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POTENTIAL OF ROSELLA FLOWER EXTRACT KEFIR (*HIBISCUS SABDARIFFA* L.) IN REDUCING PANCREATIC β-CELL DAMAGE IN DIABETES MELLITUS MODEL RATS (*RATTUS NORVEGICUS*)

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ABSTRACT
Kefir is a fermented milk product that contains probiotics, while rosella flowers (*Hibiscus sabdariffa* L.) are known to have antioxidant and antihyperglycemic activity. The combination of the two in the form of rosella flower extract kefir is expected to have a protective effect on the pancreas in diabetes. This study aims to evaluate the effect of administering rosella flower extract kefir preparations on the histopathological appearance of the pancreas of male white rats (*Rattus norvegicus*) induced by streptozotocin.This experimental study used a post-test control group design using 30 male white rats divided into several groups, namely normal controls who were given Na CMC 0.5% orally for 21 days, negative controls (induced by STZ without treatment) given Na CMC 0.5% orally for 21 days, positive controls (induced by STZ) and given glibenclamide suspension 0.45 mg/kg WB orally for 21 days, and treatment groups with different doses from kefir rosella flower extract with graded doses of 250 mg/kg BW, 500 mg/kg BW and 750 mg/kg BW. After treatment, rat pancreas was collected and analyzed histopathologically. The results of histopathological analysis showed that the group of mice that received rosella flower extract kefir at a dose of 750 mg/kg BW experienced structural improvements in pancreatic β cells with a damage value of 0.4. Administration of rosella flower extract kefir at a dose of 750 mg/kg BW has the potential to provide a protective effect on the pancreas in rats induced by streptozotocin.

Keywords: Kefir, Hibiscus sabdariffa, pancreatic histopathology, streptozotocin, diabetes mellitus

1. INTRODUCTION

Diabetes mellitus is a metabolic disease with its prevalence increasing from year to year. The 2023 Basic Health Research (RISKESDAS) stated that the prevalence of diabetes mellitus diagnosed by doctors was in Central Sulawesi, namely 1.7%. The prevalence of diabetes mellitus diagnosed by a doctor at the age of 15 years and over according to the district/city in the province of Central Sulawesi, the highest symptom was found in Poso, which was 3.62%. Meanwhile, the prevalence of diabetes mellitus diagnosed by doctors at all ages according to the district/city of Central Sulawesi province, the highest symptoms were found in North Morowali,

namely 2.13 (Kementrian Kesehatan Republik Indonesia, 2024). One of the plants that are efficacious as antidiabetic is rosella, rosella as a herbal medicine has various benefits. This is because of the content of various important substances it has. Rosella contains various nutritious compounds, such as antioxidants, essential acids, beta carotene, potassium, iron, and various types of vitamins (Guardiola & Mach, 2014). In addition to flower petals, leaves, fruit, and seeds also act as a diuretic, anti-cancer, and pain reliever. The highest content of compounds in red rosella is the presence of anthocyanin compounds that form flavonoids that act as antioxidants. Rosella flavonoids consist of flavones and anthocyanin pigments. Anthocyanins function as antioxidants which are believed to cure degenerative diseases (Bassong et al., 2022).

Various variations of milk-based fermented products are increasingly circulating in the market today, one of which is kefir. Kefir is a fermented product from milk inoculated with kefir grains (kefir seeds) containing various lactic acids, Lactobacilli, Lactococci, Lactobacillus kefir, Lactobacillus parakefir, Lactobacillus kefiranofaciens and Lactobacillus kefirgranum, yeast, and acetic acid bacteria (Guo & Batbatan, 2023). The benefits of kefir for health include being an antioxidant, anticancer, antidiabetic, antihyperglycemic, and anti-inflammatory (Gupta et al., 2024). The pancreas is an important glandular organ in the body consisting of exocrine and endocrine tissues. The function of the pancreas is to produce pancreatic juice which contains the enzymes trypsinogen, amylase, and lipase. These enzymes mix with food in the duodenum and carry out digestive functions in the intestine, the exocrine part consists of acinar cells that secrete enzymes through the duodenal tract. While the endocrine part consists of the islets of Langerhans whose function is to produce the hormone insulin which is then absorbed into the blood. If insulin levels are sufficient or its function is not impaired, the excess blood glucose will be stored or used for metabolism. Insulin plays an important role in the metabolism of carbohydrates, fats, and proteins as well as in the transport of various substances through cell membranes. Insulin is a hormone produced by pancreatic cells. The pancreatic cells that secrete insulin are the cells of the islets of Langerhans (Tandi et al., 2023).

