

Effectiveness Test of Chia Seed (*Salvia Hispanica*) Extract Nanoemulsion Preparation on Reducing Blood Glucose Levels (Kgd), Low Density Lipoprotein (LDL) Levels and Histopathological Features of the Liver of White Rats (*Rattus Norvegicus*) Wistar Strain Induced by Alloxan

Connie Calista Tham¹, Chrismis Novalinda Ginting², Linda Chiuman³
^{1,2,3}Faculty of Medicine, Universitas Prima Indonesia, Medan, Indonesia

E-mail: connie.calista@gmail.com

ABSTRACT

This lack of physical activity is often accompanied by excessive energy intake due to the wide variety of energy-rich foods, such as fast food. This study aims to test and analyze the effectiveness of chia seed (*Salvia hispanica*) extract nanoemulsion preparations on reducing blood cholesterol, low-density lipoprotein (LDL), and liver histopathology in male Wistar rats (*Rattus norvegicus*) induced by alloxan. Twenty-six rat samples were used in four groups (positive control, negative control, treatment 1, and 2), where the extract preparation in the treatment was chia seed extract nanoemulsion with concentrations of 10% and 20%. The results of histopathological observations showed that the negative control group experienced severe liver damage (score 3.16 ± 0.40), while the treatment group given chia seed extract nanoemulsion, especially the 20% concentration, showed improved liver structure with a lower damage score (1.5 ± 0.54). This indicates that chia seeds have the potential to protect liver tissue from damage.

Keywords: *Chia Seed, Nanoemulsion, Liver, LDL, Diabetes*

INTRODUCTION

This lack of physical activity is often accompanied by excessive energy intake due to the wide variety of energy-rich foods, such as fast food. This imbalance between activity and energy intake, if left unchecked, will lead to a lipid imbalance in the body. Lipids refer to a group of organic chemicals found in all animal and plant organisms. There are several types of lipids in the human body, the most important of which are fatty acids, cholesterol, triglycerides, and phospholipids. An important characteristic of lipids is their water insolubility. To dissolve them in the blood and transport them throughout the body's cells, all lipids bind to specific proteins, forming particles called lipoproteins. The four basic types of lipoprotein particles present in everyone's blood are chylomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) (Jovandaric et al., 2020).

The liver is an organ where various chemical reactions occur and plays a crucial role in maintaining lipid metabolism. These reactions include glycogenesis, glycogenolysis, gluconeogenesis, lipid synthesis, and fatty acid oxidation. Lipid metabolism in the liver includes cholesterol metabolism, fatty acid oxidation, lipoprotein metabolism, and triglyceride metabolism. In the liver, insulin not only regulates glucose production/utilization but also influences lipid metabolism more broadly. Elevated glucose

levels will trigger oxidative stress. Oxidative stress can be addressed by increasing antioxidant intake. Therapy focused on the use of functional foods rich in various phytochemicals, mono/polyunsaturated fatty acids, antioxidants, minerals, and fiber has demonstrated antioxidant, anti-inflammatory, and lipid-lowering effects (Akbari-Fakhrabadi et al., 2018), which may be beneficial in patients with NAFLD.

Chia seeds are one source of antioxidants. Chia seeds (*Salvia hispanica*) are the richest plant source of omega-3 fatty acids, antioxidants, and fiber. Chia seeds can be consumed as whole seeds or their oil can be extracted and used in various food formulations such as salad dressings, breads, yogurts, cakes, flours, emulsions, and cereals (Borneo et al., 2010). In addition to the use of chia seeds in food formulations, chia seeds have found widespread application in the feed industry, where chia seed oil and chia seed cake obtained after oil extraction have been used to lower cholesterol levels in meat and egg products while increasing polyunsaturated fatty acid levels. As a result of the increased use of chia seeds for food and feed, there has been a growing demand for their use as functional foods due to a shift in consumer patterns towards foods with nutritional value and health benefits. Furthermore, the utilization of foods with a greater potential role in reducing the risk of chronic degenerative diseases is on the rise (Ikumi et al., 2022).

