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Activity Test of Chia Seed (*Salvia Hispanica*) Extract Nanoemulsion on Reducing Urea and Creatinine Levels in Male Rats Induced by Streptozotocin and Kidney Histopathology

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ABSTRACT

This study aims to evaluate the activity of chia seed extract nanoemulsion in reducing urea and creatinine levels in streptozotocin-induced male rats, as well as to observe the histopathological features of the kidneys. Urea and creatinine levels were measured before and after treatment. The results showed Based on the results of blood glucose level measurements, administration of *chia seed extract nanoemulsion* with a concentration of 20% showed a more significant decrease in blood glucose levels. The results of the statistical test obtained a significance value of 0.074, so it can be concluded that treatment group 2 was not significantly different from the negative control group, indicating the potential antidiabetic effect of *chia seed extract nanoemulsion* . Observations on kidney function showed an improvement in urea levels. *Chia seed extract nanoemulsion* with a concentration of 20% was more effective in improving kidney function than a concentration of 10%. The urea value of treatment group 2 approached the negative control group, indicating the potential for nephroprotection. The results of observations on creatinine levels showed that treatment group 2 with the administration of 20% *Chia seed extract nanoemulsion* obtained a significance value of 0.382, the meaning of which was not different from the negative control group. Administration of a 20% concentration of *chia seed* extract nanoemulsion was more effective in protecting the structure of the kidney glomeruli than a 10% concentration. This nephroprotective effect is thought to be due to the flavonoids, phenolic acids, and antioxidants that suppress inflammation and oxidative stress.

Keywords: *Chia seed, Ureum, Creatinine, Strptozotocin.*

INTRODUCTION

Diabetes is one of the most common diseases worldwide. Currently, approximately 422 million people aged 18 and over worldwide suffer from diabetes, representing 8.5% of the global population. Type 2 diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia due to impaired insulin sensitivity and insufficient insulin production by the pancreas. Hyperglycemia, dyslipidemia, and low-grade inflammation are considered important factors in the development of type 2 diabetes mellitus and are common in obese individuals (Krause & De Vito, 2023).

Individuals with type 2 diabetes mellitus either do not produce enough insulin (insulin deficiency) or have body cells that are unable to use insulin properly (insulin resistance)

(ADA, 2014). Insulin, a hormone produced by β-cells in the pancreas, controls blood sugar levels. Insulin resistance is associated with genetic factors, obesity, a sedentary lifestyle, and aging. Consumption of energy-dense foods and lack of physical activity are important predictors of obesity and type 2 diabetes mellitus (Dendup et al., 2018).

Initially, insulin is produced in increased amounts to maintain normal glucose levels. However, this response is inadequate to overcome insulin insensitivity, particularly in obese individuals, which leads to increased glucose production by the liver. This leads to a state of "prediabetes," in which glucose levels are high but below the range of type 2 diabetes mellitus. Carbohydrate, fat, and protein metabolism are impaired as the disease progresses. Hyperglycemia (high blood sugar levels) occurs when β-cells fail to compensate for insulin resistance by producing excess insulin. Progressive decline in β-cell function and mass over time, coupled with hyperglycemia, marks the development of type 2 diabetes mellitus (Khan et al., 2014).

Diabetes can significantly impact kidney health, potentially leading to chronic kidney disease. High blood sugar levels in diabetes can damage the small blood vessels and filters in the kidneys, making them less effective at removing waste. Over time, this can lead to kidney failure, requiring dialysis or a kidney transplant (Kumar et al., 2023). Chronic kidney failure is associated with increased oxidative stress.

Oxidative stress has been implicated in various pathological systems common to chronic kidney disease and cardiovascular disease, most importantly inflammation and fibrosis. Chronic inflammation is triggered by oxidative stress and chronic degenerative diseases. Inflammatory cells are sources of free radicals in the form of reactive oxygen species (O2) and nitrogen, while *reactive oxygen species* (ROS) are considered the most responsible factors in kidney damage. ROS can be harmful in various functional pathways and structures within cells. Oxidative stress increases free radicals, which are common products of normal aerobic cellular metabolism (Beigrezaei & Nasri, 2016).

