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Antihyperglycemic Activity of Red Fruit Oil (*Pandanus conoideus* Lam) on Improving Kidney Function in STZ- NA-Induced Nephropathy Rats

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Abstract

Background*: Type 2 diabetes mellitus can cause complications, one of which is diabetic nephropathy. Parameters that indicate damage to the kidneys are the increase in creatinine and albumin levels. One of the traditional medicines used in the treatment of DM is red fruit (Pandanus conoideus Lam).* ***Objective****: The purpose of this study was to determine the antihyperglycemic activity and the effect of red fruit oil administration on creatinine levels, microalbumin, and renal histopathology in STZ-NA-induced rats.* ***Methods****: This study used 30 male Wistar rats conditioned with type 2 DM with STZ-NA induction. The rats have grouped into 6 groups: group I, the normal control, group II, the negative control, group III, the positive control (pioglitazone 15 mg/kg BW), and groups IV, V, and VI, the red fruit oil respectively 1.35 mL/kg BW,2.7 mL/kg BW, and 5.4 mL/kg BW. Red fruit oil is made in traditional way and prepared for 2 days. Parameters tested in the study include blood glucose levels, creatinine, microalbumin, and kidney histopathology. Data analysis used the ANOVA method followed by Tukey's post hoc test.* ***Results****: The results showed that a red fruit oil dose of 5.4 mL/kg BW was an effective dose in reducing blood glucose levels, microalbuminuria, and serum creatinine, and repairing damage to the kidneys of rats. The percent activity of a red fruit oil dose of 5.4 mL/kgBW for blood glucose levels, microalbuminuria and serum creatinine were 84.69%, 76.30%, and 92.20% respectively.* ***Conclusion****: Red fruit oil can reduce blood glucose levels, creatinine levels, microalbumin and can repair kidney damage.*

Keywords*: creatinine, diabetic nephropathy, microalbumin, Pandanus conoideus Lam.*

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INTRODUCTION

Diabetes mellitus has been recognized by the WHO as one of the four non-communicable diseases and is the third highest risk factor for death worldwide. Data from the IDF in 2019 showed that approximately 463 million people were living with diabetes worldwide, and it is predicted that this number will continue to increase to 578 million by 2030 and 700 million by 2045 (Infodatin, 2020). Diabetes nephropathy is a microvascular complication caused by impaired renal function in patients with type 2 diabetes mellitus. Impaired renal function begins with a progressive hyperglycemic state that triggers changes in renal cell hypertrophy, capillary permeability, and extracellular matrix synthesis (Brownlee *et al*., 2010).

Red fruit (*Pandanus conoideus* Lam.) is a traditional plant that acts as a local biological resource for the people of the Central Highlands of Papua and has been known by the local population for generations as a natural food supplement with medicinal qualities. In addition, local people also process red fruit in the form of oil to treat degenerative diseases such as diabetes mellitus (Wawo *et al*., 2019; Keim & Sujarwo, 2020). The antioxidant role of tocopherol and carotene in red fruit is higher than that in other fruits or vegetables, such as papaya, bean sprouts, tomatoes, and carrots (Ayomi, 2015). According to previous studies, red fruit has been proven to be antidiabetic (Astuti & Dewi, 2007; Febriyanti, 2011).

According to Agnesa *et al.* (2014), red fruit extract can reduce blood glucose levels by 31.51%. Diah *et al.* (2016) showed that red fruit extract is effective in reducing blood sugar levels by 68%. Alkatiry *et al.* (2014) reported that red fruit oil could reduce blood glucose levels by 63%. Another study also found that red fruit oil has a decreasing effect on serum creatinine and urea levels in rats with maximum physical activity (Sinaga *et al*., 2019) and a reducing effect on the level of kidney cell degeneration in test animals (mice) exposed to plumbum (Pb) (Aprilianti *et al*., 2020).

Based on previous research, research was conducted on the antihyperglycemic effect of red fruit oil on rats with type 2 diabetes mellitus induced by streptozotocin-nicotinamide and the histopathological picture of the kidneys of rats conditioned with nephropathy.

