Histopathology of Pre-diabetic White Rat (*Rattus norvegicus* L.) Renal After Treatment with Turmeric Powder and Organic Quail Eggs

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Received: 18 August 2022 Accepted: 21 December 2022 Published: 31 December 2022

ABSTRACT

Pre-diabetic is a disease that is caused by insulin resistance, which is identified by higher blood glucose levels than normal. Turmeric (*Curcuma longa* L.) contains curcumin compounds that act as antioxidants to prevent damage from free radicals and to repair damaged kidney tissue from pre-diabetic condition. Organic quail eggs are supplements that can help to repair kidney tissue. This study investigated the effect of turmeric powder and organic quail eggs on pre-diabetic kidney tissue histopathology of male white rats. Twenty-five male white rats (*Rattus norvegicus* L.) were used in this study and they were divided into 5 treatment groups, namely D0 (normal white rats were given standard diet), D1 (positive control, pre-diabetic white rats were given standard diet), D2 (pre-diabetic white rats were given turmeric powder 1.35 mg/head/day), D3 (pre-diabetic white rats were given 1 organic quail egg/head/day), and D4 (pre-diabetic white rats were given turmeric powder 1.35 mg/head/day and 1 organic quail egg/head/day). This research was done within 60 days. The results of the study were analysed using the ANOVA and Duncan tests. The analysis results showed that turmeric powder and organic quail eggs treatments had a significant effect on the observed parameters. The conclusion of this study is that the effect of turmeric powder and organic quail eggs has the potential to repair the kidney tissue of pre-diabetic white rats.

Keywords: Kidney tissue, organic quail eggs, pre-diabetic, turmeric powder

INTRODUCTION

Pre-diabetic is a condition where the blood glucose level is lower than diabetes mellitus, but higher than normal value (100 – 125 mg dLG). Pre-diabetic might happen as a result of insulin resistance and later will develop to be diabetes mellitus (Saraswati *et al*., 2021). Based on International Diabetes Federation (IDF), the global estimate of diabetes prevalence in the 20 – 79 age range in 2000 was 151 million, which was close to the World Health Organization (WHO) estimate at the period (150 million). The most recent WHO estimate of 422 million diabetics (2014) was likewise nearly identical to the IDF estimate of 415 million diabetics in 2015. Since then, IDF estimates have shown worrying rises in the number of diabetics, more than triple from 2000 to the current (2021) projection of 537 million.

People with diabetes mellitus is 20 times more prone to kidney damage than people without diabetes. It has been proven by research that excess insulin exposure can result in hyperglycemia due to insulin resistance (Saraswati *et al*., 2021). Excess insulin injection can increase Reactive Oxygen Species (ROS) inside the body (Yang *et al*., 2014).

Reactive Oxygen Species (ROS) has a central role in interaction that involves oxidative stress inflammation and metabolic control (Luc *et al*., 2019). Based on research, ROS has the ability to affect the function of endotel gromerus cell and cause inflammation (Maslachah *et al*., 2008). Reactive Oxygen Species (ROS) will impact the damage of visceral gromerus cell. Cells that are damaged will influence the activation of macrophage through TLR4 (toll-like receptor 4), and hereafter will activate cytokines such as TGF-beta 1. The bonded TGF-beta 1 with renal interstitial fibroblast membrane receptor can stimulate interstitial fibrosis that affects damages on kidney (Efendi *et al*., 2016).

One of the medicinal plants that can be used to treat diabetes mellitus is turmeric (Andrie *et al*., 2014). Turmeric extract containing curcumin has an antioxidant effect that can increase the...
Curcumin is a compound from phenolic group that is obtained from turmeric. Turmeric extract also contains phenolic, flavonoid, and triterpenoid. Flavonoid, especially quercetin has been proven to have antidiabetic activity (Vessal et al., 2003). Flavonoid has beneficial effect to kidney performances as a nephroprotective compound (Rajak et al., 2016).

Flavonoid is known for its natural antioxidant agent that could bind ROS. Natural flavonoid is very useful to overcome diabetic complications that is related to oxidative stress, such as diabetic neuropathy, kidney damage, and liver damage. The effect of flavonoid as antioxidant agent on diabetes is ascribed to their ability to prevent free radicals and activating antioxidant enzyme (Chen, 2015).