LITERATURE REVIEW

The liver is composed of various types of cells that interact with each other to perform specific functions. When circulating glucose levels increase and insulin is secreted by pancreatic β -cells, insulin binding to the liver induces receptor autophosphorylation (Titchenell et al., 2017). In the bloodstream, lipids are found enclosed in lipoprotein molecules because they are insoluble in water. There are several types of lipids present in the human body, the most important of which are fatty acids, cholesterol, triglycerides, and phospholipids (Jovandaric et al., 2020).

There are five types of lipoproteins: chylomicrons, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), intermediate-density lipoprotein (IDL), and high-density lipoprotein (HDL). These lipoproteins can be detected clinically to estimate the amount of cholesterol in the blood (Huff et al., 2022).

Chia seeds are chemically composed of plant proteins, lipids, carbohydrates, and dietary fiber. Recent studies have shown that chia seeds have a strong nutritional profile and significant health-promoting properties (Grancieri et al., 2019). Chia seeds are thought to have beneficial effects on improving blood lipid profiles, through their ability to lower blood pressure and blood glucose, and their antimicrobial and immune-boosting effects. Chia seeds can be consumed whole, ground, and in powdered form. Furthermore, chia seeds have a slimy texture. This occurs when the seeds come into contact with an aqueous medium; they form a gelatinous layer of mucus around the seeds (Karel, 2017). Chia seeds have also been shown to have health benefits such as improved blood lipid profiles, hypotensive, hypoglycemic, antioxidant, antimicrobial, and immunostimulating effects (Agarwal et al., 2023).

METHODS

This study employed a quantitative experimental design, 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 employed a post-test only control group design to determine the effectiveness of chia seed extract on reducing blood glucose levels, LDL cholesterol, and liver histopathology in male Wistar rats (*Rattus norvegicus*) induced by alloxan.

The sample of this study 24 Wistar rats were used in each experimental group. The test animals were randomly divided into 4 groups. The experimental group was divided into positive and negative control groups, treatments 1 and 2. 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 with soaking 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 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 and LDL levels were then monitored, followed by histopathological examination of the liver. 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 present in chia seed extract, which are suspected to have potential as therapeutic agents. Based on the results of the phytochemical tests, it was concluded that chia seed extract contains secondary metabolites in the form of flavonoids, saponins, tannins, alkaloids, and triterpenoids.

Furthermore, in this study, the test animals received preconditioning treatment in the form of alloxan induction to induce diabetes mellitus. After being injected with alloxan, the mice were incubated for 3 days and blood glucose measurements were taken to determine whether the mice had diabetes mellitus. The mice were declared to have diabetes mellitus if their blood glucose was higher than 200 mg/dl. The successful induction condition 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 results on day 21 showed that the positive control group given metformin, treatment group 1 given chia seed extract nanoemulsion at a concentration of 10%, and treatment group 2 given chia seed extract nanoemulsion at a concentration of 20% experienced a decrease so that blood sugar levels returned to normal, namely below 135 mg/dl. Treatment group 2 experienced the most significant decrease and approached the positive control group compared to the other groups. The negative control group which was only given distilled water was the group with the least decrease compared to the other groups and blood sugar levels were still in a state of hyperglycemia. Here is the table:

Table 1 Results of Blood Sugar Level Observations

No	Group	Blood Sugar Level (mg/dL) Mean \pm SD		
		Day 1	Day 14	Day 21
1	Negative Control	245.16 \pm 9.30	222.16 \pm 11.12	196.33 \pm 7.58
2	Positive Control	240.83 \pm 7.02	185.5 \pm 9.31	101.33 \pm 6.91
3	Treatment 1	247.5 \pm 9.75	208.33 \pm 6.31	122.83 \pm 7.67
4	Treatment 2	245.33 \pm 9.2	197.5 \pm 10.01	107.16 \pm 12.15

Afterward, LDL, ALT, and AST levels, as well as liver histopathology, were observed in mice. Histopathological observations were performed using a light microscope at 400x magnification. The observations aimed to evaluate the morphological structure and possible pathological changes in liver tissue. The effects of alloxan and chia seed extract induction revealed histopathological changes such as fatty degeneration and necrosis.