MATERIALS AND METHODS

Materials

The material used in this study was red fruit obtained from the North Sorong District, West Papua. The other ingredients were STZ, nicotinamide, pioglitazone, 95% alcohol, 1% phenolphthalein (PP), NaOH, acetic acid, chloroform, potassium iodide, distilled water, sodium thiosulfate, 1% starch solution, beta carotene, butylated hydroxytoluene (BHT) 0, 1% ethyl acetate, toluene, diethyl amine, methanol, water, n-hexane, diethyl ether, acetic acid 10%, Ragendorff reagent, Lieberman Burchard, KOH, phosphate buffered saline (PBS), ether, 70%, 80%, and 90% ethanol, xylene solvent, warm water, and hematoxylin and eosin (HE).

Tools

The tools used included a pycnometer, oven, cup, desiccator, water bath, and sterile Bidwell. TLC plate, capillary tube, ruler, scissors, blood glucose meter, probe, Eppendorf tube, ice bath, centrifuge, freezer, spectrophotometer, vortex, tissue cassette, microtome, refrigerator, glass object, and light microscope Olympus CH20.

Method

Manufacture of red fruit oil

Red fruit was collected from the North Sorong District, West Papua, and was analyzed at the Natural Materials Laboratory, Department of Pharmacy, Gorontalo State University. Red fruit oil is traditionally produced. Red fruit seeds are boiled continuously until the oil produced tends to be dark red and concentrated. The first boiling step was conducted for 2 h to facilitate pounding to obtain red fruit starch. The second boil took a full day to separate the oil from the dregs. The third purpose of boiling was to make the red fruit oil last longer and free of bacteria. Red fruit oil was produced in amounts as high as 1 liter.

Characterization and analysis of red fruit oil

Oil quality tests included specific gravity, moisture content, free fatty acids, peroxide value, and total carotenoid content. Specific gravity was measured using a pycnometer with three replicates. Water content was determined using the Sterling Bidwell tool. Free fatty acid and peroxide numbers were tested using the titration method, whereas the determination of total carotenoid levels was performed using a slightly modified method from Knockaert *et al*. (2012).

Preparation and treatment of test animals

The test animals used in this study were 30 Wistar male white rats, 8 weeks old, weighing 150–200 g. The rats were acclimatized for one week before treatment. Rats were grouped into 6 treatment groups, where all treatment groups consisted of 5 rats, including K1 (normal control), K2 (negative control), K3 (positive control: pioglitazone, 1.35 mg/kg BW), K4 (MBM, 1.35 mL/kg BW), K5 (MBM, 2.7 mL/kg BW), and K6

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(MBM, 5.4 mL/kg BW). All groups, except the normal group, were induced by STZ at a dose of 45 mg/kg BW rats and NA at a dose of 110 mg/kg BW rats intraperitoneally. The induced rats were then checked for their blood glucose levels. A person with a blood glucose level ≥200 mg/dL is declared to have diabetes and left for two months to reach a state of nephropathy. The parameters of diabetic nephropathy include serum creatinine and urine albumin levels.

Measurement of rat blood glucose levels

Blood glucose levels were measured using the GOD-PAP method. This method uses enzymatic serum or plasma samples to identify the red color formed in proportion to the level of glucose formed in the sample. The serum was mixed with glucose liquid chromatography reagent and incubated for 10 min at 20−25ºC or 37ºC ℃ for 5 min. Standard and sample absorbance measurements were performed using a spectrophotometer (Rias & Sutikno, 2017).

Examination of microalbumin

Microalbumin examination using fresh urine and immunoturbidimetry Rats were orally administered 8 mL of warm water. The rats were then placed in the metabolic cage, and 20 µL of urine was collected. Subsequently, 350 µL of reagent 1 (TRIS pH 7.5 + NaCl) and 70 µL of reagent 2 (TRIS pH 8.0 + NaCl) were added, and the absorbance was read using a spectrophotometer. Then calculate the albumin ratio. The reference value for the ratio of urine albuminuria was normal (<30 mg/L) and was expressed as microalbuminuria (≥30 mg/L).

Creatinine check

The Jaffe method was used to examine creatinine levels. A normal creatinine level was 1.2 mg/dL. The serum creatinine levels were measured using a spectrophotometer. To determine the creatinine level, a mono-reagent was first prepared by mixing four parts of reagent 1 (sodium hydroxide) with one part of reagent 2 (picric acid), and 10 µL of the test serum was reacted with 1000 µL of the test reagent (creatinine reagent) and then homogenized using a vortex. The absorbance was measured with a spectrophotometer (490-510 nm) at 37ºC ℃ for 60 s (A1), and then the absorbance was measured again after 120 s (A2). The difference between A2-A1 was used to calculate creatinine levels for blanks (reagent and distilled water) and standards (reactant and creatinine standards).