Nutritional intake on pre-diabetic patient has to be balanced to avoid metabolic disturbance. Organic quail eggs can increase nutrional intake on diabetes mellitus patients. Organic quail eggs are quail eggs that are produced from quail who has been given organic feeds. The organic feeds contain cassava leaves powder and turmeric powder (Riandika et al., 2019).

Organic quail eggs contain nutrients such as protein, vitamin A, mineral, carbohydrates, and essential fatty acids that are very beneficial for the health (Basri, 2018). Essential amino acids that are contained on organic quail eggs consist of 50.36% albumen protein and 46.85% yolk protein (Genchev, 2012). Amino acids from organic quail eggs can regenerate new cells and antibody, fixing tissue structures, and balancing enzymes and hormones (Syam et al., 2015).

This research needs to be done in order to understand the histopathology of pre-diabetic male white rat renal after treatment with turmeric powder and organic quail eggs. The results of this research are expected to show that turmeric powder and organic quail eggs can fix the renal histology of pre-diabetic male white rat, and furthermore turmeric powder and organic quail eggs can be used as alternative treatment on pre-diabetic therapy. The indicator of this research can be reviewed from kidney index, daily water intake, glomerulus diameter, the width of Bowman capsule, the size of proximal convoluted tubule, and the size of distal convoluted tubule.

**MATERIALS AND METHODS**

This research was carried out for six months at Biology Animal Structure and Function Laboratorium and animal test cage at Biology Department, Faculty of Science and Mathematics, Diponegoro University. Renal histologic preparations were made at Wates Yogyakarta Veterinary Center. This research was approved by the Commision of Health Research Ethics (KEPK) of Diponegoro Medicine Faculty No.96/EC/H/FK-UNDIP/IX/2020.

**Preparation of Test Animals**

This research used 25 male white rats (*Rattus norvegicus* L.) wistar strain as test animals. Two months old male white rats with 175 – 200 g weight ranges were used in this research. The white rats were obtained from a breeder in Ngaliyan, Semarang, Central Java. White rats were acclimated on 30 x 30 x 40 cm individual cage for 7 days. The feed that was used during the acclimation was Hi-Pro-Vit A594K with a dose of 25 g/day and a drinking dose of 150 ml/day.

**Experimental Design and Procedure**

White rats were split into five treatment groups with five repetitions for each treatment. The treatment groups consist of D0 (normal white rats fed with standard feed), D1 (pre-diabetic white rats fed with standard feed), D2 (pre-diabetic white rats fed with 1.35 mg/head/day turmeric powder), D3 (pre-diabetic white rats fed with 1 organic quail egg/head/day) and D4 (pre-diabetic white rats fed with 1.35 mg/head/day turmeric powder and 1 organic quail egg/head/day). After the acclimation for seven days, groups D1, D2, D3, and D4 was given 1.80 IU kg⁻¹ weight per day of glargine insulin for 28 days. White rats were induced into pre-diabetic state with glargine insulin subcutaneously. The injection site on the subcutaneous abdominal has more adipose and less nerves. On the insulin pen, a needle was
The tiniest tip was inserted from the insulin cartridge to the pen's cartridge conserving slot. To evenly spread the insulin, the pen was spun and inverted upside down ten times. The injection button controls the insulin dose of 1.80 IU. About 1.35 mg turmeric powder dissolved in 0.2 ml was given orally with cannula tip syringe. Organic quail eggs were given orally.

Renal Histologic Preparation

Renal histology preparations were conducted with paraffin method plus Hematoxylin-Eosin staining. The time period of renal histology preparations was 30 days. Dissection steps began with anesthesia with chloroform. Anesthesia was done by putting the rats into closed containers, then a chloroform moistened cotton was given, and transferring the rats to paraffin tub was done as a final step. The whole extremities of the rats were stabbed with needles to keep the body to stay still and to position the body so it can be easily dissected. The dissection was done by slashing the lower abdomen until the upper chest. The kidney was isolated by doing necropsy and then fixated with Buffer Neutral Formalin (BNF) 10% solution to preserve the organ.

The next step was dehydration with 70%, 80% and 90% alcohol to release the water inside the tissues, continued with absolute alcohol I and absolute alcohol II with 2 hr time period in each treatment. Xylol reagent was used for the clearing process and the kidney was embedded with paraffin as the next step. The kidney embedded paraffin block was conditioned inside refrigerator (Rabiah et al., 2015). Paraffin block that contained tissues was sectioned with microtome that has five μm thickness. The ribbon of paraffin was transferred with brush into water bath that has 37 – 40 °C temperature. The ribbon of paraffin would later develop and into water again into 70%, 80%, 90% and 96% graded ethanol. The next step was dipping the tissues into xylol for 10 min and mounting was done to cover the tissues with object glass. Tissues were then dripped with entellan and covered with object glass (Zulfadhli et al., 2016).

