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Kidney histopathology of white rats (*Rattus norvegicus*) fed a high-fat diet, curcumin supplement, and turmeric powder (*Curcuma longa*)

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ABSTRACT. Excessive consumption of high fat can cause damage to organs, one of which is the explain effect of high-fat diet in kidney. Turmeric is believed to prevent kidney damage due to high-fat feeding because it has curcuminoid compounds that contain antioxidants. This study aimed to analyze the effect of a high-fat diet, curcumin supplement, and turmeric powder on kidney microanatomy of white rats (Rattus norvegicus). This study used 25 white rats divided into five groups viz. C0 (control), C1 (high-fat diet), C2 (high-fat diet + curcumin 1.35 mg/200gBW/day), C3 (high-fat diet + turmeric powder 200 mg/200gBW/day, and C4 (high-fat diet + simvastatin 0.18 mg/200gBW/day). Parameters observed were glomerular diameter, Bowman's capsule space, the diameter of the proximal convoluted tubule, a diameter of the distal convoluted tubule, size of proximal convoluted tubule cells, size of the distal convoluted tubule, kidney weight and kidney index. Data analysis was performed parametrically using ANOVA with Duncan's follow-up test, while non-parametrically using the Kruskal-Wallis test with Mann-Whitney follow-up test. The results showed that the effect of high-fat diet, curcumin supplementation, and turmeric powder had a significant effect on all parameters except Bowman's capsule space size and kidney index ($P<0.05$). The effect of high-fat diet had a significant effect on kidney weight, glomerular diameter, proximal tubular diameter, distal tubular diameter, proximal tubular cell size, and distal tubular cell size. However, the results were not significant for the kidney index and the size of Bowman's capsule space. The provision of curcumin and turmeric powder supplements was able to prevent damage to the kidneys, but based on the Duncan and Mann Whitney test in this study there was no significant difference.

Keywords: curcumin supplement; glomerular diameter; kidney microanatomy; Kruskal-Wallis test; simvastatin (Zocor)

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INTRODUCTION

The habit of eating fast food is now a lifestyle that appears in children and adolescents, has easy availability, has a delicious and savory taste (Kaushik *et al*., 2011). Good economic status causes parents to prefer buying fast food or junk food because it is more accessible and practical (Budiarti, 2021). The prevalence of hyperlipidemia in both men and women worldwide is very high. Kamso doing research on 656 respondents conducted in four major cities in Indonesia namely Jakarta, Bandung, Yogyakarta, and Padang, the results obtained the most hyperlipidemia in the city of Padang and Jakarta by > 56% (have total cholesterol > 240/mg/dl), followed by Bandung city of52.2% and Yogyakarta 27.7% (Kamso *et al*., 2005). Based research data conducted in Beijing in 2006 showed that about 65% of the population aged 45 years suffer from hyperlipidemia (Wang *et al*., 2011).) In Indonesia, it is noted that hyperlipidemia sufferers are increasing from year to year. In 2008 it was recorded at 35.1%, while in 2013, it increased to 35.9% (WHO, 2013).

Higher fat consumption results in higher triacylglycerol synthesis in the liver, leading to an increase in triglyceride levels in the blood. High-fat diets can reduce HDL levels by increasing lipid intake and absorption, so the amount of lipids, including triglycerides and cholesterol in small-density lipoproteins and peripheral cells increases (Olivia & Radita, 2019). Fat that accumulates in the tissue can lead to changes in both kidney physiology and glomerular structure, tubular necrosis, inflammation (inflammation), degeneration and organ weight loss (Eghziaber *et al*., 2013). A high-fat diet for eight weeks increased LDL levels (Heriansyah, 2013). Chade (2013) research proved that lipid abnormality is due to a high-fat diet that results in renal endothelial dysfunction, intrarenal

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inflammation, fibrosis, significant vascular dysfunction, damage and remodeling of renal blood vessels. Feeding a high-fat diet can significantly increase glucose levels. This happens because of insulin resistance, and cells cannot respond to glucose in high levels so that levels remain high (Azhari *et al*., 2016). High glucose levels can cause damage to the blood vessels in the kidneys. Damaged blood vessels are not able to work appropriately (Thomas *et al*., 2015). The selection of the kidney as a research target because it is one of the vital organs in humans and there are many cases of kidney disease. Excessive consumption of high-fat diet is one of the triggers for kidney disorders, so it is necessary to prevent this damage, one of which is by giving curcumin and turmeric powder.

The prevention of hyperlipidemia can do by changing lifestyles, including by following a low-fat diet or adopting a healthy diet and exercise; if it does not work with non-pharmacological treatment, it is necessary to give anti-dyslipidemia anti-hyperlipidemic drugs that can reduce lipid levels (Delima *et al*., 2012). Statin drugs have a mechanism to reduce lipid levels in the blood. However, if consumed for an extended period, it can cause side effects such as decreased kidney function, myalgia, tendon rupture, and increased hemorrhagic effects (Thompson *et al*., 2016).

In addition to chemical drugs such as statins, traditional medicines are developed from herbal ingredients, one of which is the turmeric powder. Turmeric (Curcuma longa) is a spice containing curcuminoids, which consist of curcumin compounds and their derivatives, including desmethoxycurcumin and bisdemethoxycurcumin (Shimizu *et al*., 2019). Giving a high-fat diet can result in the formation of reactive oxygen species (ROS) in excess which causes oxidative stress (Jasda *et al*., 204). Stress caused by ROS can cause lipid peroxidation, DNA damage, protein modification, and other pathological effects that can trigger cell damage and death (Alsharif *et al*., 2021). Research has shown that curcumin can affect lipid metabolism and inhibit lipid peroxidation (Kohli *et al*., 2005). Turmeric contains antioxidant compounds that can overcome the damage caused by ROS. Inhibition of ROS is done by inhibiting the signal itself, signal inhibition is done by giving one oxygen atom of curcumin to stabilize the reaction (Kocaadam & Sanlier 2015).