Reporting Research Results

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

Table 2 Results of Observation of LDL Levels After Treatment

No	Group	LDL Level (mg/dl) Mean ± SD		
		Day 1	Day 14	Day 21
1	Negative Control	34.02 ± 1.12	31.81 ± 0.64	28.99 ± 0.57
2	Positive Control	33.59 ± 1.35	28.95 ± 0.78	9.27 ± 0.74
3	Treatment 1	33.77 ± 1.24	30.96 ± 1.13	14.48 ± 0.95
4	Treatment 2	33.79 ± 1.10	29.56 ± 0.71	9.42 ± 0.75

Treatment was administered daily at 10:00 a.m. Observations on LDL levels on the first day showed that the negative control group had an average value of 34.02 ± 1.12 mg/dl. The positive control group showed an average value of 33.59 ± 1.35 mg/dl. Treatment group 1 had an average value of 33.77 ± 1.24 mg/dl and treatment group 2, namely, 33.79 ± 1.10 mg/dl. Based on the results of LDL level measurements on the first day, it can be seen that the alloxan-induced group experienced an increase in LDL levels.

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

Table 3 Results of ALT Level Observations After Treatment

No	Group	ALT Level (IU/L) Mean ± SD		
		Day 1	Day 14	Day 21
1	Negative Control	62.50 ± 0.97	57.98 ± 0.97	46.51 ± 0.94
2	Positive Control	62.0 ± 0.83	35.55 ± 0.79	24.59 ± 1.07
3	Treatment 1	62.43 ± 0.99	41.48 ± 0.88	27.93 ± 0.72
4	Treatment 2	62.55 ± 0.78	36.90 ± 0.56	24.66 ± 0.90

Treatment was given daily at 10:00 a.m. Observations on ALT levels on the first day showed that the negative control group had an average value of 62.50 ± 0.97 IU/L. The positive control group showed an average value of 62.0 ± 0.83 IU/L. Treatment group 1 had an average value of 62.43 ± 0.99 IU/L and treatment group 2, namely, 62.55 ± 0.78 IU/L. Based on the results of ALT level measurements on the first day, it can be seen that the alloxan-induced group experienced an increase in ALT levels.

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

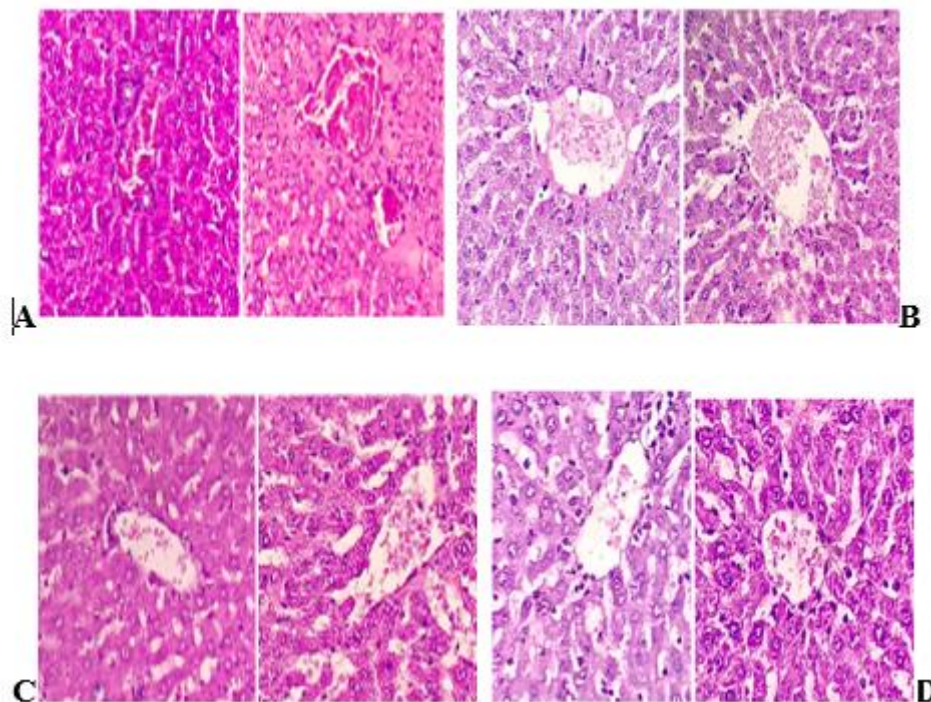
Table 4 Results of Observation of AST Levels After Alloxan Induction