Sample Collection

The rats were weighed with digital scale. The data of rats weight were collected for two times in a week (Arief et al., 2010). Daily water intake was measured by filling 150 ml water into the water bottle on day one. The remaining water inside the water bottles were then calculated on day two. The total daily drinking intake was calculated by subtracting the volume of water on the second day from the volume of water on the first day (Kuncarli & Djunarko, 2014). The right and left renal were weighed with digital scale (Mappa et al., 2013). Glomerulus diameter was examined with Olympus BX51 microscope and photomicrograph. The data of glomerulus diameter was calculated by summing up the widest diameter and the narrowest diameter of glomerulus and then divided by two (Wiladipta et al., 2021).

The distance of Bowman’s capsule chamber was examined with Olympus BX51 microscope and photomicrograph. The data of the distance of Bowman capsule’s chamber was collected by measuring the edge of glomerulus until the edge of Bowman capsule. The thickness of proximal convoluted tubule and distal cells were examined with Olympus BX51 microscope and photomicrograph and measured from the outer edge until the inside of tubules (Kotyk et al., 2016). Kidney index was calculated with Eq. (1)

\[ \text{Renal weight} \times 100 \]
\[ \text{Rat weight} \]  

Eq. (1)

Statistical Analysis

The data from the study were tested for homogeneity and normality using the Lavene test and then analysed using ANOVA that is
based on Completely Randomized Design (CRD) on a 95% (α = 0.05) confidence level and continued with Duncan test (Isadiyanto, 2018). Total analysis was calculated using SPSS 24 software (Tandi et al., 2017).

RESULTS AND DISCUSSION

The results of ANOVA test on kidney index and daily water intake and the results of Duncan test on glomerulus diameter, the distance of Bowman’s capsule chamber, the size of distal convoluted tubule and the size of proximal convoluted tubule, of pre-diabetic male white rats fed with turmeric powder and organic quail egg are shown in Table 1.

The treatment of turmeric powder and organic quail eggs on the kidney index of pre-diabetic white rats showed no significant difference (p>0.05), according to the result of the ANOVA test (Table 1). Based on these results, it was shown that turmeric powder and organic quail eggs fed to pre-diabetic white rats gave insignificant changes.

Based on a study conducted by Azhari et al. (2017), it is evident that the purpose of measuring organ index is to see the effect of a compound to an organ, but the organ index cannot be used as the main benchmark of organ damage. Organ histological observation need to be done to complete the analysis, in order to understand the influence of the compound to the organ. Research done by Khalishah et al. (2021) proves that the standard value of the kidney organ index is 0.7.

Table 1. The results of ANOVA test on kidney index and daily water intake and the results of Duncan test on glomerulus diameter, the distance of Bowman’s capsule chamber, the size of distal convoluted tubule and the size of proximal convoluted tubule, of pre-diabetic male white rats (Rattus norvegicus L.) fed with turmeric powder and organic quail egg

<table>
<thead>
<tr>
<th>Parameter</th>
<th>D0 (X ± SD)</th>
<th>D1 (X ± SD)</th>
<th>D2 (X ± SD)</th>
<th>D3 (X ± SD)</th>
<th>D4 (X ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney index</td>
<td>0.356 ± 0.032</td>
<td>0.335 ± 0.035</td>
<td>0.428 ± 0.147</td>
<td>0.405 ± 0.025</td>
<td>0.364 ± 0.061</td>
</tr>
<tr>
<td>Glomerulus diameter (µm)</td>
<td>76.836± 4.213</td>
<td>80.283± 1.659</td>
<td>75.646± 5.910</td>
<td>77.099± 1.934</td>
<td>69.119± 1.775</td>
</tr>
<tr>
<td>The distance of Bowman’s capsule chamber (µm)</td>
<td>9.590± 1.092</td>
<td>6.675± 1.381</td>
<td>7.311± 0.968</td>
<td>7.626± 1.234</td>
<td>12.610± 0.965</td>
</tr>
<tr>
<td>The size of proximal convoluted tubule cell (µm)</td>
<td>10.718± 0.891</td>
<td>13.647± 1.171</td>
<td>10.684± 1.675</td>
<td>11.436± 0.802</td>
<td>10.336± 1.172</td>
</tr>
<tr>
<td>The size of distal convoluted tubule cell (µm)</td>
<td>6.818± 0.435</td>
<td>7.633± 0.575</td>
<td>6.233± 0.462</td>
<td>6.873± 0.564</td>
<td>5.486± 0.165</td>
</tr>
</tbody>
</table>