Histopathological test

Experimental animals were anesthetized first with ether, then sacrificed, the kidney organs were taken, and histopathological preparations were made. Kidney organs were fixed in formalin in phosphate-buffered saline (PBS) at pH 7.4. The next step is embedding. The embedding procedure consists of several stages, including dehydration, clearing, and paraffin block preparation. The tissue pieces were then removed, placed on a glass slide, and stained with hematoxylin and eosin or HE. Kidney tissue preparations were observed under a microscope at 400x magnification. An analysis was carried out on the changes that occurred, and the average of these changes was calculated.

Data analysis

The data obtained in this study were statistically analyzed. If the data were normally distributed (p> 0.05), then the parametric test was continued with the post hoc test, namely Tukey, to determine whether there was a difference between each treatment group.

RESULTS AND DISCUSSION

Characterization and oil quality test

The characterization and quality testing of red fruit oil were carried out on the parameters of specific gravity, moisture content, free fatty acids (FFA), peroxide number, and total carotenoids. The characterization and quality test results for the red fruit oil are shown in Table 1.

The specific gravity obtained in this study is 0.93. This result is similar to that of a previous study by Widowati *et al.* (2009), who reported that the specific gravity of red fruit oil was 0.92. Specific gravity describes the number of components contained in a substance. The more chemical components in the oil, the higher the specific gravity (Kristian *et al.*, 2016).

Table 1. Characterization and oil quality test results

No.	Parameters	Result	Requirements	Literature
1.	Specific gravity	0.93±0.002	-	-
2.	Moisture content	4.98%±0.008	0.5%	(CPO SNI, 2006)
3.	Free fatty acids	0.62%	0.5%	(CPO SNI, 2006)
4.	Peroxide number	7.95 meq O2/kg	10 meq O2/kg	(CPO SNI, 2013)
5.	Total carotenoids	4889.32 mg/kg	-	-

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The obtained red fruit oil had an average water content of 4.98% on average. The standard for red fruit oil has not yet been established, but according to a previous study by Pratiwi *et al.* (2020), the moisture content obtained was 0.92%. Meanwhile, when compared to the SNI for CPO, the maximum moisture content was 0.5%. The high water content of the oil causes hydrolysis reactions to occur. The very high water content in red fruit oil can be caused by the extraction process of red fruit oil using a wet extraction method, where it is suspected that the water separation process does not take place properly, so that the amount of water included in the oil is still quite high (Pratiwi *et al*., 2020). More attention will be given to the process of separating water and oil, which can be done so that the water contained in the oil is not too high.

Free fatty acids are an important parameter for determining the quality standards of oils and fats because they can indicate the rancidity of oil during storage. The obtained result was 0.62%. The FFA level of CPO according to the SNI 2006 was 0.5%, which means that the FFA level of the research results is still unqualified when referring to the standard value for CPO. The results of this study are in line with those of previous studies that reported FFA values higher than SNI. Pratiwi *et al.* (2020) reported that the FFA content produced was higher than the specified standard of 0.66% for crude oil. The high FFA content of red fruit oil can be caused by the extraction process of red fruit oil, hydrolysis reactions that are influenced by temperature, air, heating time, and the container used (packaging) (Pratiwi *et al.*, 2020; Sarungallo *et al.*, 2011). Previous research also reported FFA levels of red fruit oil ranging from 7.24-8.5%. FFA values depend on the extraction method used. The wet extraction method had a higher FFA value than the dry extraction method because water was added to the extraction process to allow hydrolysis. Red fruit oil with a high FFA value requires a refining process with four general stages: degumming, neutralization, bleaching, and deodorization (Sarungallo *et al*., 2014). The peroxide number is an important parameter that can indicate damage to oil owing to the rancidity of fat due to oxidation. The peroxide number measured in this study was 7.95 meq O2/kg. This peroxide value meets the peroxide number required for cooking oil, which is 10 meq O2/kg (SNI 2013). According to Pratiwi *et al.* (2020), the low peroxide number value can be due to the presence of active components, such as carotenoids and tocopherols, which are quite high in red fruit oil. The peroxide number of red fruit oil in this study was lower than that reported by Sarungallo *et al.* (2011), who reported the traditional extraction of red fruit oil (17.6 mg O2/100 g) and commercial oil (16.62 mg/kg). The difference in the peroxide number was mainly due to the length of the cooking process. Merdey's traditional extraction, with a total extraction process time of more than 30 h, causes the peroxide number of the oil to be very high. Their findings revealed that by shortening the extraction time, both dry and wet extraction could reduce the level of oxidative damage manifested by lower peroxide values (Sarungallo *et al*., 2011).