No	Group	AST Level (IU/L) Mean ± SD		
		Day 1	Day 14	Day 21
1	Negative Control	103.25 ± 1.21	96.9 ± 0.96	81.00 ± 0.57
2	Positive Control	102.85 ± 1.22	82.17 ± 0.66	61.18 ± 0.77
3	Treatment 1	103.08 ± 1.56	84.89 ± 0.51	74.47 ± 0.74
4	Treatment 2	103.31 ± 1.52	85.61 ± 0.97	61.68 ± 0.68

Treatment was administered daily at 10:00 a.m. Observations on AST levels on the first day showed that the negative control group had an average value of 103.25 ± 1.21 IU/L. The positive control group showed an average value of 102.85 ± 1.22 IU/L. Treatment group 1 had an average value of 103.08 ± 1.56 IU/L and treatment group 2, namely, 103.31 ± 1.52 IU/L. On the first day, it was seen that the alloxan-induced group experienced an increase in AST levels.

Based on histopathological observations of the liver, each preparation was assessed and scored according to the degree of tissue damage. The assessment used the following categories: a score of 0 indicates normal liver tissue with no pathological changes; a score of 1 indicates degeneration or necrosis focused on one site; a score of 2 indicates degeneration

or necrosis occurring in multiple sites; and a score of 3 indicates generalized necrosis throughout the liver tissue. The following is an illustration:



Description: A= negative control group, B= Positive Control, C= Treatment 1, D= Treatment 2

Figure 1 Liver Histopathology

In the negative control group, the results showed an average score of 3, indicating severe liver tissue damage in the form of widespread necrosis throughout the liver. This finding indicates that the mice in the negative control group experienced severe oxidative stress and tissue damage, likely caused by alloxan exposure in the absence of protective intervention or antioxidant therapy.

Treatment group 1 was induced by alloxan and a 10% concentration of chia seed extract nanoemulsion. Rat liver tissue showed significant damage, characterized by hepatocyte necrosis in several areas. This necrosis was manifested by the loss of cell boundaries, shrinking nuclei (pyknosis), and nuclear lysis (karyolysis). The structure of the liver lobules appeared irregular, with disorganized hepatocyte arrangement around the central vein. Hepatic sinusoids were dilated and irregular, and there was mild to moderate infiltration of inflammatory cells around the necrotic areas. These findings indicate significant liver dysfunction due to cellular damage.

Treatment group 2 was induced by alloxan and a 20% concentration of chia seed extract nanoemulsion. Treatment group 2 showed signs of improved liver tissue structure, although degeneration was still observed in hepatocyte cells. Hepatocytes began to reorganize radially from the central vein, indicating restoration of lobular architecture. Little necrosis was found in this group. Sinusoids began to show normal morphology, and the central vein appeared intact without any signs of inflammation.