Note: Numbers followed by different superscripts in the same row showed significant differences at the 95% confidence level (p<0.05). Different letter superscripts in the same column indicate significant differences between treatment groups. D0 (normal white rats fed with standard feed), D1 (pre-diabetic white rats fed with standard feed), D2 (pre-diabetic white rats fed with 1.35 mg/head/day turmeric powder), D3 (pre-diabetic white rats fed with 1 organic quail egg/head/day) and D4 (pre-diabetic white rats fed with 1.35 mg/head/day turmeric powder and 1 organic quail egg/head/day).

The results of the ANOVA test for daily water intake for all treatments (Table 1) showed no significant difference (p>0.05). Based on the research, it can be proven that the induction of curcumin and organic quail eggs in pre-diabetic white rats does not affect daily drinking water intake. Based on Table 1, the D0 and D4 groups showed a smaller average number than the other treatment groups (D1, D2, and D3). The treatment groups D1, D2, and D3 showed a decrease in the amount of daily water intake, while in the D5 group there was a decrease in the amount of daily water intake.

Pre-diabetic state can cause a high rate of filtration in the glomerular tissue to excrete excessive amounts of glucose through the urine. The condition of polyuria might dehydrates the body that results in increasing water intake. Research conducted by Nugroho (2012) proved that the condition of polydipsia (increased drinking water intake) caused by polyuria (increased amount of urine due to pre-diabetes) can be an early diagnosis of diabetes.

A study conducted by Franco-Robles et al. (2014) proved that curcumin could reduce urine production in diabetic rats. Polyuria is reversible within glycemic control. Research done by
A study by Mediani et al. (2016) showed that curcumin has the ability to lower blood glucose levels by increasing the sensitivity of cells to insulin. According to Sovia et al. (2011), curcumin can boost adipocyte differentiation stimulation and exhibit activity against PPAR-γ, which contributes to improve the sensitivity of insulin receptors.

The results of Duncan test showed a significant difference (p<0.05) in the glomerular diameter of pre-diabetic rats (Table 1). This indicates that the treatment of group D4 has significant difference (69.119 ± 1.775). According to the results of the study, dosing white rats with 1.8 IU/head/day of human insulin for 28 days will cause a pre-diabetic condition that impacts glomerular tissue’s structure. Based on the data, group D1 has the largest average diameter due to glomerular hypertrophy. The D4 group had a smaller average diameter than the D1 group (Figure 1).

Ezelin brand Glargine insulin was produced using recombinant DNA from Escherichia coli. Human insulin therapy at 1.80 IU kg⁻¹ weight per day for 14 days elevated glucose levels in male Wistar mice blood and induced insulin resistance. Insulin resistance is produced in male mice by administering 1.80 IU kg⁻¹ weight per day of human insulin for 14 days, resulting in a rise in blood glucose levels. Glargine insulin is a human insulin derivative that is soluble in neutral OH. Glargine insulin is soluble in acid after injection of glargine insulin (pH 4). In terms of the kinetics of insulin receptor attachment, glargine insulin is identical to human insulin. Glargine insulin and human insulin both have the same potential at the same dose in pharmacologist clinical studies. A diabetic mouse animal model is essential for hyperglycemic experiments (Saraswati et al., 2021).

The results of the research are caused by the ability of turmeric powder and organic quail eggs that could improve the structure of the glomerular tissue. According to studies done by Celsi et al. (1989), it was shown that the normal diameter of the glomerulus of white rats is 65.2 ± 2.4 μm. Based on research conducted by Rohman et al. (2021), it was found that the average glomerular diameter of 60-day-old rats is from 77 μm to 151 μm.

Recent research suggests that insulin may dynamically re-model the actin cytoskeleton of podocytes and influence podocyte process retraction. Insulin also increases the production of hydrogen peroxide and superoxide anion. The NAD(P)H oxidase NOX4 subunit may also be a cause of fast ROS production in insulin-stimulated cells (Piowskowska et al., 2013).