Red fruit oil has been reported to contain carotenoids as one of its most important active components (Pratiwi *et al*., 2020). Carotenoids are natural pigments that give red color to red fruit oil, and are antioxidants that are needed by humans (Santoso *et al.*, 2018). This study produced carotenoid levels of 4889.32 mg/kg. This result is still lower than those reported in previous studies. According to Pratiwi *et al.* (2020), the total carotenoid content in crude red fruit oil is 6299 ppm. Santoso *et al.* (2018) reported a total carotenoid content of 6398 ppm in crude red fruit oil. Sarungallo *et al.* (2018) reported a total carotenoids of 7857 ppm. The difference in carotenoid content can be attributed to the differences in the extraction process. This study used a wet extraction method (boiling) in which a large amount of water was used. Excess water during the extraction process is thought to cause oxidation and hydrolysis reactions in oil. These reactions can trigger damage to the carotenoid components. In addition, the processing process using a heating process is also a factor in the damage to carotenoid components (Sarungallo *et al*., 2014). Wilska and Jeszka (2002) stated that carotenoids are very sensitive to oxygen, light, and temperature because they have conjugated double bonds that contain many reactive electrons and are easily oxidized (Pratiwi *et al.*, 2020; Sarungallo *et al.*, 2018).

Blood glucose levels of rats

Blood glucose levels were measured six times at different times on days 0, 3, 24, 31, 38, and 45. Day 0 (T0) was the day the rats were acclimatized for 7 days. Blood glucose levels measured in this study ranged from 60 to 70 mg/dL. This is because T0 has not been given any treatment, so it can be ascertained that: the Test animals were healthy. All groups except the normal group were then given STZ-NA intraperitoneally to condition the test animals to experience diabetes mellitus (DM). The results of measuring Blood glucose levels in rats are shown in Figure 1.

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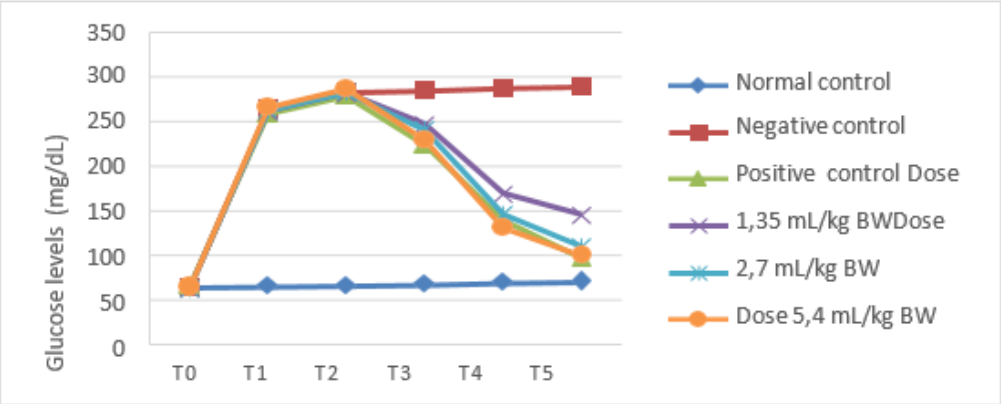

Figure 1. Average blood glucose levels of rats

Blood glucose levels were measured on days three (T1) and 24 (T2). At this time, the test animals had already experienced DM and were conditioned for 21 days to induce nephropathy. The measurement of blood glucose levels showed an increase in blood glucose levels due to STZ-NA induction. This is due to the mechanism of STZ, which can damage pancreatic beta cells via nitric oxide (NO) production, the formation of reactive oxygen species (ROS), and DNA alkylation, which can cause cell death or total cell damage. Therefore, NA induction was also performed to partially protect pancreatic beta cells from STZ exposure (Ghasemi *et al.*, 2014; Kishore *et al.*, 2017; Szkudelski, 2012).

