 3 came from populations that had the same variance, or were homogeneous. The results of the One-Way ANOVA test on the observation of amialse and lipase showed that the resulting significance value was 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. In the LSD test, these results indicate that the positive control group and treatment 3 showed a response closer to normal conditions compared to the other treatment groups. The following is the scoring table:

Table 3 Pancreatic Histopathology Score

Group	Mean Score ± SD	Interpretation
Negative Control	0.2 ± 0.44	The island boundaries are still clear with a slight decrease in the number of cells and mild degeneration without necrosis.
Positive Control	1.6 ± 0.54	Blurred boundaries, decreased cell count, cell degeneration, and some changes in cell shape
Treatment 1	2.4 ± 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	1.4 ± 0.89	Blurred boundaries, decreased cell count, cell degeneration, and some changes in cell shape

DISCUSSION

This study aims to test and analyze the effectiveness of Chia seed (Salvia hispanica) extract nanoemulsion preparations on blood sugar and pancreas in male Wistar rats (Rattus norvegicus) induced by Alloxan. The sample of this study was Wistar rats (Rattus norvegicus) weighing 160-200 grams and aged 2-3 months.The total sample taken by researchers was 25 Wistar rats for 5 experimental groups.

Nomor ISSN: 3124-1115

Diabetes mellitus is one of the most common metabolic disorders worldwide, and its development is primarily due to a combination of two main factors: defective insulin secretion by pancreatic β-cells and the inability of insulin-sensitive tissues to respond to insulin. Insulin release and action must precisely meet metabolic needs; therefore, the molecular mechanisms involved in insulin synthesis and release, as well as insulin response in tissues, must be tightly regulated. Therefore, impairment in any of these mechanisms can lead to metabolic imbalances that lead to the pathogenesis of type 2 diabetes mellitus (Galacia-Garcia et al., 2020).

First, a phytochemical test was conducted on the chia seed extract nanoemulsion. Based on the results of the phytochemical test, it can be concluded that chia seed (Salvia hispanica) extract contains secondary metabolites in the form of flavonoids, saponins, tannins, alkaloids, and triterpenoids. These results are consistent with previous research conducted by Motyka et al. (2023) who found flavonoids, saponins, tannins, alkaloids, and triterpenoids in chia seed extract. The next step was the research procedure.

The research procedure began with alloxan induction. Each white rat was weighed to meet the criteria, and their blood sugar levels were checked using a glucose stick. Alloxan was then administered intraperitoneally at a dose of 150 mg/kg body weight. After 72 hours or 3 days, the blood sugar levels of the alloxan-induced rats were re-examined. The purpose of alloxan administration was to induce diabetes in the rats. Blood measurements were performed using a glucometer.

The results of blood sugar level measurements showed that all treatment groups, including the positive control group that received metformin, treatment group 1 that was given chia seed extract nanoemulsion at a concentration of 5%, treatment group 2 with a concentration of 10%, and treatment group 3 with a concentration of 20%, experienced a decrease in blood glucose levels to reach normal values, namely below 135 mg/dL. The most significant decrease occurred in the positive control group and treatment group 3, whose average value was close to the negative control group. Meanwhile, treatment group 1 showed the lowest decrease in blood glucose levels compared to the other groups, but remained within the normal range.

The results of amylase level measurements on day 21 showed that all treatment groups, including the positive control group given metformin, as well as the treatment groups receiving chia seed 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%, with an average value close to that of the negative control group. Conversely, the group with a concentration of 5% showed the smallest decrease in amylase levels compared to the other groups. This decrease in amylase levels indicates recovery of pancreatic function, considering that amylase is an enzyme produced by the pancreas and its increased levels are generally associated with inflammation or damage to the organ.

Lipase levels were measured on day 21, showing that all treatment groups, including the positive control group given metformin and the treatment groups receiving chia seed 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%, with an average value close to that of the negative control group. Conversely, the group with a concentration of 5% showed the smallest decrease in lipase levels compared to the other groups. This decrease in lipase levels indicates the recovery of pancreatic function.

Histopathological observations of pancreatic tissue showed that the negative control group was in normal condition without any cell damage, with an average damage score of 0.2 ± 0.44. In contrast, the positive control group given metformin showed mild changes in the form of blurred islet boundaries, decreased cell numbers, and mild cell degeneration, with an average score of 1.6 ± 0.54. In treatment group 1 (5% chia seed), more severe tissue damage was found with a score of 2.4 ± 0.54, characterized by unclear islet boundaries, the

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appearance of necrosis, and many cells with abnormal morphology. Treatment group 2 (10% chia seed) also showed similar damage, with a score of 2.2 ± 0.44, indicating that this concentration was not able to provide maximum protection to pancreatic tissue. Therefore, treatment group 3 which received the highest concentration (20% chia seed) showed better tissue condition compared to the previous two treatment groups.

CONCLUSION

1. Chia seed extract nanoemulsion demonstrated the ability to lower blood glucose levels in alloxan-induced diabetic rats. The 20% concentration produced the most significant reduction, approaching normal levels, comparable to the positive control (metformin).
2. The 20% concentration also resulted in a decrease in amylase and lipase enzyme levels closer to the negative control group, indicating the potential for restoration of pancreatic function.
3. Based on observations of pancreatic tissue, treatment group 3 (chia seed 20%) showed the mildest damage among the treatment groups, characterized by mild degeneration and no severe necrosis.

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