The dose of turmeric consumption in humans is 500 mg – 8000 mg turmeric powder every day (Alschuler, 2013). Giving turmeric powder at a dose of 50-200 mg/kgBW has an anti-inflammatory effect (Jurenka, 2009). Curcumin has a renoprotective effect on the kidneys, hemodynamic changes in the glomerular microcirculation, injury due to kidney inflammation, and functional damage related to the reduction of kidney mass and increased activity of antioxidant enzymes (Tapia *et al*., 2012). This study aimed to analyze the effect of a high-fat diet, curcumin supplement, and turmeric powder on kidney microanatomy of white rats (*Rattus norvegicus*). The safe dose of curcumin for human consumption is 10 mg/day as for rats 5 g/day.

MATERIALS AND METHODS

The research was conducted at the Structural and Animal Biology Laboratory, Test Animal Maintenance Cages, Department of Biology, Faculty of Science and Mathematics (FSM), Universitas Diponegoro. The implementation of this research has been approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Diponegoro, with no 95/EC/H/FK-UNDIP/IX/2020.

Turmeric powder making. Turmeric powder is made from 3 kg of turmeric rhizome, peeled, cut transversely, dried for three days, and baked at 450°C. Then it was blended for 10 minutes and sieved using a sieve size of 200 mesh (Saraswati, 2014).

Research design. The study used a completely randomized design (CRD). The study used 25 white rats (*Rattus norvegicus*) aged 21 days with body weights ranging from 30-50 grams, then divided into five treatments. Each treatment unit contained five white male rats. The treatments for each treatment were: C0 (rats given standard diet or control), C1 (high-fat diet), C2 (high-fat diet + curcumin supplement), C3 (high-fat diet + turmeric powder), and C4 (high-fat diet + simvastatin). The Treatment was carried out for 51 days.

Acclimation for research animals. White rats (*Rattus norvegicus*) aged 21 days were acclimatized for seven days to adapt to their new environment (Hasanah, 2015). Rats in this process are fed standard feed and drink *ad libitum*.

Treatments giving. In this study there were 5 treatments where the treatment was C0 (given standard feed brand Hi-Pro-Vite A594K 20 g/head/day); C1 (given high-fat diet 20g/head/day); C2 (given with high-fat feed + curcumin supplement brand KGaA 64271 Darmstadt 1.35 mg/200gBW/head/day); C3 (given high-fat feed + turmeric powder 200 mg/200gBW/head/day; C4 (given high-fat feed + simvastatin 0.18 mg/200gBW/head/day). The simvastatin used is manufactured by Dexa Medika with a weight of one tablet, which is 10 mg. The tablets were crushed, taken and weighed as much as 0.18 mg. Standard feed has composition: maximum water content 13%, minimum protein 18.5%, fat minimum 3%, fiber minimum 8%, ash maximum 8%, calcium 0.9%, phosphorus 0.6%, and aflatoxin maximum 50 ppb. High-fat diet obtained from the medical faculty of Gadjah Mada University with a composition of 29.7% cornstarch, 14% casein, 25% fructose, 21.4% solid oil, 5% -cellulose, 3.5% mineral mix, 1% vitamin mix, 0.18% methionine, and choline chloride 0 ,25% The treatment was given for 56 days.

Histology slide preparation. Preparation of histology slide was carried out at the Veterinary Center, Yogyakarta. Slides were made using the paraffin method with hematoxylin and eosin staining. The first stage is the fixation process using 10% BNF (*Buffer Neutral Formalin)* for 24 hours; then, washing is carried out to remove the fixative solution. The following process is dehydration using graded alcohol in 70%, 80%, 90%, 96%, absolute alcohol for 30 minutes. Clearing process using toluol and continued with paraffin infiltration process. The infiltration process was carried out by inserting a tissue sample into a mixture of toluol: paraffin of 3:1, 1:1, and 1:3 each for 30 minutes and then put into pure paraffin I, pure paraffin II, and pure paraffin III, respectively for 30 minutes at 56^0C. The next stage is the embedding process into the paraffin block. The following process is slicing or sections with a thickness of 6 µm using a rotary microtome. After that, the process of attaching or affixing the paraffin slices to the glass object is carried out. The next stage is staining using hematoxylin and eosin. This process begins with deparaffinization or removing the paraffin contained in the tissue slices by soaking the preparations using xylol for 24 hours. Furthermore, it is dipped in graded alcohol (96%, 90%, 80%, 70%, 50%, 30%) to approach the atmosphere of distilled water, each for 1-2 minutes, then put in hematoxylin dye for 15-20 minutes. minutes ago rinsed with distilled water. After being stained, it was followed by immersion with graded alcohol 30%, 50%, 70% and continued with eosin staining for 5 minutes after the color was even then soaked with graded alcohol (70%, 80%, 90%, 96%) and put into xylol for 24 hours. The next stage is mounting or closing the preparation with a cover glass. The stained preparations were covered with Canada balsam and labeled and then the preparations could be observed under a light microscope.

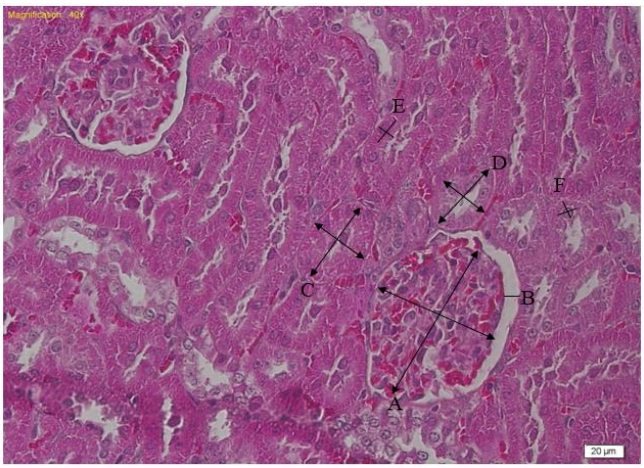

Fig. 1. Measurement of parameters. A: glomerular diameter; B: Bowman's capsule size; C: proximaltubules diameter,D:distaltubulesdiameter, E: proximal tubule cell size, F: distal tubule cell size (Magnification: 400x).