Blood glucose levels were measured at T3, the first week (day 31); T4, the second week (day 38); and T5, the third week (day 45). As shown in Figure 1, the measurement of blood glucose levels at T3, T4, and T5 showed a decrease in blood glucose levels in the group administered pioglitazone (the positive control) and the test preparation. The decrease in blood glucose levels indicated that there was an effect on the blood glucose levels after treatment. The 5.4 mL/kgBW red fruit oil group was not significantly different from that in the positive control group. This proves that the administration of red fruit oil group at 5.4 mL/kg BW decreased blood glucose, which continued to increase. This proves that the longer the administration time, the higher is the lowering effect shown by the test preparation.

Statistical analysis showed significant differences in the measurement of blood glucose levels in each group ($P<0.05$). Blood glucose levels at T5 in the positive group (pioglitazone) were closest to normal when compared to the test preparation group, while in the test preparation group, the dose of K6 (red fruit oil, 5.4 mL/kg BW) was closest to the positive and normal control groups.

The positive control (pioglitazone) reduces blood glucose levels by acting on peroxisome proliferator-activated receptor agonist (PPAR) to increase insulin stimulation, thereby increasing glucose uptake in peripheral tissues. Pioglitazone can reduce blood sugar levels due to its mechanism of increasing insulin sensitivity in the liver and adipose tissue and with a lower risk of hypoglycemia-related side effects (Ulfa & Arfiana, 2020).

The test preparation can reduce blood glucose levels because of its chemical content, which acts as an antihyperglycaemic agent. According to previous research, red fruit oil contains flavonoid compounds, which are antioxidants that can prevent the formation of AGE chains that cause pathological changes under hyperglycemic conditions. Flavonoids reduce blood glucose levels through direct and indirect protection of pancreatic beta cells from damage and oxidative stress, which can increase insulin secretion (Ghasemi *et al.*, 2014). Red fruit oil contains carotenoids. Carotenoids can improve pancreatic function so that insulin secretion by the beta islets of Langerhans can increase (Heriyanto *et al.*, 2021; Stahl & Sies, 2005).

Microalbuminuria levels in rats

Microalbuminuria measurement is the initial examination to detect the occurrence of diabetes nephropathy. Microalbuminuria is characterized by higher than normal albumin excretion of more than 30 mg/day (Natesan & Kim, 2021; Rivandi & Yonata, 2015; Verdiansah, 2016). Figure 2 shows the results of measuring microalbumin levels in rats.

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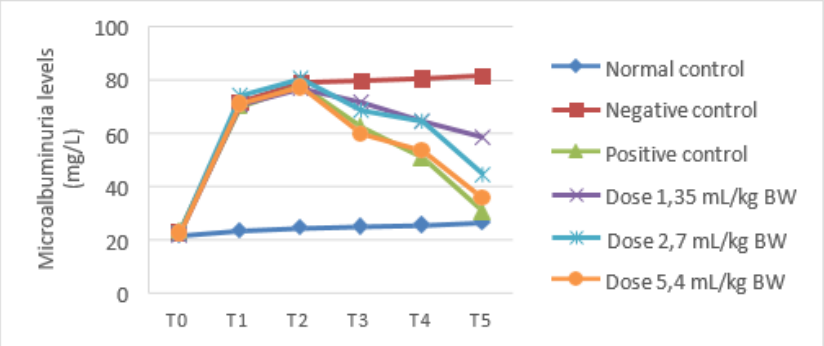

Figure 2. Average microalbuminuria level of rats

The measurement of microalbuminuria levels in the test animals began on day 0 (T0). The average albumin level in the urine of test animals at T0 was <30 mg/L. In contrast to the urine albumin levels measured at T1 (day 3) and T2 (day 24), which revealed that they had experienced microalbuminuria, with urine albumin levels exceeding the normal value of >30 mg/L. The increase in urinary albumin levels is due to STZ-NA induction, which causes blood glucose levels to exceed normal so that diabetic nephropathy occurs, indicating disruption of kidney function.

Examination for microalbuminuria is very important for detecting impaired renal function. The initial stage begins with renal hypertrophy, hyperfunction, and thickening of glomerular and tubular membranes. The process of glomerulosclerosis that continues to occur results in increased glomerular permeability, which causes albumin to escape glomerular filtration and be found in the urine (Verdiansah, 2016).