The condition of hyperglycemia in chronic DM can trigger many complications, both macrovascular and microvascular, one of which is diabetic nephropathy. Diabetic nephropathy or diabetic kidney disease (PGD) is one of the complications that often occur in diabetics. In this disease, there is damage to the kidney filter, known as the glomerulus. Due to glomerular damage, several same many blood proteins are excreted in the urine abnormally (Joni et al., 2022). Under normal conditions, the glomerulus cannot be passed by large molecular proteins, but in pathological conditions, these proteins can pass. Tubular cells in addition to functioning reabsorb, also add chemicals such as iodine, ammonia, and hippuric acid. At the glomerulus, foreign materials reach the tubules at abnormal rates through Bowman's space. This causes tubular epithelial cells to undergo ischemia, degeneration, and even death if too much material must be reabsorbed (Slamet et al., 2023). Previous research stated that infusion of the effectiveness of roselle tea with active ingredients such as flavonoids, anthocyanins, and polyphenols in roselle tea contribute to antihypertensive effects through vasodilation mechanisms and increasing blood vessel elasticity (Anggi et al., 2023). This research concludes that rosella tea is effective as a safe additional therapy in treating hypertension in the elderly and lower sucrose. Previous studies using water extract of rosella petals at a dose of 250 mg/kg BW, 500 mg/kg BW and 750 mg/kg BW have antidiabetic activity comparable to glibenclamide 0.45 mg/kg BW. Based on the above background, it is necessary to research the effect of giving kefir preparations of rosella flower extract on decreasing blood glucose levels, regenerating pancreatic cells, and repairing kidney tubular injury by observing the histopathological picture in male white rats (*Rattus norvegicus*) induced by streptozotocin 40 mg/kg. BW

intraperitoneally (IP), with a dose of 250 mg/kg BW, a dose of 500 mg/kg BW, and a dose of 750 mg/kg BW in the form of kefir.

2. METHODS

2.1 Tool and Material

2.1.1. Tool

Watch, autoclave, blender (kirin), kefir holder bottle, mouse drinking bottle, petri dish, porcelain dish, glass funnel, Erlenmeyer, freeze dryer, measuring cup 25 ml, 50 ml, 100 ml (pyrex®), 100 ml beaker (pyrex®), glucometer (Accu-Chek®), glukotest test strip (Accu-Chek®), incubator, needle loop, test animal cage, filter cloth, stove, flask (pyrex®), mortar and stamper, Olympus microscope Cx-21, oven, stirrer, dropper, water bath, test tube (pyrex®), tube rack, surgical kit (Renz), 1 ml syringe, 3 ml (One Med Health Care), styrofoam, marker (Snowman) organ tube 3 ml (VacuTube), test tube (pyrex®), cutting board, glass jar, thermometer, analytical balance (Ohaus), a scale for mice (Cook Master).

2.1.2. Material

Kefir seeds, rosella flower (*Hibiscus sabdariffa* L), 70% alcohol, 90% alcohol, 95% alcohol, 100% alcohol, aqua distillate (aqua), aqua pro injection (Otsuka), hydrochloric acid (Merck), Citrate-buffer saline (Sodium Citrate, Citric Acid), ether, formalin 10% Buffered Neutral Formalin (BNF), FeCl3, glibenclamide (PT. Indo Farma), handspun (Sensi), cotton, label paper, filter paper, duct tape, Liebermann Burchard, mask, Na CMC 0.5%, sodium chloride, standard feed, Hematoxilyn Eosin may solution, magnesium powder (Merck), streptozotocin (Bioworld USA), milk, tissue, and Xylol.

2.2. Procedures

2.2.1. Making Rosella Flower Petals Water Extract

The dried rosella petals are then ground into powder. The making of rosella flower water extract was carried out using the infusion method. The rosella flower powder is then sieved using a sieve until it becomes a fine powder (flour), then the rosella flower powder is dissolved in water in a ratio of 1000 grams/5000 ml, then heated at a temperature of 50°C for 30 minutes, after that it is filtered and then cooled then frozen in the refrigerator/freezer, then freeze dryer to get the desired juice or extract.