Data retrieval. Olympus BX5 photomicrographs assisted histological observations of the kidneys. This study uses 5 fields of view and 5 repetitions. Magnification used is 200x and 400x. The measurement parameters include glomerular diameter, Bowman's capsule size, proximal and distal tubule diameter, proximal convoluted tubule cell size and distal tubular cell size, kidney weight, and kidney index. The diameter is calculated by multiplying by the shortest diameter and dividing by two (Figure 1). The resulting data is processed using Microsoft Excel software. Calculate the weight of the kidneys by dividing the weight of the left and right kidneys by two using a digital scales. The kidney index was derived by multiplying the kidney weight by the bodyweight of white rats (*Rattus norvegicus*).

RESULTS AND DISCUSSION

High-fat diet has been shown to cause kidney damage if consumed continuously. Efforts that can be made to prevent kidney damage due to high-fat diets are one of them by giving curcumin and turmeric powder supplements. This was proven in this study by collecting data on the kidneys of white rats (*Rattus norvegicus*) both histologically, kidney weight and kidney index**.**

Kidney weight. Observations of the kidneys were also carried out macro-anatomically through their morphology Morphological observations were made by looking at the kidneys' shape, color, and size. Mappa*et al.* (2013) stated that to see the macroscopic picture of the rat kidney, it can be known based on the color, consistency and weight of the right kidney and left kidney. Morphological observations can be seen in Fig. 2.

Fig. 2. Kidney microanatomy of white rats.C0: Control, C1: high-fat diet, C2: high-fat diet + curcumin supplement, C3: high-fat diet + turmeric powder, C4: high-fat diet + simvastatin.

Fig. 2 shows that the kidneys do not change in color or shape, but there is a change in the size of the kidneys. High-fat diet (C1) treatment resulted in smaller kidney size; after supplementation with curcumin, turmeric powder and simvastatin, there was a slight increase in kidney size in rats. According to Polzin (2011), the reduction in kidney size can be caused by atrophy of the kidneys and is one of the characteristics of suspected chronic kidney disease (CKD). Based on the results of ANOVA, the effect of a high-fat diet, curcumin supplements and turmeric powder significantly ($P<0.05$) was able to reduce kidney weight of white rats (*Rattus norvegicus*). A decrease in body weight triggered the decrease in kidney weight. Tumbol *et al.* (2018) stated that variations in kidney weight are strongly influenced by body weight, food and drink intake, age and exposure to toxic substances, and factors of kidney abnormalities themselves.

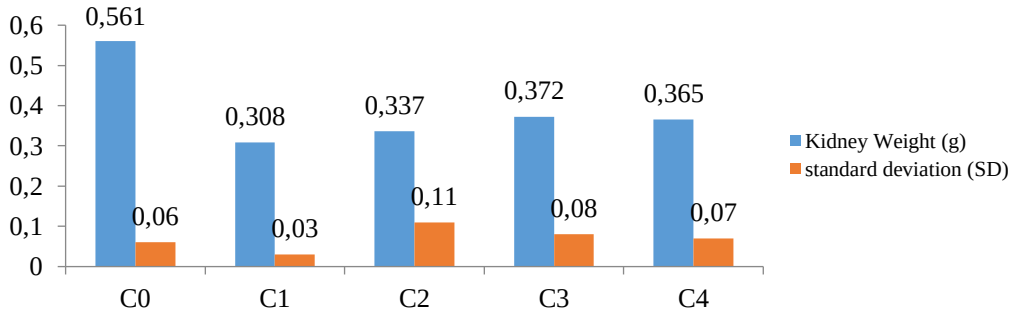

Fig. 3. Kidney weight of white rats (*Rattus norvegicus*) given high-fat diet, curcumin supplement, and turmeric powder. C0: Control, C1: high-fat diet, C2: high-fat diet + curcumin supplement, C3: high-fat diet + turmeric powder, C4: high-fat diet + simvastatin.

The addition of curcumin 1.35 mg/200gBW/head/day and turmeric powder 200 mg/200gBW/head/day had not been able to increase kidney weight significantly (Figure 3). This is triggered because this treatment's increase in body weight has not occurred significantly. Suwarta*et al*. (2021) stated that the curcumin content of turmeric could improve appetite, digestibility, increase nutrient absorption from the gastrointestinal tract and maintain beneficial microflora. The same results were shown in the addition of simvastatin (C4), which did not result in a significant increase in kidney weight. According to Elvannudin *et al*. (2016), the average kidney weight decreased, indicating that the last detoxification activity in the body was reduced.

Kidney index. The results of the ANOVA showed that the high-fat diet, curcumin supplements and turmeric powder did not affect the kidney index of white rats (*Rattus norvegicus*). Kidney index calculation is influenced by white rats' kidney weight and body weight. Lewis (2005) explained that a high-fat diet increased hepatic lipase activity. Lipase enzyme is a lipolytic enzyme synthesized by hepatocytes, increased hepatic lipase activity in white rats can result in reduced HDL levels and small HDL size so that the cholesterol degradation process is reduced, including triglyceride levels in it and accumulation in the tissues will be excessive and can cause body weight increases. This study's decrease in body weight could be due to the toxic effects of continuous high-fat feeding for 56 days. This statement is by Sireeratawong *et al*. (2010), which explains that toxicity studies show that the bodyweight of experimental animals receiving high doses will generally lose weight due to decreased appetite.

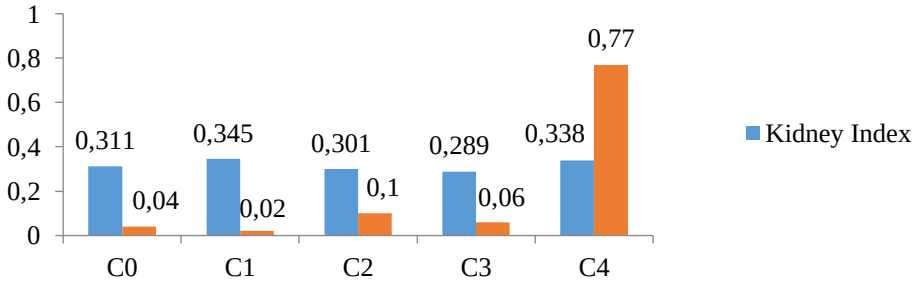

Fig. 4. Kidney index of white rats (*Rattus norvegicus*)given high-fat diet, curcumin supplement, and turmeric powder. C0: Control, C1: high-fat diet, C2: high-fat diet + curcumin supplement, C3: high-fat diet + turmeric powder, C4: high-fat diet + simvastatin.