Urine albumin levels were measured on days 31, 38, and 45. Based on the average graph in Figure 2, urine albumin levels at the three measurement times showed a decrease compared to the negative control. The decrease in urine albumin levels was because, on day 24, each group was given treatment, except for the normal group and the negative control. As shown in the graph, the decrease from T3 to T4 and from T4 to T5 was different for each treatment group. Data on urine albumin levels at T5 were subjected to statistical analysis. Statistical analysis from T5, namely the 45th day, showed a significant value (P <0.05) in the one-way ANOVA test. Testing was continued using a post-hoc Tukey test. The results showed that the positive control group was closest to the normal group. Then the red fruit oil group 5.4 mL/kg BW is not different from the positive control so the positive control group (pioglitazone) has proven its effectiveness in reducing urinary albumin levels, and the red fruit oil group 5.4 mL/kg BW is the dose closest to the positive group, so it can be said to have the effect of reducing urinary albumin levels best compared to the red fruit oil groups 1.35 mL/kg BW and 2.7 mL/kg BW. However, when compared to normal urine albumin levels, normal urine albumin levels were not reached.

The development of microvascular complications in renal mesangial cell growth that occur during diabetic nephropathy results in increased levels of AGEs in the blood. AGEs bind to the AGEs receptor (RAGE), which then triggers the generation of ROS and activates NF-κB in target cells, mesangial cells, the endothelium, and macrophages, resulting in increased vascular permeability. This results in transvascular albumin leakage that causes microalbuminuria. The decrease observed in the test preparation treatment group could be due to the presence of antioxidant compounds from flavonoids and carotenoids. Antioxidants reduce the production of ROS-modified proteins in mitochondria to prevent the progression of diabetic nephropathy through the effect of ROS scavenging on mesangial cell mitochondria (Murnah & Indranila, 2014).

Serum creatinine levels of rats

High creatinine levels in blood indicate weak kidney function (Aditya *et al.*, 2018; Aji *et al.*, 2019; Alfarisi *et al.*, 2012; Martono & Satino, 2014; Verdiansah, 2016). Creatinine levels above normal (<1.2 mg/dL) indicate impaired renal function (Alfarisi *et al.*, 2012). The following is a graph of the results of creatinine level measurements in rats.

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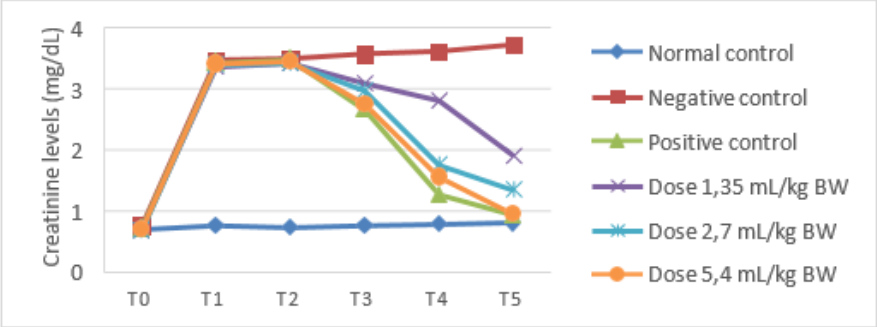

Figure 3. Average rat creatinine levels

Creatinine levels increased on days 3 (T1) and 24 (T2). According to Aditya *et al.* (2018), creatinine levels in the blood that increase above 1.5 mg/dL indicate weak kidney function. Increased creatinine levels are caused by damage to glomerular filtration. The damage that occurs can be caused by exposure to toxic substances, such as STZ-NA. STZ is a diabetic agent that causes pancreatic beta cell death and increases blood glucose levels through an increase in ROS. High blood glucose levels can cause oxidative stress, which triggers the production of NO to increase, resulting in the release of vasoconstrictive mediators that can then affect kidney function, namely, a decrease in glomerular filtration in rats (Aji *et al*., 2019). Glomerular damage can be determined by measuring creatinine levels because one of the factors that affects kidney function is determined by serum creatinine levels (Martono & Satino, 2014).