2.2.2. Making of Kefir

Milk is pasteurized at a temperature of 85°C for 30 minutes, lowering the temperature to ± 27°C, then weighing the rosella juice for 3 concentrations each, then mixing the milk with rosella juice at each concentration, inoculation or inoculation of 5% starter for each concentration, incubation at a temperature of 25°C, then filtering between kefir grains and kefir, after which the finished kefir is stored at a temperature of 5°C.

2.2.3. Preparation of 0.5% NaCMC suspension

As much as 0.5 grams of sodium carboxymethyl cellulose (Na CMC) was weighed, sprinkled in a mortar containing 10 ml of heated distilled water, allowed to stand for 15 minutes until a transparent mass was obtained, and then stirred until homogeneous. The Na CMC suspension was transferred to a 100 ml volumetric flask. The volume is made up of distilled water up to 100 ml.

2.2.4. Preparation of 0.45 mg/kg BW mg Glibenclamide suspension

The dose of glibenclamide in adult humans is 5 mg per day, if it is converted to a rat weighing 200 grams, it is multiplied by a conversion factor of 0.018 so that the dose of glibenclamide for rats is 0.45 mg/kg BW. The equivalent of 3.6 mg of glibenclamide tablet powder was weighed and then suspended in 0.5% CMC to 100 ml then shaken until homogeneous.

2.2.5. Preparation of Streptozotocin induction solution

Streptozotocin was weighed as much as 0.08 grams and then dissolved using citrate-buffer saline with a pH of 4.5 to 25 ml, then induced in rats via intraperitoneal (IP). The dose of streptozotocin is 40 mg/kg BW.

2.2.6. Test Animal Experiment

The experimental animal protocol was approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Tadulako University with number 7665/UN 28.1.30/KL/2020. Male Wistar rats weighing 200-250g were obtained from Vaila Wistar and acclimatized in local animal cages for 2 weeks.

2.2.7. Process of Kefir from Rosella flower petals (Hibiscus sabdariffa L.)

This study used rosella flower petals (*Hibiscus sabdariffa* L.) and the addition of kefir seeds (kefir seeds). Rosella flower extract was obtained by extraction using the infusion method. The infusion method was chosen because the tools used are simple, the method of manufacture is easy and the operational costs are relatively low. In addition, the solvent used in the infusion method is using water solvent, where the solvent is used as a liquid filter because it is polar, universal, easy to obtain, and also safe to use in the manufacture of formula preparations such as beverages, this process can be seen in **Figure 1.**

Figure 1. The process of making rosella flower petal infusion

2.2.8. Pancreatic Histopathological Testing

Pancreatic histopathological testing was carried out on the 28th day after treatment. The test animals were sacrificed using anesthesia, that is, the test animals were placed in a glass jar containing cotton after being given ether. Then wait until the rat loses consciousness by stimulating pain in the soles of the rat's feet, if it doesn't respond then the anesthetic effect has worked. The surgical process on mice was carried out on the skin of the stomach until the internal organs of the mouse's stomach were visible. The pancreatic organ is then taken and placed in a special container containing 10% formalin (Anggi et al., 2020)

2.2.9. Making Pancreatic Histopathology Preparations

Once the rat pancreas organ has been removed, pancreatic preparations are then made using the following steps:

Fixation

Tissue samples are fixed with Buffered Neutral Formalin (BNF), the volume of Buffered Neutral Formalin (BNF) is at least 10 times the tissue volume. In general, the time required for complete fixation is 48 hours.

Specimen Cutting

Specimens selected for examination are cut 0.5-1 cm thick. Specimen cutting is done to reduce the size of the specimen so that it is easy to process. The specimen pieces are placed in the processing basket accompanied by a specimen number label written in pencil.

Processing and Embedding

The embedding cassette filled with tissue specimens is inserted into the tissue processor with a set time. Processing is carried out starting from the fixation process to preserve and harden the organ, the dehydration process to remove all the fluid contained in the tissue that has been fixed, the clearing process to remove alcohol from the organ, and the impregnation process to remove toluene from the organ and replace it with liquid paraffin for the immersion process. Embedding or impegmentation is done to harden the organ so that it can be easily cut using a microtome.