Based on Fig. 4, the curcumin supplement and turmeric powder in this study have not affected the kidney index of white rats. The curcumin supplement and turmeric powder have not increased body weight by stimulating appetite significantly. According to Affairs and S. Canan (2017), *Curcuma longa* powder added to feed results in better nutrient absorption because the number of pathogenic bacteria decreases so that the administration of 2 g/kg *C. longa* powder is suggested to increase body weight and conversion rate because of the positive effect of microflora on the gut. The

same results were shown in the treatment with simvastatin which did not show significant results in the control or high-fat diet treatment.

Glomerular diameter. The analysis results were not homogeneous, so it needed to be analyzed non-parametrically using Kruskal Wallis, from these results showed significance on the glomerular diameter ($P < 0.05$). The provision of high-fat feed 20 g/head/day (C1) for 56 days caused glomerular diameter atrophy. This can happen because high-fat feeding results in toxic waste products of metabolism in reactive oxygen species (ROS). Purba *et al.* (2021) explained that ROS compounds would interact with cell molecules that will cause damage to the glomerulus so that the filtration organ experiences impaired function, which results in glomerular atrophy. Feeding a high-fat diets can cause the formation of ROS that trigger oxidative stress which can lead to cell death, which is one of the causes of atrophy. Glomerular atrophy is a decreased tissue size due to a decrease in the number of cells due to reduced cell numbers due to necrosis or reduced cell size which may occur due to slow circulation or oxygen deprivation tissue (Al-Tamemeemi *et al.*, 2016). Based on a case-control study in vitro, histology of the kidneys due to the administration of a high-fat diet showed significant results, there were structural changes in the kidneys, one of which was glomerular atrophy and necrosis (Altunkaynak *et al.*, 2008). Consumption of dietary fat for a long time will result in the accumulation of fat in the kidneys, induce glomerular retraction, increase inflammatory cytokines, and dysfunction in the kidneys (Muller *et al.*, 2019).

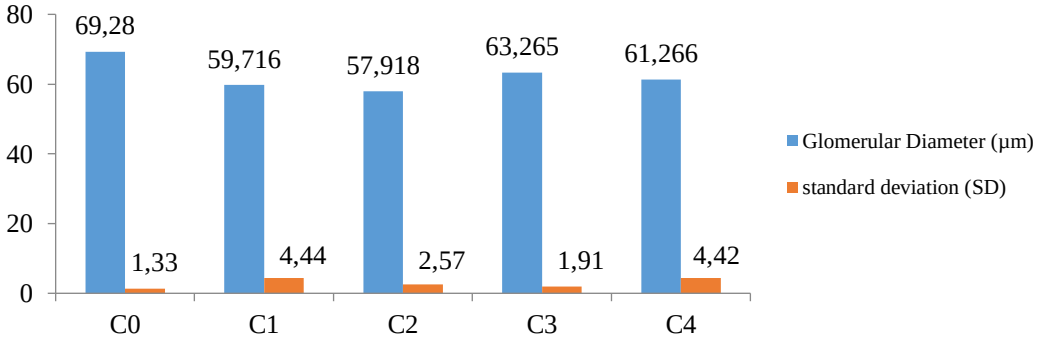

Fig. 5. the effect of high-fat diet, curcumin and turmeric powder on the diameter of the glomerulus. C0: Control; C1: high-fat diet; C2: high-fat diet + curcumin supplement; C3: high-fat diet + turmeric powder; C4: high-fat diet + simvastatin.

Based on the statistical results (Figure 5), high-fat feeding (C1) resulted in a significant decrease in diameter size. This decrease in size indicates atrophy of the glomerulus. Glomerular constituent cells experience atrophy in response to cytotoxic compounds to meet the need for damaged tissue to maintain organ integrity (Helal, 2011). Treatment of a high-fat diet and given curcumin 1.35 mg/200 gBW/head/day showed results that were not significantly different compared with the administration of high-fats (C1). This shows that the curcumin content has not been able to restore the size of the glomerulus (Figure 6). The same thing happened in the treatment of high-fat feeding and given turmeric powder as much as 200 mg/200 gBW/head/day (C3), but it has not been able to prevent glomerular damage and restore the size of the glomerulus to a normal state. This happens because the damage in the form of atrophy in the glomerulus is irreversible, so it is not easy to return to its original state even though it has been given curcumin and turmeric powder with antioxidant compounds. Nurdiniyah *et al*. (2015) explained that severely damaged glomeruli could disrupt the peritubular vascular system so that toxic substances have the potential to flow into the tubules. On the other hand, tubules that are severely damaged due to increased intraglomerular pressure can result in irreversible cell atrophy or shrinkage.