For 21 days, measurements of creatinine levels in test animals were carried out three times, namely, on day 31 (T3), day 38 (T4), and day 45 (T5), to observe the effect of decreasing creatinine levels in each treated group. The measurement results at T3, T4, and T5 based on the graph in Figure 3 show that there was a decrease in creatinine levels in the positive control and treatment groups compared to before the administration of the test preparation. The measurement data of creatinine levels at the three time points were then subjected to statistical analysis. Statistical test results using one-way ANOVA showed a significance value of 0.000 ($P<0.05$). Furthermore, to determine the difference in the decrease in creatinine levels in each group, a post-hoc Tukey test was conducted on the 45th day. The test results showed that the administration of pioglitazone as a comparison drug provided the best reduction in creatinine levels because it was closest to the normal control. The results also showed that there was no difference between the positive control and 5.4 mL/kgBW red fruit oil groups. Therefore, the 5.4 mL/kgBW red fruit oil group had reduced creatinine levels and improved kidney function in test animals experiencing diabetic nephropathy.

The effect of decreasing creatinine levels that occurred in the 5.4 mL/kg BW red fruit oil group could be due to red fruit oil containing antioxidants, such as flavonoids and carotenoids, which indirectly reduce creatinine levels in test animals with impaired kidney function. Flavonoids can capture free radicals by releasing hydrogen atoms from their hydroxyl groups. Carotenoids can quench singlet oxidation and their antioxidant content binds to free radicals. The bond does not eliminate the electrons but reduces the energy possessed so that it is not able to induce other cells (Palupi & Martosupono, 2009).

Rat kidney pathology

The histopathology of kidney organs aims to determine the damage that occurs in the tubules and glomeruli of the kidneys. Renal histopathological observations were performed using hematoxylin-eosin (HE) staining. Necrosis was also observed. Each group was administered three preparations to read the histopathological picture of the kidneys. Observations were made using a light microscope at 400X magnification.

Histopathological changes were evaluated according to the Arshad system by classifying histopathological changes in rat kidneys as no change (0), mild damage (1), moderate damage (2), and severe damage (3). Percentage of mild damage (changes <30%), moderate damage (changes <50%), and severe damage (changes >50%) (Jannah & Budijastuti, 2022). The results of the histopathological observations and percentage of kidney damage are shown in Figure 4 and Table 2.

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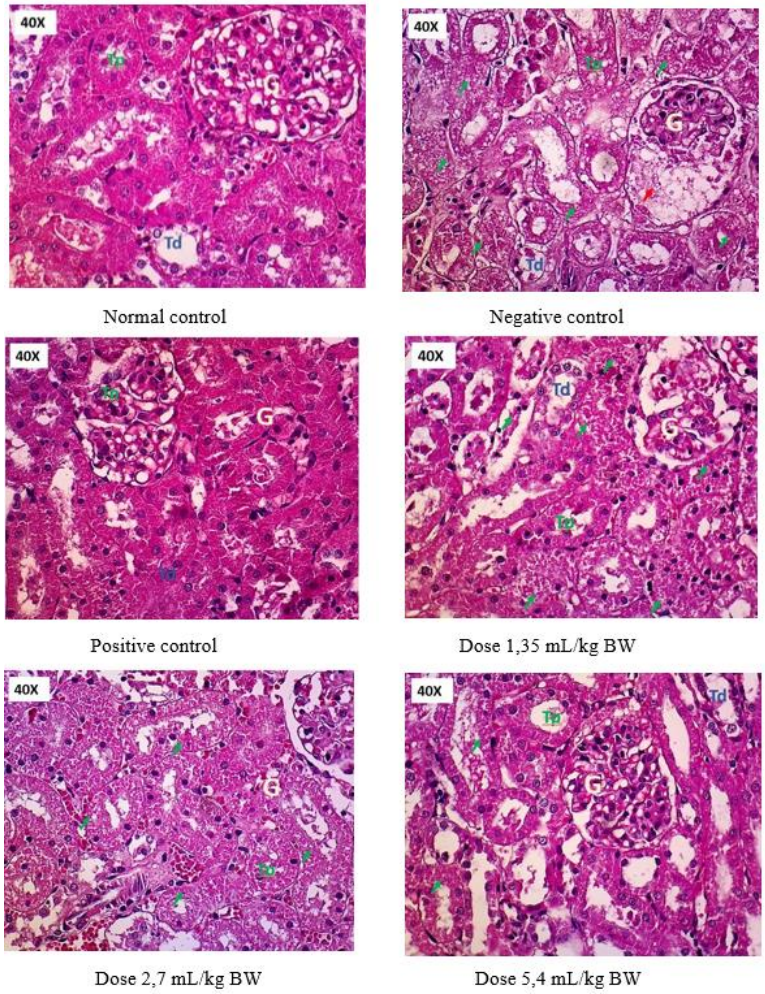