The human body has an innate defense mechanism against oxidative stress, namely antioxidants. Antioxidants inhibit some damaging oxidation reactions by oxidizing themselves. This defense system operates through a cascade of blocking the initial production of free radicals and scavenging oxidants, where oxidants are converted to less toxic compounds and the secondary production of toxic metabolites is blocked. Furthermore, the defense system aims to repair molecular injuries or enhance the endogenous antioxidant defense system, which consists of enzymatic and non-enzymatic antioxidants (Daenen et al., 2018). One plant-based source of antioxidants is *chia seeds.*

Chia seeds (Salvia hispanica) are a rich source of fiber, an essential nutrient for the kidneys. Consuming adequate fiber helps lower blood urea levels. Chia seeds are an excellent source of polyphenols and antioxidants, such as caffeic acid, rosmarinic acid, myricetin, quercetin, and others (Knez Hrnčič et al., 2019). This research is developing a chia seed extract preparation in the form of a nanoemulsion, which is expected to have better solubility and absorption properties.

Objective in study is to know the characteristics of the *Chia seed* extract nanoemulsion preparation *(Salvia hispanica)* through phytochemical tests , to determine kidney function by observing urea and creatinine levels in male white rats (*Rattus norvegicus*) Wistar strain induced by streptozotocin after administration of nanoemulsion preparations of Chia seed extract *(Salvia hispanica)* at concentrations of 10% and 20%. And to see the histopathological picture of kidney tissue in white mice given a nanoemulsion preparation of *Chia seed extract (Salvia hispanica)* with concentrations of 10% and 20% .

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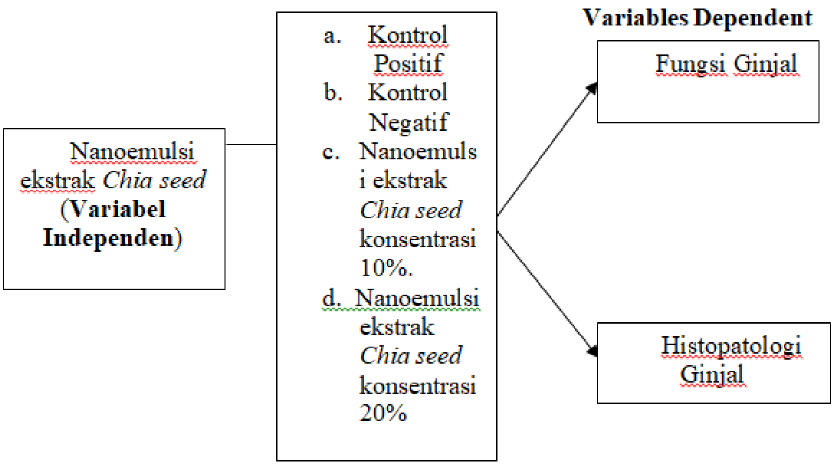

Figure 1. Conceptual Framework

METHODS

The type of research used in this study was 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* . This research was conducted in the Department of Pharmaceutical Pharmacology Laboratory, Faculty of Medicine, University of North Sumatra, and the Anatomical Pathology Laboratory, University of North Sumatra, from June to August 2025 .

Variables refer to characteristics or attributes that can be measured or observed and vary among the people or organizations being studied.

A. Variables Free :	Giving nanoemulsi extract *Chia seeds*
B. Variables Bound :	Kidney Function and Kidney Histopathology
C. Precondition Variables:	Streptozotocin Induction

The tools used in this research are needles, rotary vacuum evaporator, measuring flask, autoclave, tip, Bunsen burner, matches, test tube rack, test tube, beaker, measuring cylinder, caliper, hot plate, vortex, petri dish, alcohol spray bottle, dropper, cutting board, knife, grinder, measuring cylinder, Erlenmeyer flask, scales, magnetic stirrer, funnel, filter paper, *rotary evaporator, water bath*, vial, EDTA, Reflovet Plus.