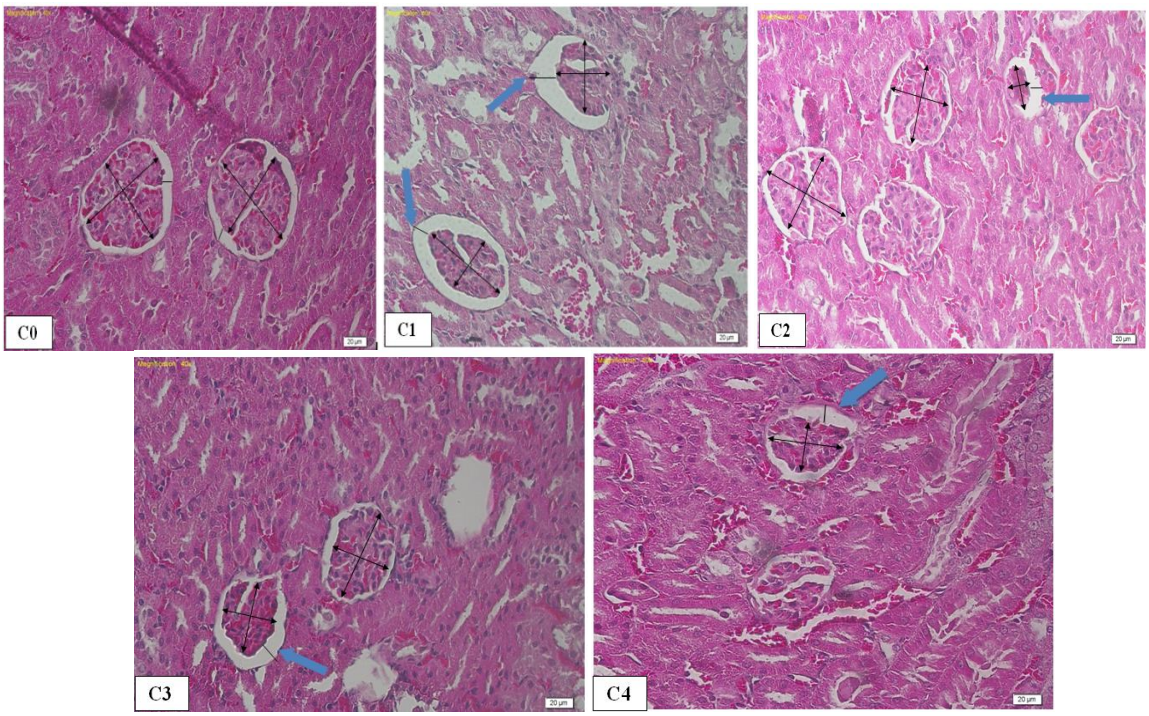

Fig. 6. Glomerular and Bowman's capsule space microanatomy of white rats (*Rattus norvegicus*). (H&E staining, M= 400x). C0: Control, C1: high-fat diet, C2: high-fat diet + curcuminsupplement, C3: high-fat diet + turmeric powder, C4: high-fat diet + simvastatin. arrow: dilation of bowman's capsule.

The feeding of high-fat + simvastatin 0.18 mg/200gBW/head/day (C4) was not significantly different when compared to the treatment that was only fed with high-fat (C2) so that in this treatment, the condition of the glomerulus was still abnormal (atrophying). A high-fat diet can highly increase HDL levels and decrease LDL levels so that simvastatin has not been able to inhibit cholesterol synthesis in the liver. The high-fat feed group experienced increased energy or fat intake, so that lipogenesis activity could increase, and the free fatty acids (FFA) formed would also increase. Mobilization of FFA from fat tissue will occur towards the liver and bind to glycerol to form triacylglycerol (TG). Consumption of foods with a high-fat content will increase triacylglycerol synthesis in the liver and increase triglyceride levels in the blood (Mayer, 2003).

Bowman's capsule. The results of the ANOVA analysis showed that the high-fat diet, curcumin supplementation and turmeric powder did not affect the size of Bowman's capsule space because the results were not significant (P > 0.05).

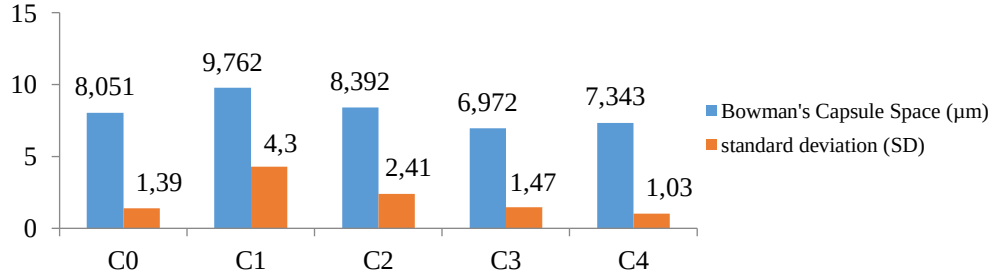

Fig. 7. Diagram of the effect of feeding high-fat, curcumin and turmeric powder on the Bowman's capsule space. C0: Control, C1: high-fat diet, C2: high-fat diet + curcumin supplement, C3: high-fat diet + turmeric powder, C4: high-fat diet + simvastatin.

Fig. 7 shows that the high-fat diet was not significantly different from the control treatment (C0), so that in the C1 treatment, the Bowman's capsule space size was still in average condition. Statistically, a high-fat diet had no effect, but some glomeruli were still dilated (Figure 6). This happens because high-fat feeding for 56 days causes reactive oxygen species (ROS), triggering

oxidative stress. The widening of Bowman's capsule space occurs due to glomerular atrophy, which is a decrease in tissue size due to necrosis or slow circulation or oxygen deprivation tissue (Al-Tameemi *et al.*, 2016). The addition of 1.35 mg/200gBW/head/day of curcumin and 200 mg/200gBW/day of turmeric powder did not affect Bowman's capsule space. However, Figure 6 shows that curcumin and turmeric powder slightly decreased compared to the treatment that was only given a high-fat diet (C1). The content of curcumin can reduce levels of NF-kB and cytokines. Inhibition of NF-Kb improved kidney injury related to inflammation, oxidative stress, and increased proinflammatory insulin in high-fat diet (HFD) rats (Costa *et al*., 2020).The same results were also shown in the addition of simvastatin 0.18 mg/200gBW/head/day, the results were not significant, so in this treatment, there was no difference between the control treatment (C0) and the high-fat diet (C1). The damage that occurred to the glomerulus did not damage to the Bowman's capsule space as a whole. Simvastatin at a dose of 5 mg/kg/day could significantly reduce LDL cholesterol levels (Jabeen*et al.*, 2015). It can maintain normal cholesterol limits even though it is given a high-fat diet.

Proximal tubules diameter. The ANOVA shows that a high-fat diet, curcumin supplementation, and turmeric powder significantly affected the proximal convoluted tubule diameter (P<0.05).