The results of liver histopathology observations in the negative control group, positive control, treatment 1, and treatment 2 were then scored. For each preparation, the average value of liver damage was calculated according to category. With the following score: 0 = Normal, no pathological changes; 1 = Degeneration or necrosis focused on one site; 2 = Degeneration or necrosis in several sites; 3 = Necrosis throughout the site.

Here is the scoring table:

Table 5 Liver Histopathology Score

Group	Mean Score \pm SD	Interpretation
Negative Control	3.16 \pm 0.40	Necrosis all over the place.
Positive Control	1.16 \pm 0.75	Degeneration or necrosis in one area
Treatment 1	2.16 \pm 0.75	Degeneration or necrosis in several places
Treatment 2	1.5 \pm 0.54	Degeneration or necrosis in one area

Then continued with data analysis, Based on the results of the normality test that has been carried out using the Shapiro Wilk test, the results of significance >0.05 were obtained in the LDL, ALT, and AST examinations so that all data from the negative, positive, treatment 1 and 2 control groups in the examination were normally distributed. In the homogeneity test, the significance value of the LDL examination was 0.570, ALT = 0.747, and AST = 0.357. The homogeneity test between groups was carried out using the Levene test with a significance level of 5%. it can be concluded that the negative control group, positive control, treatment 1, and treatment 2 came from a population that had the same variance, or was homogeneous. Thus, the data from all groups in this study met the assumption of homogeneity of variance and further analysis can be continued. The following are the results of the one-way ANOVA test at a significance value of 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 test and analyze the effectiveness of chia seed (*Salvia hispanica*) extract nanoemulsion preparation on reducing blood glucose (BGD), LDL and liver histopathology in male Wistar rats (*Rattus norvegicus*) induced by Alloxan. Administering alloxan at a dose of 120 mg/kgBW not only causes hyperglycemia through damage to pancreatic β cells, but can also have systemic effects, including causing damage to the liver. After alloxan induction, a sustained increase in blood glucose levels causes oxidative stress due to the accumulation of reactive oxygen species (ROS).

Chia seeds are one source of antioxidants. Chia seeds (*Salvia hispanica*) are the richest plant source of omega-3 fatty acids, antioxidants, and fiber. Researchers conducted phytochemical tests to determine the content of chia seed extract nanoemulsions. Based on the results, it was concluded that chia seed extract contains secondary metabolites in the form of flavonoids, saponins, tannins, alkaloids, and triterpenoids.

Observation of blood sugar levels shows a decrease. Based on the results of blood sugar level measurements on the 21st day, it can be concluded that the positive control group given metformin, treatment group 1 given chia seed extract nanoemulsion at a concentration of 10%, and treatment group 2 given chia seed extract nanoemulsion at a concentration of 20% experienced a decrease so that blood sugar levels returned to normal, namely below 135 mg / dl. Treatment group 2 experienced the most significant decrease and approached the positive control group compared to the other groups. The negative control group which was only given distilled water was the group with the least decrease compared to the other groups and blood sugar levels were still in a state of hyperglycemia.

Based on the results of LDL level measurements on the 21st day, it can be concluded that the positive control group given metformin, treatment group 1 given chia seed extract nanoemulsion at a concentration of 10%, and treatment group 2 given chia seed extract nanoemulsion at a concentration of 20% experienced a decrease. Treatment group 2 experienced the most significant decrease and was closer to the negative control group

compared to the other groups. The negative control group experienced the least decrease compared to the other groups. LDL levels were still in the high category.

Based on the results of ALT level measurements on the 21st day, it can be concluded that the positive control group given metformin, treatment group 1 given 10% chia seed extract nanoemulsion, and treatment group 2 given 20% chia seed extract nanoemulsion experienced a decrease. Treatment group 2 experienced the most significant decrease and was closer to the positive control group compared to the other groups. The negative control group experienced the lowest decrease and was still in the high ALT level category.