Cutting

The cutting process is carried out to obtain thin slices using a microton. The process of cutting network blocks is as follows: 1) Tissue blocks were fixed using a microtome. The tissue block is then cut to obtain a flat surface. 2) Cutting is carried out using a sharp microtome knife, with a cut thickness of 5-6 microns. Select the best piece of tissue from the formed ribbon. 3) The selected pieces are stretched in a floating out at a temperature of about 40°C. The ideal temperature will cause the tissue pieces to stretch perfectly and not shrink. 4) Sprinkle 5 grams of gelatin powder for 100 cc of distilled water and let it dissolve completely. 5) A good piece, not scratched, not shriveled is selected and taken using a glass slide that has been numbered according to the epi/pathology number. 6) Slides containing tissue pieces are placed on a slide heating plate for a minimum of two hours.

Coloring

The staining process is carried out to provide color to the tissue that has been cut so that the tissue becomes contrasting so that it can be recognized and observed with a microscope. Before coloring is carried out, all coloring materials must be checked for clarity. After completing the coverslip coloring, prepare enough coverslips according to the number of preparations that have just been colored, then drop 1-2 drops of "entellan" on the coverslip, turn it over and cover it on the slide of the preparation that has just been colored, to prevent air bubbles from forming, the preparation that has been covered with the coverslip is left until it dries completely. The slides were then cleaned with xylol and labeled accordingly and ready to be examined under a light microscope. Observations are carried out under a microscope to see changes in the morphology of the pancreatic organ being observed. Observations were made using an Olympus Cx-21 microscope with 400x magnification, with A = Score 0 (Normal), B = Score 1 (damage to ¼ of the cells), C = Score 2 (damage to ½ of the cells), D = Score 3 (damage to ¾ of the cell) and E = Score 4 (damage to almost all parts of the cell).

2.3. Data Analysis

The results of a microscopic examination that can be obtained are in the form of scoring data on the level of pancreatic damage of male white rats. First, the normality test and homogeneity test were carried out. If the data obtained is not normal or homogeneous, then it is analyzed using the Kruskal Wallis non-parametric test to determine the significant difference between the treatment group and the control group with a p-value of <0.05 chosen as the level of significance. If there is a significant difference, then proceed with the Mann-Whitney test to

see a significant difference in each treatment group. Data processing was carried out using the SPSS 25 software program.

3. RESULTS AND DISCUSSION

3.1 Content of secondary metabolite compound of Extract Kefir Rosella Flower Extract (*Hibiscus sabdariffa* L.

Phytochemical testing is an important initial step in identifying the content of secondary metabolite compounds in a plant extract. These compounds often play a role in providing potential pharmacological activity. **Table 1**, presents the results of phytochemical tests on Rosella flower extract (Hibiscus sabdariffa L.), which includes several groups of bioactive compounds such as alkaloids, flavonoids, saponins and tannins. These results provide an initial picture of the chemical components contained in the extract and are the basis for supporting its therapeutic potential in pharmacological studies, these results can be seen in **Table 1.**

Table 1. Phytochemical Test Results of Rosella Flower Extract (*Hibiscus sabdariffa* L.)

Test	Reactor	Observation	Results
Flavonoid test	Magnesium and HCl	Formed color purple red	+
Saponin test	HCl 2N	Foam is still formed ±1 minute	+
Tannin test	$FeCl_3$	Formation of dark blue color	+