The black arrow in Figure 1 shows the glomerular cells that have necrosis. The necrosis condition is due to exposure to free radicals due to pre-diabetic conditions. This can be characterized by the loss of image from chromatin in the cell nucleus, the cell changes color to dark, solidifies, wrinkles, experiences cariorex, namely the presence of torn fragments.

Research conducted by Wibawa et al. (2019) showed that curcumin in turmeric extract might decrease blood glucose levels while improving insulin sensitivity at cell receptors. Research done by Jacob et al. (2013) has shown that curcumin can protect kidney function by inhibiting cell infiltration, inflammation, and the production of fibronectin that induces fibrosis.

According to a study conducted by Pivari et al. (2011) to promote adipocyte differentiation stimulation and to possess PPAR-γ action. PPAR-γ has a role in increasing the sensitivity of insulin receptors. These mechanisms can prove that curcumin is potential as an antidiabetic agent. Peroxisome Proliferator-Activated Receptor (PPAR) is a transcription factor that binds to the nuclear membrane of cells and has anti-inflammatory properties (Hidayat, 2015).

Research done by Saraswati and Tana (2016) has shown that quail fed with organic diet, such as turmeric powder, will produce egg yolk that is rich in β-carotene. According to Gumolung (2017), β-carotene prevents free radicals from entering the tissues when the pressure of partial oxygen is low. The reaction of free radicals with β-carotene produces a more stable compound that lacks energy to bind with other molecules. According to Lismawati et al. (2021), antioxidant substances such as β-carotene can reduce the risk of chronic diseases caused by oxidative stress, such as diabetes mellitus.
Figure 1. Histological cross-section of glomerular diameter in all treatment groups (HE staining; magnification 200 times). Description: yellow line (glomerular diameter), red arrow (glomerulus), black arrow (necrotic cells)

Duncan test of Bowman’s capsule width showed that the treatment of turmeric powder and organic quail eggs had a significant effect (p<0.05) on the width of Bowman's capsule in pre-diabetic white rats (Table 1). The results of treatment group D0 differed significantly from those of groups D1, D2, D3, and D4. These results showed that human insulin injection of 1.8 IU/head/day in male white rats resulted in pre-diabetes, which affected the wide structure of Bowman's capsule. This also showed that the presence of a protective mechanism against pre-diabetic Bowman’s capsule that is given by turmeric powder and organic quail eggs has the most effect on the D4 group. According to Septiva et al. (2019), the average width of Bowman’s capsule in normal rats ranged from 9.63 ± 1.93 to 12.96 ± 3.70.

Research conducted by Al-Malki and El-Rabey (2015) reported that in patients with diabetes, there is an abnormality in Bowman’s capsule in the form of narrowing. Research done by Fahrimal et al. (2016) showed that narrowed...
Bowman’s capsule is caused by glomerulus hypertrophy. Research conducted by Gómez and Lorido (2018) showed that exogenous insulin is degraded in the kidney, filtered at the glomerulus, and reabsorbed in the proximal convolute that will later influence the tissue structure.

Based on histological observations of the width of the bowman’s capsule presented in Figure 2, there is a narrowing in the bowman’s capsule space. The size of the normal bowman’s capsule is also obtained from the histological observations. Based on this, it can be proven that giving turmeric flour and organic quail eggs can protect the bowman’s capsule of pre-diabetic white rats. Based on Figure 2, there was a narrowing of bowman capsules in group D1 given human insulin treatment at a dose of 1.80 IU/tail/day induced in white rats for 28 days. The D2, D3, and D4 groups have a size of the width of the bowman’s capsule that is close to the control group. Based on this, it can be proven that giving turmeric flour and organic quail eggs can improve the bowman’s capsule tissue of pre-diabetic white rats.

Figure 2. Histological structure of Bowman’s capsule width in each treatment (HE staining; magnified 400 times). black arrows (normal Bowman's capsule space), yellow arrows (narrowing of Bowman's capsule space)
A study conducted by Mayori (2013) showed that the shrinking of the Bowman’s capsule chamber is caused by glomerular inflammation or by the proliferation of Bowman’s capsule epithelium. Those conditions might stimulate cell hypertrophy, which causes enlargement in glomerular cell. Glomerulonephritis is a condition that is caused by the proliferation of the glomerular cells that leads the Bowman’s capsule to bind with glomerulus.