Figure 4. Histopathology of rat kidney

Description:
Tp: Proximal tubule
Td: Distal tubule
G: Glomerulus
Green arrow: Tubular necrosis
Red arrows: Glomerular necrosis

Table 2. Average and scoring of rat kidney damage

Group	Mean percentage of tubule necrosis ± SD (%)	Scoring
I	0	0
II	41.7 ± 14.34	2
III	5 ± 0	1
IV	35 ± 8.50	2
V	16.7 ± 4.71	1
VI	6.7 ± 2.36	1

Description:

I: Normal control
II: Negative control
III: Positive control (pioglitazone)
IV: Red fruit oil 1.35 mL/kg BW
V: Red fruit oil 2.7 mL/kg BW
VI: Red fruit oil 5.4 mL/kg BW

(0) No changes occurred
(1) Mild cell damage with less than a 30% change
(2) Moderate cell damage with less than a 50% change
(3) Severe cell damage with changes > 50%

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Damage to the kidneys can be caused by several factors, including toxic substances that enter the body and interfere with kidney function. Damage to the kidneys due to toxic substances can be identified through structural changes in histology, including cell necrosis, which is morphologically characterized by deconstruction of proximal tubule epithelial cells. Proximal tubule epithelial cells are sensitive to anoxia and are easily destroyed in cases of poisoning owing to metabolic waste excreted by the kidneys. Therefore, histological changes that occur in the kidneys can be ascertained from the number of compounds that enter the body. Necrosis is a cell tissue that undergoes death due to injury while the individual is still alive. Changes occur in the nucleus in the form of loss of chromatin images: the nucleus appears denser and becomes wrinkled, no longer vascular, the color becomes dark (picnosis), the nucleus is divided into torn fragments (cariorexis), and the nucleus appears pale or unreal because it no longer takes up much color (karyolysis). no longer takes up much color (karyolysis) (Jannah & Budijastuti, 2022).

The improvement in kidney damage closest to that of the normal control was a positive control. Histopathological examination of the kidneys in the positive control group showed that the glomeruli and tubules were relatively normal. Likewise, the scoring was 1, with mild cell damage. This is because the positive control was administered with a comparator drug, pioglitazone. This drug acts on PPAR and reduces the amount of glucose in the blood stream. Pioglitazone has strong antihyperglycemic action by reducing insulin resistance (Schernthaner *et al.*, 2013).

The test preparation group showed the best dose VI group in repairing kidney damage, and approaching the positive control was the 5.4 mL/kg BW red fruit oil group. The damage that occurred in dose group VI was less than that in the other dose groups, with an average damage score of 1 for mild damage. The histopathological picture of the kidneys in red fruit oil (5.4 mL/Kg BW) also shows that necrosis was reduced and observed better than in other dose groups.

The ability of red fruit oil to reduce or repair cell damage through the improvement of cell metabolism and cell division means that cells that experience necrosis can be replaced with new cells. This ability can be attributed to the content of red fruit oil, which is rich in antioxidants, proteins, and unsaturated fatty acids, such as oleic acid and linoleic acid. Antioxidants, such as carotenoids, counteract free radicals, which are highly reactive and harmful to cell life (Suastika, 2011).

CONCLUSION

Based on this study, the physical characteristic and mechanical properties of the chitosan-PVA-*Aloe vera* films were affected by both polymers: chitosan (1%, 1.5%) and PVA (0.5%, 1%, 1.5%). The increased concentrations of chitosan and PVA caused an increase in the mechanical properties. However, there was a decrease in the swelling index. Based on the results, films with chitosan 1.5% and PVA 1.5% had the best characteristics and mechanical properties (swelling index, tensile strength, and elongation at break) compared to the other formulations. Therefore, this film has the potential to be developed as a wound dressing material.

AUTHOR CONTRIBUTIONS

Conceptualization, A. C. K., T. W., G. P. W.; Methodology, A. C. K., T. W., G. P. W.; Software, A. C. K.; Validation, A. C. K.; Formal Analysis, A. C. K., T. W., G. P. W.; Investigation, A. C. K.; Data Curation, A. C. K., T. W., G. P. W.; Writing - Original Draft, A. C. K.; Writing - Review & Editing, T. W., G. P. W.; Visualization, A. C. K.; Supervision, T. W., G. P. W.; Project Administration, A. C. K.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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