A total of 100 grams of streptozotocin was dissolved using 1.5 ml of citrate buffer pH 4.5 and vortexed until homogeneous. The streptozotocin solution was stored at 4oC and used for injection into mice according to the dose adjusted to body weight. Mice. The streptozotocin to be injected was taken from the streptozotocin stock. The dose used was 20 mg/kg body weight five times in a row for 5 days. The mice were positioned supine so that their abdomens were visible. The abdomens of the mice were sprayed with 70% alcohol to prevent infection, then pinched until the muscles were felt, then streptozotocin was injected into the abdomens. After being injected with streptozotocin, the mice were incubated for 1 week and blood glucose measurements were taken to determine whether the mice had diabetes mellitus. Mice were declared diabetic if their blood glucose was higher than 200 mg/dl.

Blood from mice was drawn before streptozotocin induction to determine baseline blood glucose levels before hyperglycemia. Blood glucose levels were measured using a glucometer. Blood glucose is an important parameter in the study of metabolism and diabetes. Blood glucose levels measured by laboratory biochemical tests are used for the diagnosis of diabetes and for therapeutic monitoring of diabetic patients. A glucometer is used to monitor blood glucose levels. Glucometers are commonly used to measure blood glucose levels in animal models, especially rodents, because measurements can be easily performed with just a few µL of blood (Togashi et al., 2016).

Data from histopathological observations through microscopic examination were collected and then scored. The research data were tabulated, then the changes found were analyzed and presented descriptively. The observation data were analyzed using SPSS *(Statistics of Package for Social Science)* 25.0 *for Windows* . The normality test for the data was analyzed using *the Kolmogorov-Smirnov test approach* ($p > 0.05$). To test the significance between the experimental groups, a one-way analysis of variance technique or *One Way ANOVA was used* at a 95% confidence level ($p < 0.05$). Further analysis or testing was carried out using *the Post Hoc Test* with the LSD technique.

RESULTS

Phytochemical Test Results

Phytochemical testing was carried out to identify the content of secondary metabolite compounds contained in *chia seed extract nanoemulsion. (Salvia hispanica)* is suspected to have potential as a natural therapeutic agent. The following screening results were obtained:

Table 1. Test Phytochemicals

Secondary Metabolites	Color	Results	Note
Flavonoid	Pink	+	It is indicated that there is
Saponin	Yellow and foamy	+	It is indicated that there is
Tannin	turquoise	+	It is indicated that there is
Alkaloid	Yellowish white, brown, and orange deposits	+	It is indicated that there is
Steroids/Triterpenoids	Purplish red	+	It is indicated that there is

Information: (+) = Contain group compound Which tested
(-) = Does not contain the tested compound

Blood Sugar Level Observation Results

In this study, the test animals received preconditioning treatment in the form of streptozoticin induction to induce diabetes mellitus. Streptozoticin induction was performed on each test animal. The first stage was that the mice were fasted for 18 hours and then induced with streptozoticin. The dose used was 20 mg/kgBW five times in a row for 5 days. The mice were positioned supine so that their abdomen was visible. The abdomen of the mice was sprayed with 70% alcohol to prevent infection, then pinched until the muscle was felt, then streptozoticin was injected into the abdomen. After being injected with streptozoticin, the mice were incubated for 1 week and blood glucose measurements were taken to determine whether the mice had diabetes mellitus. The mice were declared diabetic if their blood glucose was higher than 200 mg/dl. Blood sugar levels were measured by drawing 1 mL of blood from the rat's tail after first cleaning it with alcohol. The blood was then dripped onto a glucometer strip, which was then inserted into the device to read the results. Successful induction was indicated by a blood sugar level of 200 mg/dL or greater. Blood sugar levels were measured on days 1, 14, and 21. The changes observed were as follows:

Table 2. Results of Blood Sugar Level Observations

No	Group	Blood Sugar Level (mg/dL) Mean ± SD		
		Day 1	Day 14	Day 21
1	Negative Control	91 ± 7.34	92.5 ± 7.84	93.5 ± 7.91
2	Positive Control	279.16 ± 8.68	215.66 ± 9.50	110.5 ± 7.89
3	Treatment 1	281.33 ± 7.91	232.83 ± 5.74	121 ± 7.82
4	Treatment 2	282.16 ± 7.11	185.5 ± 9.31	103.16 ± 11.33

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 (Salvia hispanica) 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 negative control group compared to the other groups. Treatment group 1 given *chia seed extract nanoemulsion (Salvia hispanica)* with a concentration of 10% was the group with the least decrease compared to the other groups but was still in the normal category.

Urea Level Observation Results

Observations of changes in urea levels were carried out periodically, namely on day 1, day 14, and day 21.

Table 3. Results of Observation of Urea Levels After Treatment

No	Group	Ureum Levels (mg/dl) Mean ± SD		
		Day 1	Day 14	Day 21
1	Negative Control	16.22 ± 0.80	16.23 ± 0.81	16.2 ± 0.81
2	Positive Control	41.76 ± 1.03	32.60 ± 0.86	18.35 ± 0.64
3	Treatment 1	41.88 ± 0.82	37.73 ± 0.64	22.33 ± 0.66
4	Treatment 2	41.91 ± 0.90	34.79 ± 1.07	17.06 ± 0.95

Based on the results of measuring urea levels on the 21st day, it can be concluded that the positive control group was given metformin, treatment group 1 was given *chia seed extract nanoemulsion (Salvia hispanica*) concentration of 10%, and treatment group 2 which was given nanoemulsion of *Chia seed extract (Salvia hispanica*) at a concentration of 20% decreased. Treatment group 2 experienced the most significant decrease and approached the negative control group compared to the other groups. Treatment group 1 was given a nanoemulsion of *chia seed extract. (Salvia hispanica*) with a concentration of 10% was the group with the least decrease compared to the other groups.

Creatinine Level Observation Results

Observations of changes in creatinine levels were carried out after streptozotocin induction, namely on day 1, day 14, and day 21.

Table 4. Results of Creatinine Level Observations After Streptozotocin Induction

		Creatinine Levels (mg/dL) Mean ± SD		
No	**Group**	**Day 1**	**Day 14**	**Day 21**
1	Negative Control	0.29 ± 0.07	0.30 ± 0.07	0.31± 0.07
2	Positive Control	1.32 ± 0.05	0.98 ± 0.08	0.41 ± 0.07
3	Treatment 1	1.30 ± 0.05	1.02 ± 0.09	0.47 ± 0.07
4	Treatment 2	1.32 ± 0.06	0.77 ± 0.09	0.35 ± 0.09

Based on the results of measuring creatinine levels on the 21st day, it can be concluded that the positive control group was given metformin, treatment group 1 was given *chia seed extract nanoemulsion. (Salvia hispanica)* concentration of 10%, and treatment group 2 which was given nanoemulsion of *Chia seed extract (Salvia hispanica)* at a concentration of 20% decreased. Treatment group 2 experienced the most significant decrease and approached the negative control group compared to the other groups. Treatment group 1 was given a nanoemulsion of *chia seed extract. (Salvia hispanica)* with a concentration of 10% was the group with the least decrease compared to the other groups.