3.2 Results of Average Blood Glucose Level of Extract Kefir Rosella Flower

Measurement of blood glucose levels is an important parameter in evaluating the effects of a treatment of Extract Kefir Rosella Flower Extract (*Hibiscus sabdariffa* L.) on glucose regulation in the body, especially in studies related to antidiabetic activity. **Table 2 and Figure 2**, presents data on average blood glucose levels in each treatment group. These data reflect the extent to which the extract or compound being tested is able to lower or stabilize blood glucose levels compared to the control group or other comparison groups. Through this analysis, the hypoglycemic potential of the tested material can be identified, as well as its effectiveness in lowering blood glucose levels in the model used. Based on the results of tests carried out where the administration of kefir preparations of rosella flower extract (*Hibiscus sabdariffa* L.) there are 2 pathways, namely the first pathway inhibits the entry of methyl groups into DNA molecules and then inhibits ribosomal poly ADP or cell death so that NAD + and ATP increase (converted to energy) then there is an improvement in the DNA molecule, causing blood glucose levels to decrease, malondialdehyde levels decrease, 8-OhdG levels decrease, pancreatic beta-cell regeneration occurs, insulin levels increase and insulin sensitivity in pancreatic beta cells increases (Handayani et al., 2022). Doses of 250, 500, and 750 mg/kg BW have a therapeutic effect effectiveness in lowering blood glucose levels and regenerating cells in the kidneys, and a dose of 750 mg/kg BW has a better effect with 175.6 mg/dL than doses of 250 and 500 mg/kg BW. Then the second pathway is to inhibit the immune response (inflammation) as a result of which reactive oxygen species (ROS) are not formed which causes NFKB to be inactivated and transcriptional genes, cytokines, and chemotactic factors are also not activated, so there is no insulitis or inflammation in the pancreas, causing glucose levels to rise. decreased blood levels, decreased malondialdehyde levels, decreased 8-OhdG levels, pancreatic beta-cell regeneration occurred, insulin levels increased and insulin sensitivity in pancreatic beta cells increased (Tandi, 2018). This beverage product is believed to have a positive effect on health because it contains microbes that can inhibit the growth of Gram-positive and Gram-negative pathogenic bacteria, and when consumed can maintain the balance of intestinal microbes and

stimulate gastrointestinal peristalsis. Kefir seed microflora can function as an inhibitor of pathogenic bacteria. The role of lactic acid bacteria in kefir can suppress the colonization of pathogenic bacteria in the digestive tract/so that it has potential as a health drink (Sunarti et al., 2015), these results can be seen in **Table 2.**

Table 2. Average Blood Glucose Level

Days to	Normal control	Control negative	Positive control	Dosage 250 mg/kg BW	Dosage 500 mg/kg BW	Dosage 750 mg/kg BW
0	89.0±4.1	89.8±8.6	94.4±14.8	96.6±4.0	97.2±9.1	95.0±9.6
7	96.8±3.5	340.6±85.2	242.0±97.8	328±117.6	415.2±60.3	390.0±166.4
14	103.0±11.3	363.2±60.1	187.0±4.1	251.6±57.6	298.4±43.0	278.2±94.6
21	92.2±5.1	369.6±59.9	132.0±66.5	294.8±136.2	201.2±124.5	195.4±54.6
28	84.2±9.5	383.0±55.9	76.0±17.2	255.6±125.8	205.2±134.8	175.6±58.2

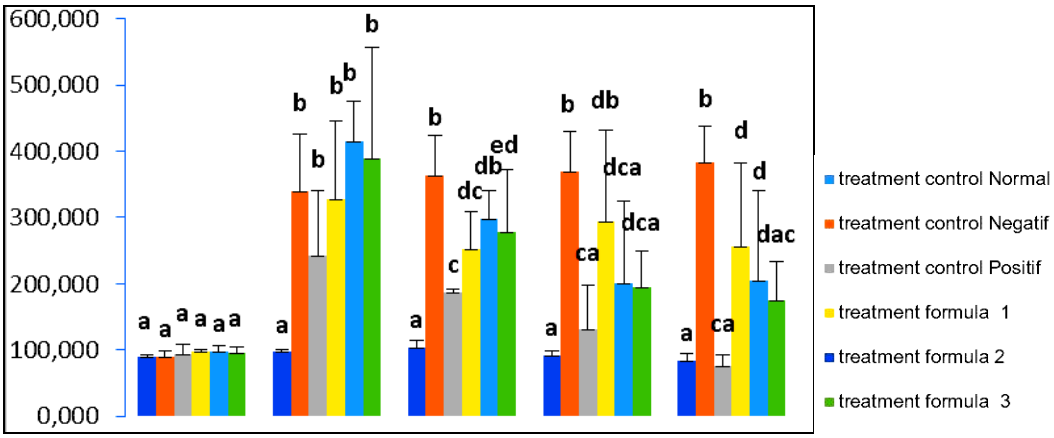

Figure 2. Blood glucose levels of male white rats

Information:
"a" → means there is no significant difference between all groups at that time.
"b" → significantly different from group "a".
"db" or "dca" → not significantly different from groups labeled