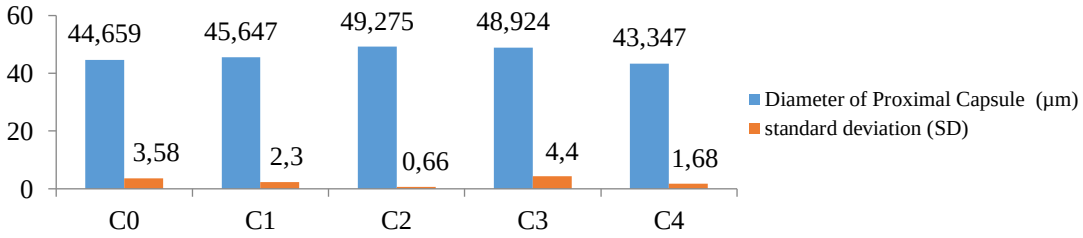

Fig 8. Effect of high-fat diet, curcumin supplementation, and turmeric powder gave the significant result to the diameter of proximal convoluted tubule. C0: Control, C1: high-fat diet, C2: high-fat diet + curcumin supplement, C3: high-fat diet + turmeric powder, C4: high-fat diet + simvastatin.

A high-fat diet did not significantly affect the diameter of the proximal tubule.Based on Figure 5, there was an increased diameter of the proximal tubule in normal limits. It is caused by a high-fat diet that led to hyperglycemia condition. According to Iskandar *et al*. (2021), a high-fat diet will cause lipogenesis of fat and cholesterol and increase blood sugar levels. Curcumin 1.35 mg/200gBW/head/day and turmeric powder 200 mg/200gBW/head/day also had no effect on high-fat feeding. However, based on the statistical results (Figure 8), the size of the diameter of the proximal tubule increased but was still within acceptable limits. The increase in the diameter of the proximal tubule is triggered by a high-fat diet resulting in hyperglycemia which increases the absorption process in the proximal tubule. Andrade *et al.* (2014) revealed that due to beta-cell dysfunction and insulin resistance, hyperglycemia occurs, which causes in the early stages of deglycation to experience nephron hypertrophy as an adaptive or compensatory process to prevent glucose loss. It is characterized in the proximal tubule has increased glucose reabsorption, and urate retention has increased.

The addition of simvastatin did not show a significant difference compared with the treatment that was only given a high-fat diet and the control treatment so that the diameter of the proximal tubule was in normal conditions. This was caused byhigh-fat feeding has not resulted in a significant increase in triglycerides and cholesterol. According to research, Kurniawati *et al.* (2021) stated that high-fat diet 30g/head/day for 56 with a feed composition of 55% bio rat, 10% margarine, 20% beef fat, and 15% coconut milk powder, cholesterol levels did not show a significant difference. This study found that the cells in the proximal tubule were necrotic (Fig. 9). Necrosis is a degeneration process that occurs after the blood supply is lost with marked cell swelling, protein denaturation and drug metabolism and body metabolism with biomolecules that make up kidney cell membranes causing necrosis (Alamsyah *et al.*, 2021).

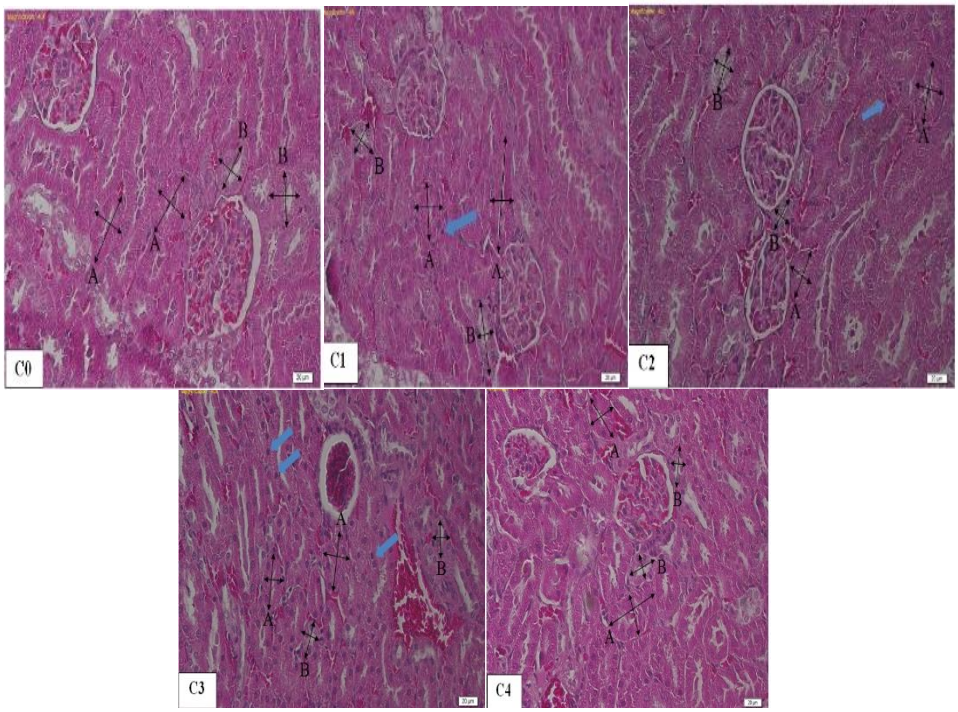

Fig. 9. Proximal convoluted tubule and distal convoluted tubulemicroanatomy of white rats (*Rattus norvegicus*). (H&E staining, M= 400x).C0: Control, C1: high-fat diet, C2: high-fat diet + curcumin supplement, C3: high-fat diet + turmeric powder, C4: high-fat diet + simvastatin. Line Arrow: necrosis cell. A: proximal tubule, B: distal tubule.

Diameter of distal tubules. The ANOVA shows that a high-fat diet, curcumin supplementation, and turmeric powder significantly affected the diameter of the distal convoluted tubule ($P<0.05$).