Histopathological Observation Results

The following is a histological image of the kidney tissue of each treatment group:

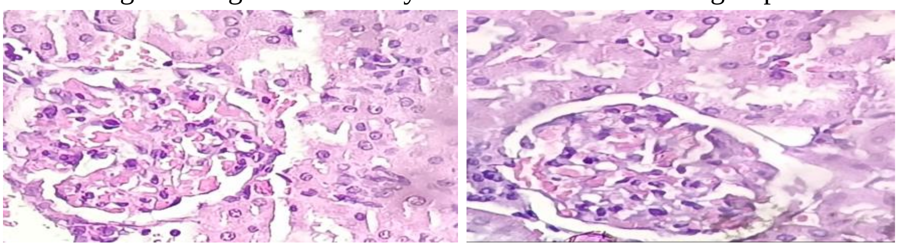

Figure 2. Histopathology of the Kidneys of the Negative Control Group

The positive control group was given streptozotocin and metformin induction. Glomerular basement membrane thickening and slight mesangial proliferation were observed in the glomerulus. Glomerular cell dilation resulted in adhesions between the glomerulus and Bowman's capsule. This condition is related to the effects of the previous streptozotocin induction. The histological condition of the kidneys in the positive control group differed slightly from the negative control group due to glomerular thickening.

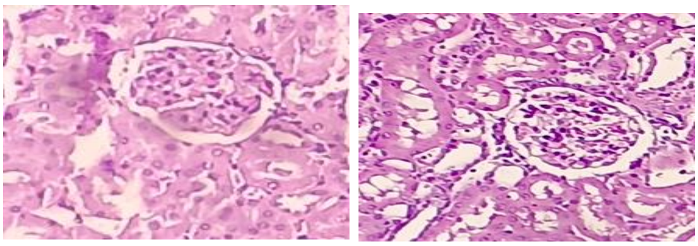

Figure 3. Histopathology of the Kidneys of the Positive Control Group

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Treatment group 1 was induced by streptozotocin and a 10% *chia seed extract nanoemulsion* . Glomeruli exhibited hypertrophy and hypercellularity, characterized by an increase in the number of mesangial and endothelial cells. Furthermore, there was thickening of the glomerular basement membrane. This widening of the glomerular cells resulted in adhesions between the glomerulus and Bowman's capsule. This condition is related to the effects of streptozotocin induction.

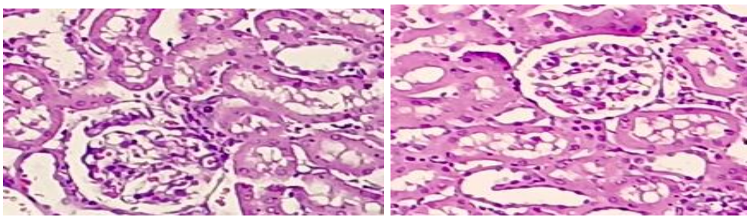

Figure 4. Kidney Histopathology of Treatment Group 1

Treatment group 2 was induced with streptozotocin and a 20% concentration of *chia seed extract nanoemulsion* . The glomeruli experienced slight thickening of the glomerular basement membrane. This dilation of the glomerular cells resulted in adhesions between the glomeruli and Bowman's capsule. This condition is related to the effects of streptozotocin induction during preconditioning. However, this dilation was smaller than that of treatment group 1 and the positive control group.

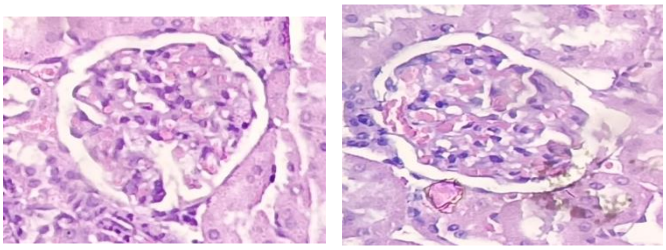

Figure 5. Kidney Histopathology of Treatment Group 2

The results of histopathological observations of the kidneys in the negative control group, positive control, treatment 1, and treatment 2 were then scored. The scoring system used was based on glomerular cell damage, namely: score 0 = no histopathological damage, score 1 = focal (mild) damage, score 2 = multifocal (moderate) damage, and score 3 = diffuse (severe) damage.