3.3 Results of Histopathological of Extract Kefir Rosella Flower

Histopathological evaluation is an important method in assessing the level of microscopic tissue damage due to certain treatments or inductions. **Table 3 and Figure 3**. shows the histopathological damage score observed in the target organ tissue, based on parameters such as necrosis, inflammation, cell degeneration, or other structural changes. **Table 4 and Figure 4** shows of Scoring of the Damage Level of Rat Kidney Tubules. This score provides a quantitative picture of the degree of tissue damage and allows comparison between treatment groups. Through this analysis, it can be seen to what extent the test agent is able to provide a protective effect or is actually toxic to the tissue structure, thus becoming an important indicator in assessing the safety and biological effectiveness of a compound or kefir extract

Based on the score of pancreatic cell damage in the 6 sample groups, it was found that the normal control group had a damage score of (0) which can be seen in the picture where there was no damage to Langerhans cells of exocrine cells. This is because the normal group was not given streptozotocin which can damage the pancreas and only NaCMC suspension which functions as a solution stabilizer and has no impact on blood glucose levels. The negative control group has a moderate damage score with and average damage is (2) which can be seen in the picture where Langerhans cells are necrotic and exocrine cells are lysed. This proves that giving streptozotocin to rats can experience impaired insulin secretion which can lead to disruption of blood glucose homeostasis due to damage to pancreatic cells so that rats suffer from diabetes. The positive control group has a score with an average value (0.6) and can be seen in the figure which is still mildly damaged. Langerhans cells appear swollen and degenerative, while exocrine cells remain normal. Overall, the pancreatic tissue shows improvement compared to the negative control, indicating the therapeutic effect of glibenclamide.

Based on the score value data in the experimental group of kefir preparations rosella flower extract at a dose of 250 mg/kg BW had an average score of damage (1.4), it can be seen in the picture where the pancreas is moderately damaged, namely exocrine cells are necrotic and Langerhans cells occur necrotic and lysed cells. In this case, it can be seen that a dose of 250 mg/kg BW showed a slight improvement compared to the negative control but the improvement had not reached the level of positive control and normal control. At a dose of 500 mg/kg, BW has the same thing, namely the average score of damage (1,2) this can be seen in the picture where the pancreas is lightly damaged, namely Langerhans cells degenerative cells occur, and in exocrine cells appear normal. In this case, it can be seen that at a dose of 500 mg/kg BW the rate of improvement was better than the negative control, but the improvement had not yet reached the state of normal control and positive control. At a dose of 750 mg/kg BW has an average score of damage (0.4), this can be seen in the picture where the pancreas is lightly damaged, in Langerhans cells there are still degenerative cells and in exocrine no degeneration or cells look normal. In this case, it can be seen at a dose of 750 mg/kg BW where the improvement rate is better than the negative control and the improvement rate is almost the same as the normal and positive control. Based on the scoring data, it can be seen that a dose of 750 mg/kg BW is more effective in regenerating pancreatic cells compared to a dose of 250 mg/kg BW and 500 mg/kg BW. At a dose of 750 mg/kg BW has an average score of damage (0.4), this can be seen in the picture where the pancreas is lightly damaged, in Langerhans cells there are still degenerative cells and in exocrine no degeneration or cells look normal. In this case, it can be seen at a dose of 750 mg/kg BW where the improvement rate is better than the negative control and the improvement rate is almost the same as the normal and positive control. Scoring data indicate that a dose of 750 mg/kg BW is the most effective in regenerating pancreatic cells compared to doses of 250 mg/kg BW and 500 mg/kg BW. At this dose, the average damage score is 0.4, with histological observations showing only mild degeneration in Langerhans cells and normal exocrine cells. The degree of improvement is significantly better than the negative control and closely resembles that of the normal and positive controls."