Based on the results of AST level measurements on the 21st day, it can be concluded that the positive control group given metformin, treatment group 1 given 10% chia seed extract nanoemulsion, and treatment group 2 given 20% chia seed extract nanoemulsion experienced a decrease. Treatment group 2 experienced the most significant decrease and was closer to the negative control group compared to the other groups. The negative control group was the group with the smallest decrease compared to the other groups and AST levels were still high.

The results showed that administration of chia seed extract nanoemulsion has protective potential against alloxan-induced liver tissue damage. The group given a higher concentration (20%) showed more significant histological liver improvements compared to the 10% concentration, and approached the effectiveness of metformin, a standard drug. This indicates that chia seeds have potential as a natural hepatoprotective agent in reducing the impact of oxidative stress and liver cell degeneration in type 2 diabetes.

The improvement in liver function and histological structure observed in the treatment group given chia seed extract nanoemulsion is strongly suspected to be related to its bioactive compounds, such as flavonoids, saponins, tannins, and triterpenoids. Flavonoids are known to have high antioxidant activity, which can ward off oxidative stress and prevent damage to hepatocyte cell membranes. Based on various scientific studies, chia seeds have significant therapeutic potential in improving liver function, normalizing lipid profiles, and controlling blood sugar levels, making them very useful in preventing and managing metabolic conditions such as dyslipidemia and type 2 diabetes.

CONCLUSION

1. *Chia seeds* contains various phytochemical compounds such as flavonoids, tannins, saponins, alkaloids, and triterpenoids, which have antioxidant and anti-inflammatory activities, as well as the ability to stabilize cell membranes and inhibit lipid peroxidation.
2. Administration of chia seed extract nanoemulsion significantly reduced blood glucose levels in diabetic model mice, showing an effective hypoglycemic effect comparable to the positive control (metformin).
3. There was a decrease in LDL (Low Density Lipoprotein) levels in the group given chia seed extract, indicating the hypolipidemic effect of the active compounds in chia seeds, especially soluble fiber and omega-3 fatty acids.
4. Decreased levels of the liver enzymes ALT (Alanine Aminotransferase) and AST (Aspartate Aminotransferase) indicate improved liver function. This is supported by liver histopathology results, which showed less damage in treatment group 2 (20% chia seeds).
5. Histopathological observations showed that the negative control group experienced severe liver damage (score 3.16 ± 0.40), while the treatment group given chia seed extract nanoemulsion, especially at a concentration of 20%, showed improved liver structure with a lower damage score (1.5 ± 0.54). This indicates that chia seeds have the potential to protect liver tissue from damage.

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REFERENCES

- Agarwal, A., Rizwana, Tripathi, A.D., Kumar, T., Sharma, K.P., & Patel, S.K. (2023). Nutritional and Functional New Perspectives and Potential Health Benefits of Quinoa and Chia Seeds. *Antioxidants*, 12(7), 1413.
- Feingold, K. R. (2022). Lipid and lipoprotein metabolism. *Endocrinology and Metabolism Clinics*, 51(3), 437-458.
- Guido, M., Sarcognato, S., Sacchi, D., & Ludwig, K. (2019). The anatomy and histology of the liver and biliary tract. *Pediatric Hepatology and Liver Transplantation*, 41-55.
- Jovandacic, M., & J. Milenkovic, S. (2020). Significance of Lipids and Lipoproteins in Organisms. *IntechOpen*. doi: 10.5772/intechopen.91407.
- Titchenell, P. M., Lazar, M. A., & Birnbaum, M. J. (2017). Unraveling the regulation of hepatic metabolism by insulin. *Trends in Endocrinology & Metabolism*, 28(7), 497-505
- Krause, M., & De Vito, G. (2023). Type 1 and Type 2 Diabetes Mellitus: Commonalities, Differences and the Importance of Exercise and Nutrition. *Nutrients*, 15(19), 4279. <https://doi.org/10.3390/nu15194279>
- Muriel, P. (2009). Role of free radicals in liver diseases. *Hepatology international*, 3(4), 526-536.