Table 5. Renal Histopathology Score

Group	Mean Score ± SD	Interpretation
Negative Control	0.16 ± 0.40	No damage
Positive Control	1.66 ± 0.51	Minor damage
Treatment 1	2.00 ± 0.63	Moderate damage
Treatment 2	1.33 ± 0.51	Minor damage (structural repairs)

These findings suggest that administration of a 20% *chia seed extract nanoemulsion* tends to be more protective against glomerular structural damage than a 10% concentration, and even has a protective effect comparable to that of metformin. This nephroprotective effect is likely related to the active compounds in chia seeds, such as flavonoids, phenolic acids, and other antioxidants, which can suppress inflammation and oxidative stress in kidney tissue.

DISCUSSION

This study was conducted to test and analyze the effectiveness of *Chia seed (Salvia hispanica) extract nanoemulsion preparation* on reducing urea, creatinine, and kidney histopathology levels in male Wistar rats (*Rattus norvegicus*) induced by streptozotocin. A total of 100 grams of streptozotocin was dissolved using 1.5 ml of citrate buffer pH 4.5 and vortexed until homogeneous. The streptozotocin solution was used for rat injection according to the dose adjusted to the rat's body weight. The streptozotocin to be injected was taken from the streptozotocin stock. The dose used was 20 mg/kgBW five times in a row for 5 days. After being injected with streptozotocin, the rats were incubated for 1 week and blood glucose measurements were taken to determine whether the rats had diabetes mellitus. Rats were declared to have diabetes mellitus if their blood glucose was higher than 200 mg/dl.

Type 2 diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia due to impaired insulin sensitivity and insufficient insulin production by the pancreas (Krause & De Vito, 2023). Diabetes can significantly impact kidney health, potentially leading to chronic kidney disease. High blood sugar levels in diabetes can damage the small blood vessels and filters in the kidneys, making them less effective at removing waste. Over time, this can lead to kidney failure, requiring dialysis or a kidney transplant (Kumar et al., 2023). This condition can be treated with *chia seed extract.*

Chia seeds are known as one of the richest plant sources of omega-3 (ω-3) fatty acids, particularly α-linolenic acid (ALA). Research over the past two decades, in both human and animal models, has shown that *chia seeds* can have a positive effect on insulin resistance, lipid profiles, glucose tolerance, and body fat levels (Fateh et al., 2024). To determine the content of *chia seed extract nanoemulsions* , researchers conducted phytochemical tests, including flavonoids, saponins, tannins, alkaloids, and steroids/triterpenoids.

Based on the results of the phytochemical tests carried out, it can be concluded that *Chia seed extract (Salvia hispanica)* contains secondary metabolites in the form of flavonoids, saponins, tannin, alkaloids, And triterpenoids. These results are consistent with previous research conducted by Velix et al. (2023). This study found that *chia seed (Salvia hispanica) extract* Contains flavonoids, saponins, tannins, and alkaloids. Nanoemulsion of *chia seed extract* (*Salvia hispanica)* also contains omega-3 fatty acids which are beneficial for the human body. The benefits include lowering triple glyceride and cholesterol levels, anti-inflammatory activity, cardioprotective and hepatoprotective activity, antidiabetic activity, and protection against cancer, arthritis, and autoimmune diseases (Grancieri et al., 2019; Ullah et al., 2016; de Falco et al., 2018). The content and benefits of *chia seed* (*Salvia hispanica*) extract nanoemulsion were then tested for their effectiveness in lowering blood sugar levels and improving kidney function in male Wistar rats (*Rattus norvegicus)* induced by streptozotocin.

On the first day, the positive control group's blood sugar levels were 279.16 ± 8.68 mg/dL. Treatment group 1 had an average blood sugar level of 281.33 ± 7.91 mg/dL, and treatment group 2 had an average blood sugar level of 282.16 ± 7.11 mg/dL. mg/dl. The blood sugar measurement results indicate that the test animals fall into the hyperglycemic category and are then given treatment according to their respective groups.