Statistical results *Kruskal Wallis* histopathological scoring showed p-value = 0.001 that is ($p<0.05$) for histopathological scoring which showed there were significant differences in the 3 treatment groups giving rosella flower extract at a dose of 250 mg/kg BW, a dose of 500 mg/kg BW and a dose of 750 mg/kg. kg body weight, with normal control, negative control, and positive control. So, a further test of Mann Whitney was carried out to see significant differences between treatment groups. The results of the Mann-Whitney analysis showed that there was a significant difference in the histopathological scoring of pancreatic cells from each

treatment group. Among them were the treatment group giving kefir extract of rosella flower at a dose of 250 mg/kg BW, a dose of 500 mg/kg BW, a dose of 750 mg/kg BW was significantly different ($p<0.05$) with a negative control which stated that the dose variation was no damage occurred as in the negative control, meaning that the kefir preparation of rosella flower extract can regenerate pancreatic cells because the dose group gets a therapeutic effect from the active substance of rosella flower petals.

Improvements in pancreatic cells were due to the active substance in rosella flower extract in the form of flavonoids, tannins, and saponins that act as antioxidant activity (Oktapiya et al., 2022). Flavonoids can act as antioxidants in inhibiting the formation of free radicals by neutralizing the increase in reactive oxygen species (ROS) due to diabetes so that they can regenerate damaged pancreatic cells and insulin deficiency can be overcome (Falyani et al., 2016). Saponins are compounds that have a variety of chemical properties and properties that can be used in the manufacture of traditional medicines, saponins can lower blood glucose levels by inhibiting glucose transport in the gastrointestinal tract and stimulating insulin secretion in cells. Tannins are compounds that have various benefits, tannins can also cause some plants and fruits to have an astringent and bitter taste (Fachreza Erdi Pratama & Rina Fajri Nuwarda, 2018).

Based on the score data in the experimental group of kefir preparations of rosella flower extract at a dose of 250 mg/kg BW, mild damage occurred with an average damage score of 1.4 this level of damage was higher than the normal control while compared to the negative control group the level of damage was lower. In the group giving kefir preparations of rosella flower extract at a dose of 500 mg/kg, BW suffered minor damage with an average damage score of 1 where the level of damage was higher than the normal control, while compared to the negative control group the level of damage was lower. In the group giving rosella flower extract kefir at a dose of 750 mg/kg BW, mild damage occurred with an average score of 0, 4 where the level of damage was higher than the normal control while compared to the negative control group the damage was still lower. Based on the scoring data in table 3, it can be seen that doses of 250, 500, and 750 mg/kg BW have a therapeutic effect in regenerating cells in the kidneys, and a dose of 750 mg/kg BW has a better effect than doses of 250 and 500 mg/kg BW. This is because a dose of 750 mg/kg BW has a better effect in preventing damage to kidney tubule injury in diabetic rats.

These three therapeutic doses showed no statistically significant difference but showed a clinically significant difference at a dose of 750 mg/kg BW. 350 and 750 mg/kg BW had a therapeutic effect in regenerating cells in the kidneys and a dose of 750 mg/kg BW had a better effect than 250 and 500 mg/kg BW. This is because a dose of 750 mg/kg BW has a better effect in preventing damage to kidney tubule injury in diabetic rats. These three therapeutic doses showed no statistically significant difference but showed a clinically significant difference at a dose of 750 mg/kg BW. 350 and 750 mg/kg BW had a therapeutic effect in regenerating cells in the kidneys and a dose of 750 mg/kg BW had a better effect than 250 and 500 mg/kg BW. This is because a dose of 750 mg/kg BW has a better effect in preventing damage to kidney tubule injury in diabetic rats. These three therapeutic doses showed no statistically significant difference but showed a clinically significant difference at a dose of 750 mg/kg BW. The repair effect caused by the administration of rosella flower extract kefir on kidney tubular injury in diabetic rats was caused by the presence of flavonoid compounds, saponins, and tannins. This is by following the results of the phytochemical screening test. The compounds contained in the kefir preparations of rosella flower extract that play a role in lowering blood glucose levels are flavonoids which act as antioxidants so that they can inhibit the formation of free radicals Reactive Oxygen Species (ROS) due to diabetes and can regenerate damaged pancreatic cells (Gonzalez et al., 2023;Elbatreek et al., 2019), these results can be seen in **Table 3 and Table 4.**

Table 3. Histopathological Damage Score

Sample	Average Damage Value
A	0 ± 0
B	2 ± 0
C	0.6±0.489
D	1.4±0.489
E	1.2±0.4
F	0.4±0.489