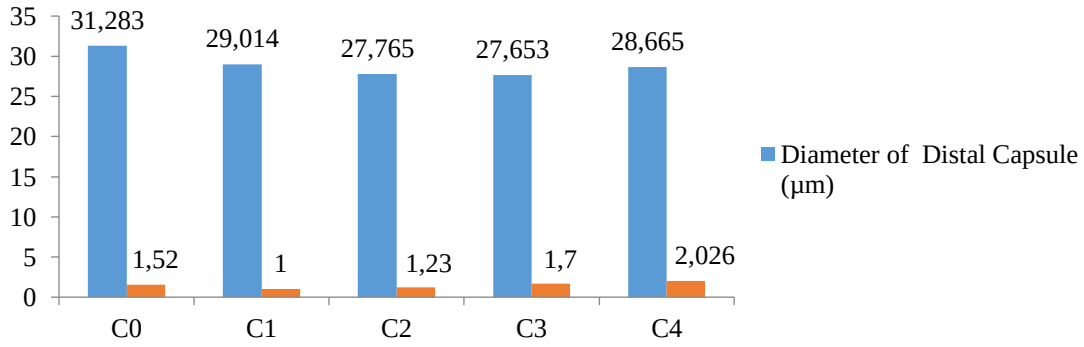

Fig. 10. Effect of high-fat diet, curcumin supplementation, and turmeric powder to the diameter of distal convoluted tubule. C0: Control, C1: high-fat diet, C2: high-fat diet + curcumin supplement, C3: high-fat diet + turmeric powder, C4: high-fat diet + simvastatin.

The high-fat diet (C1) was not significantly different from the control treatment (C0). The control treatment had a diameter of 31, 238 µm, while the high-fat treatment was 29,014 µm. This happens because the distal tubule absorbs water and NaCl so that the effect of a high-fat diet does not affect the diameter of the distal tubule. According to Hammer and McPhee (2014), the distal convoluted tubule can reabsorb water and NaCl. Reabsorption can occur through active and passive transport, using the energy of ATPase in active transport or a type of Na+/k+ pump to carry molecules while in passive transport using diffusion and osmosis. Different results showed that the addition of curcumin 1.35 mg/200gBW/head/day, turmeric powder 200 mg/200gBW/head/day showed no significant difference compared to the treatment that was only given high-fat feed. H However, the results were significantly different when compared with the control treatment. This may be due to increased inflammation in the distal tubule. According to Morgan and Liu (2011), increased levels of ROS can

be involved in the activation of nuclear factor B (NF-kB), which can increase the expression of proinflammatory cytokines. The same results were shown in simvastatin 0.18mg/200gBW/head/day, which showed significantly different results from the control treatment (C0), resulting in abnormal tubular diameter conditions. This happens because simvastatin is excreted mainly in the liver and a small part in the kidneys, so it is still possible for damage to occur due to the administration of high-fat diets and simvastatin (Adelina & Arifayu, 2018).

Cell size of proximal tubules. The analysis results show that the data is not homogeneously distributed, so it needs to be tested non-parametrically with the Kruskal Wallis test with the Mann Whitney further test. Based on the analysis results, the results are as shown in Figure 11.

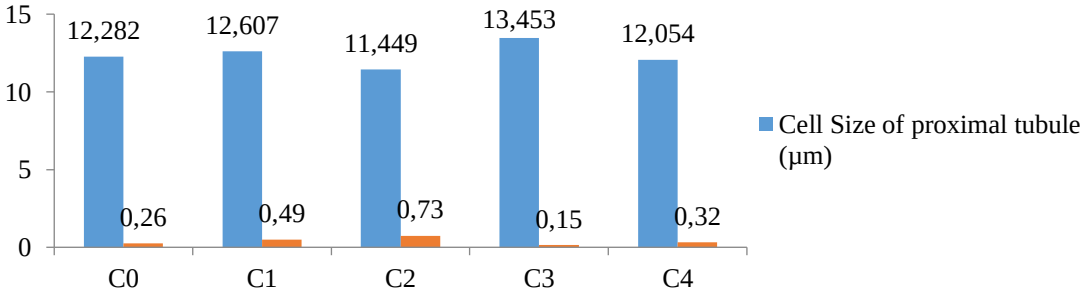

Fig. 11. Effect of high-fat diet, curcumin supplementation, and turmeric powder to cell size of proximal tubules. C0: Control, C1: high-fat diet, C2: high-fat diet + curcumin supplement, C3: high-fat diet + turmeric powder, C4: high-fat diet + simvastatin.

Based on the further test of Mann Whitey, it showed that there was no difference between the treatment of high-fat feeding, but when viewed from statistical calculations (Figure 11), the cell size increased slightly; this could be due to the inhibition of the Na+ pump which increased in cell size. Na+ is a cell volume setting (Latif, 2019). The addition of curcumin showed the same result compared to the control treatment (C0) and the high-fat feeding treatment.The absence of a significant difference could have occurred because feeding high-fats for 56 days has not resulted in fatal damage such as atherosclerosis and hyperlipidemia, so disturbances due to high-fat feeding can still be prevented with the antioxidant content present in curcumin. The addition of curcumin also showed no difference with turmeric powder. However, the addition of turmeric powder treatment of 200 mg/200gBB/head/day showed a significant increase in the size of proximal tubule cells. According to Saraswati *et al.* (2013), the turmeric powder increased lipid metabolism. An increase in fat metabolism could increase the production of Reactive Oxygen Species (ROS) in the circulation and adipose cells. Increased ROS in adipose cells can cause an imbalance of oxidation-reduction reactions so that antioxidant enzymes decrease circulation (Pillon and Christophe, 2012). Simvastatin treatment showed no significant difference, but statistically, there was a decrease in the size of the proximal tubule cells but still within normal limits. This can happen because simvastatin administration for 56 days has not significantly increased triglyceride and cholesterol levels, so it was still within normal limits in this study. Research by Heriansyah (2013) gave a high-fat diet for eight weeks triglyceride levels were still in normal condition 124 mg/dL (<150 mg/dL).