Treatment in the form of administering *chia seed extract nanoemulsion* for 21 days showed differences in response between treatment groups to induced hyperglycemia. The negative control group, which did not undergo induction and was only given distilled water, showed blood glucose levels within the normal range of 93.5 ± 7.91 mg/dL. Meanwhile, the positive control group that was induced with hyperglycemia and received metformin showed blood glucose levels of 110.5 ± 7.89 mg/dL, indicating a decrease in blood glucose in response to standard therapy.

Treatment group 1, which received chia seed extract nanoemulsion with a concentration of 10%, showed the highest blood glucose levels, namely 121 ± 7.82 mg/dl, indicating that this concentration was not able to reduce blood glucose levels effectively. In contrast, treatment group 2 with a concentration of 20% showed blood glucose levels of 103.16 ± 11.33 mg/dl, which was lower than the positive control group and close to normal levels.

One-Way Anova test of blood sugar levels showed that the significance value produced 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. The results of the LSD post hoc test analysis showed that the significance value of the negative control group with the positive control was 0.003. The negative control group with treatment group 1 was 0.000. The negative control group with treatment group 2 was 0.074. Based on these data, it can be concluded that the negative control group and treatment group 2 were not significantly different. While the negative control group with treatment groups 1 and 2 were significantly different (p <0.05).

These results indicate that administering a 20% *chia seed extract nanoemulsion* has a potentially better antidiabetic effect than a 10% concentration. This effect is likely related to the active compounds in *chia seeds,* such as flavonoids, fiber, omega-3 fatty acids, and antioxidants, which play a role in enhancing glucose metabolism and improving insulin sensitivity.

Further observations were made on kidney function and urea levels. Observations of urea levels on the first day showed that the negative control group, which was not streptozotocin-induced, had an average value of 16.22 ± 0.80. mg/dl. The positive control group showed an average value of 41.76 ± 1.03 mg/dL. Treatment group 1 had an average value of 41.88 ± 0.82 mg/dl and treatment group 2, namely, 41.91 ± 0.90 mg/dl. After 21 days of treatment, the rats' urea levels were re-measured to see the changes that occurred in all treatment groups. The negative control group had an average result of 16.2 ± 0.81 mg/dl. The positive control group had an average result of 18.35 ± 0.64 mg/dl. Treatment group 1, which was given 10% *Chia seed (Salvia hispanica*) extract nanoemulsion , had a result of 22.33 ± 0.66 mg/dL. Treatment group 2, which was given nanoemulsion of *Chia seed (Salvia hispanica*) extract with a concentration of 20%, obtained an average result of 17.06 ± 0.95 mg/dl.

One-Way Anova test on urea levels showed that the significance value produced 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. Further *Post-hoc* LSD tests were conducted to analyze the average differences between groups. The results of the analysis showed that there was a significant difference between the negative control group and the positive control group (p = 0.000) and treatment group 2 (p = 0.000). There was no significant difference between the negative control group and treatment group 1 (p = 0.083).

The highest decrease in urea levels occurred in treatment group 2 which was given a 20% concentration of *Chia seed extract nanoemulsion* , where the value was close to the urea levels of the negative control group that did not undergo induction. This indicates that the 20% concentration has a more optimal nephroprotective potential in reducing urea levels as an indicator of kidney function. In contrast, treatment group 1 which received a 10% concentration showed the lowest decrease in urea levels among the other treatment groups, indicating that its effectiveness is still limited at that concentration. Overall, these results indicate that the administration of *Chia seed extract nanoemulsion* , especially at higher concentrations, has the potential to provide protection against impaired kidney function characterized by a significant decrease in urea levels.

The third observation was creatinine levels. Observations on creatinine levels on the first day showed that the negative control group that was not streptozotocin-induced had an average value of 0.29 ± 0.07 mg/dL. The positive control group showed an average value of 1.32 ± 0.05 mg/dL. Treatment group 1 had an average value of 1.30 ± 0.05 mg/dL and treatment group 2, namely, 1.32 ± 0.06 mg/dL.