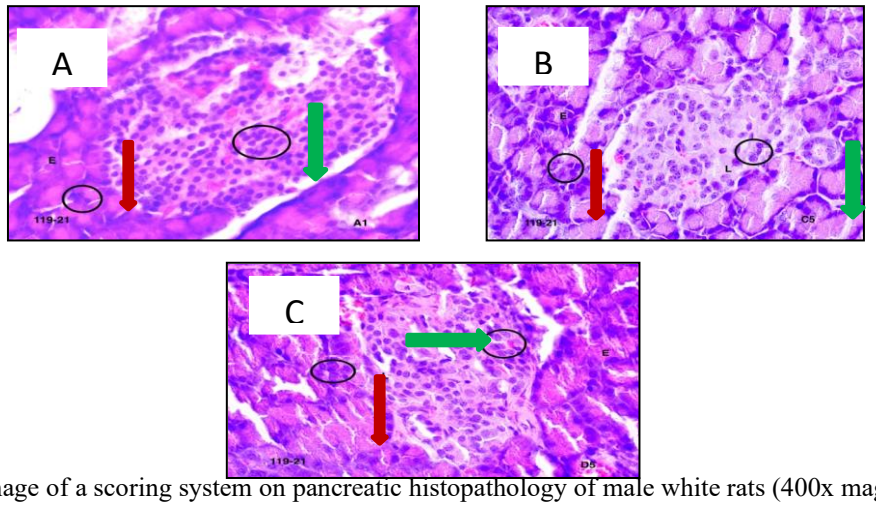

Figure 3. Image of a scoring system on pancreatic histopathology of male white rats (400x magnification with HE staining)

Information:
A. Score 0: Normal
B. Score 1: Swelling and Degenerative 25%
C. Score 2: Necrotic and Lysis 25%-50%

Exocrine island Langerhans Island

Table 4. Scoring of the Damage Level of Rat Kidney Tubules

Sample	Average Damage Value
A	0 ± 0
B	2 ± 0
C	0.6±0.547723
D	1.4±0.547723
E	1±0
F	0.6±0.54723

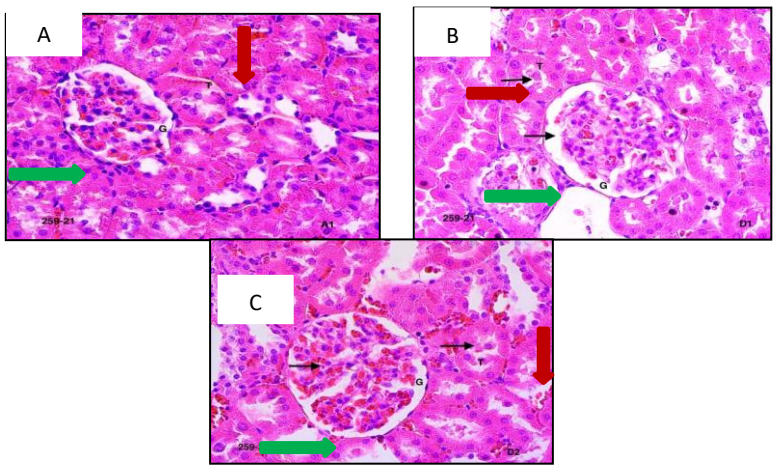

Figure 4. Histopathological assessment with a scoring system on renal tubular injury with HE staining of DM rat model 400 times magnification

Information:
A. Score 0 : Normal
B. Score 1: Light Damage 25%
C. Score 2: Moderate Damage 25-50%

Tubules Glomerulus

4. CONCLUSION

Based on the results of research on kefir preparations of rosella flower extract on blood glucose levels, pancreatic histopathology, and diabetic nephropathy in male white rats (*Rattus* norvegicus) induced by streptozotocin, it can be concluded that: Rosella flower extract kefir (*Hibiscus sabdariffa* L.) contains secondary metabolites, namely flavonoids, saponins, and tannins. The kefir preparation of rosella flower extract (*Hibiscus sabdariffa* L.) affects decreasing blood glucose levels, regenerating the pancreas, and repairing renal tubular injury.

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6. CONFLICT OF INTEREST

The author states that there is no conflict of interest in this study.

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