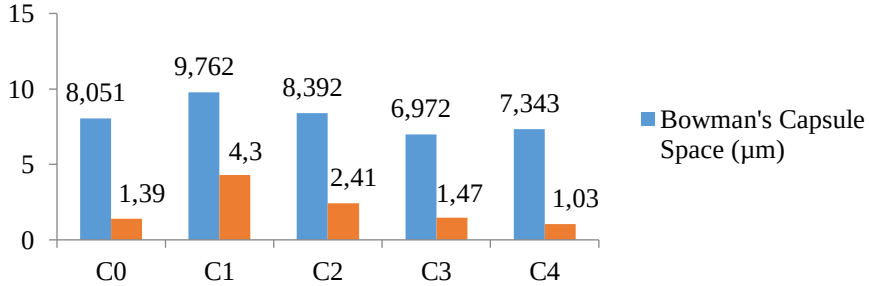

Fig. 12. Effect of high-fat diet, curcumin supplementation, and turmeric powder to cell size of distal tubules. C0: Control, C1: high-fat diet, C2: high-fat diet + curcumin supplement, C3: high-fat diet + turmeric powder, C4: high-fat diet + simvastatin. Line Arrow: necrosis cell. A: proximal tubule, B: distal tubule.

Cell size of distal convoluted tubules. The normality test results on the distal convoluted tubule cell size data showed abnormal results, so a non-parametric analysis was carried out using Kruskal Wallis. The test results showed significant results ($P < 0.05$). The results of further tests using Mann whitey showed that there was no significant difference between the treatment of high-fat diet 20g/head/day (C1) and the control treatment (C0); treatment with the addition of curcumin (1.35 mg/200gBW/head/day) (C2) with high-fat diet (C1) and control treatment (C0); the addition of simvastatin 0.18mg/200gBW/head/day with treatments that were only given a high-fat diet (C1) or with control treatments (C0) (Figure 12). This could be because the high-fat diet given to young rats for 56 days does not cause fatal damage. After all, cholesterol and triglycerides are still very much needed at the age of growth. According to Divayantiand Birkah (2021), triglycerides and phospholipids function as providers of energy for daily activities, while cholesterol plays an essential role in regulating body functions. Another result was found that the addition of turmeric powder 200 mg/200gBW/head/day experienced an increase in cell size, which was significantly different compared to the treatment given a high-fat diet and the control treatment (Figure 13). This happens because cells have swelling because the injury has not eliminated water, so water is retained in the cells (Alamsyah *et al.*, 2021). Damage in the proximal tubule can impact damage that occurs in the distal tubule because the distal tubule is the site of urine concentration (Ervina & Sukarjati, 2017).

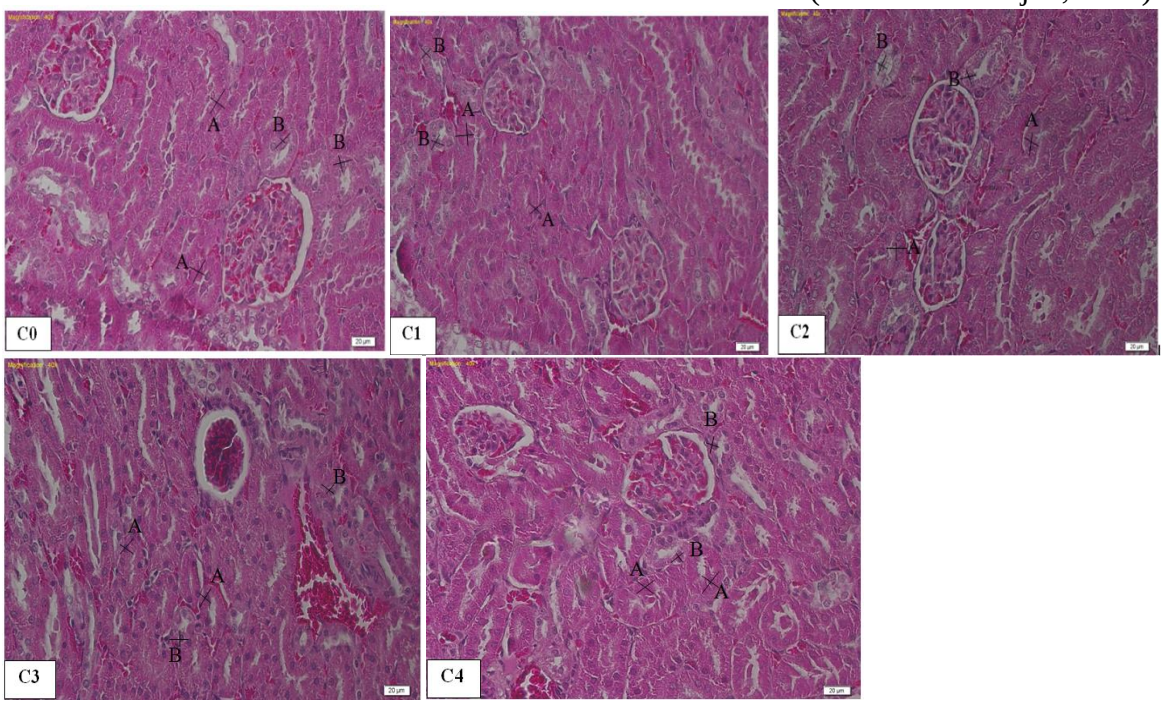

Fig. 13. Proximal convoluted tubule cellsand distalconvoluted tubule cellsmicroanatomyof white rats (Rattus norvegicus). (H&E staining, M= 400x). C0: Control, C1: high-fat diet, C2: high-fat diet + curcumin supplement, C3: high-fat diet + turmeric powder, C4: high-fat diet + simvastatin.

CONCLUSION

The effect of high-fat diet had a significant effect on kidney weight, glomerular diameter, proximal tubular diameter, distal tubular diameter, proximal tubular cell size, and distal tubular cell size. However, the results were not significant for the kidney index and the size of Bowman's capsule space. The provision of curcumin and turmeric powder supplements was able to prevent damage to the kidneys, but based on the Duncan and Mann Whitney test in this study there was no significant difference.

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