After administering *chia seed extract nanoemulsion* for 21 days, differences in creatinine levels were observed between groups, reflecting the effect of the treatment on kidney function. The negative control group, which was not induced, showed creatinine levels within normal limits, at 0.31 ± 0.07 mg/dL. The positive control group, which was induced and given metformin, experienced a decrease in creatinine levels to 0.41 ± 0.07 mg/dL.

In the treatment group given *Chia seed* (*Salvia hispanica*) extract nanoemulsion, there was a difference in effectiveness between concentrations. Treatment group 1 given *Chia seed* (*Salvia hispanica*) extract nanoemulsion at a concentration of 10% showed the highest creatinine levels, namely 0.47 ± 0.07 mg/dL. Treatment group 2 given a concentration of 20% showed a decrease in creatinine levels to 0.35 ± 0.09 mg/dL, which is close to the value of the negative control group. These results indicate that administration of *Chia seed extract nanoemulsion* at a concentration of 20% is more effective in reducing creatinine levels, and thus has nephroprotective potential for rats experiencing hyperglycemia.

Based on the results of histological observations of the renal glomeruli, it can be concluded that there are differences in the level of tissue damage between treatment groups. The negative control group showed normal glomerular structure without any pathological changes, while the positive control group given metformin showed mild damage in the form of thickening of the glomerular basement membrane and slight proliferation of mesangial cells (score 1). Treatment group 1 given a 10% concentration of Chia seed (Salvia hispanica) extract nanoemulsion experienced moderate damage (score 2), characterized by thickening of the basement membrane and an increase in the number of mesangial and endothelial cells. In contrast, treatment group 2 given a 20% concentration showed milder damage (score 1), with minimal morphological changes.

Overall, administration of chia seed extract nanoemulsion at a concentration of 20% showed a better nephroprotective effect than the 10% concentration, and its protective effect on the structure of the kidney glomerulus was almost equivalent to that of metformin. This effect is thought to be related to the bioactive content of chia seeds, such as flavonoids, phenolic acids, and other antioxidant compounds that play a role in suppressing oxidative stress and inflammatory processes in kidney tissue. Flavonoids have many positive health effects on metabolic disorders, such as cardiovascular disease, cancer, obesity, and diabetes. Flavonoids also function as antioxidants that modulate oxidative stress in the body by neutralizing the effects of nitrogen and oxygen species, thereby preventing disease (Kawser et al., 2016). The antidiabetic activity of flavonoids supports the regulation of carbohydrate digestion, insulin signaling, insulin secretion, glucose absorption, and adipose deposition (Vinayagam et al., 2015). They target many molecules involved in the regulation of several pathways, such as increasing β-cell proliferation, promoting insulin secretion, reducing apoptosis, and improving hyperglycemia by regulating glucose metabolism in the liver.

CONCLUSION

1. Based on the results of blood glucose level measurements, administration of *chia seed extract nanoemulsion* with a concentration of 20% showed a more significant decrease in blood glucose levels. The statistical test results obtained a significance value of 0.074, so it can be concluded that treatment group 2 was not significantly different from the negative control group, indicating the potential antidiabetic effect of *chia seed extract nanoemulsion* .
2. Observations on kidney function showed improvements in urea levels. *Chia seed extract nanoemulsion* at a 20% concentration was more effective in improving kidney function than the 10% concentration. The urea levels in treatment group 2 were close to those of the negative control group, indicating potential nephroprotection.
3. The results of observations on creatinine levels showed that treatment group 2 with the administration of 20% *Chia seed extract nanoemulsion* obtained a significance value of 0.382, the meaning of which was not different from the negative control group.
4. *chia seed* extract nanoemulsion was more effective in protecting the structure of the kidney glomeruli than a 10% concentration. This nephroprotective effect is thought to be due to the flavonoid, phenolic acid, and antioxidant content, which can suppress inflammation and oxidative stress.

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