Based on a research conducted by Nugroho et al. (2019), curcumin can be used to prevent complications in pre-diabetic patients. Curcumin can decrease blood glucose levels by increasing GLUT4, GLUT2, and GLUT3 gene expression; increasing AMP kinase activation; increasing PPAR-γ activity and ligand binding; and suppressing pre-diabetic inflammation. Based on the obtained results, organic quail eggs can improve the structure of Bowman capsule.

A study conducted by Syam et al. (2015) discovered that amino acids in organic quail eggs are effective for cells regeneration and repairing damaged tissues structure. According to Saraswati and Isdadiyanto (2018), the following composition of organic quail eggs, such as: carbohydrates (4.01 g/dl), protein (12.7 g/dl), fat (9.89 g/dl), iron (1.06 g/dl), and low cholesterol (767.77 mg/dl) can reduce the risk of tissue degeneration.

The results of Duncan test (Table 1) showed a significant (p<0.05) difference in the size of the proximal convoluted tubule cells of pre-diabetic white rats fed with turmeric powder and organic quail eggs. Based on this finding, it is possible to conclude that feeding turmeric powder and organic quail eggs has a significant effect on the size of the proximal convoluted tubule cells. The control group (D0) had significantly different results from the D1 group. Group D0 also had results that were not significantly different from groups D2, D3, and D4.

The D1 group had significantly different results from the D2, D3, and D4 groups. The results of Duncan’s test for group D2 showed no significant difference to groups D3 and D4. In the D3 group, the results were not significantly different from the D4 group. According to Figure 3, there is a proximal tubular epithelial cells thickening in group D1. Based on this finding, it can be proven that the treatment of turmeric powder and organic quail eggs improve the proximal convoluted tubule tissue of pre-diabetic white rats. Studies conducted by Singh and Farrington (2010) finds that pre-diabetic conditions affect the performance of proximal tubular cells in glucose reabsorption, resulting in proximal tubular epithelial cell hypertrophy.

Based on research conducted by Vallon (2011), the increased performance of proximal tubule in glucose reabsorption is caused by an increase of expression in mRNA that express SGLT1 and SGLT2 as protein transports. These transporters control the transport of glucose from the lumen into the tubular cells through sodium transport.

Research conducted by Schrijvers et al. (2004) showed that prediabetes and high blood sugar levels increase mRNA expression and angiotensin synthesis in proximal tubular epithelial cells. Those process is accompanied by polyol pathway activation. Polyol pathway is one of the alternative mechanisms in glucose metabolism. During normal conditions, the concentration of sorbitol in the cell is low. The amount of sorbitol in cells increase along pre-diabetic state. Sorbitol is then converted to fructose by SDH enzyme (sorbitol dehydrogenase). Sorbitol compound is degraded slowly in pre-diabetic state. As a result, the osmotic pressure inside the cell increases, causing damage to the proximal tubular cells.
Based on the results of histological observations, the treatment of turmeric flour and organic quail eggs could decrease the thickness of proximal tubule cells in pre-diabetic white rats. Curcumin is capable of activating PPAR, according to the research conducted by Malik et al. (2021). PPAR activation decreases inflammation through the NF-KB signal transduction mechanism, as well as cell death that is caused by ROS activity.

NF-KB is a transcription factor that is involved in a cell's apoptotic pathway (Ratnasari et al., 2016). Curcumin can serve as a PPAR-γ ligand, improving insulin sensitivity. Curcumin inhibits NF-KB and decreases IκBA kinase activity. Inhibiting NF-KB and IκBA suppresses proximal tubular cell inflammation. IκBA functions as a negative feedback regulator, limiting NF-KB activity (Ando et al., 2021).
The nutrients contained in organic quail eggs are able to repair proximal tubular cells. According to Gultom and Supratman (2012), nutritional intake is required for tissue growth, cell regeneration, tissue structure improvement, and enzyme structure formation. Protein is one of the required nutrients. Sudargo et al. (2021) shown that pre-diabetic patients can prevent diabetes mellitus by maintaining their nutritional intake.

Research conducted by Wahyuni and Hermawati (2017) shows that nutrition is a crucial physiological and metabolic need in healing. According to Saraswati and Tana (2016), higher nutritional needs can be provided with organic quail eggs. Carbohydrates, fatty acids, protein, beta-carotene, antioxidants, and vitamins contained in organic quail eggs are required to fulfill the nutrients that is needed by the body. Research conducted by Riandika et al. (2019) showed that the consumption of organic quail eggs had no significant effect on blood glucose levels.

The results of Duncan test analysis (Table 1) showed that the treatment of turmeric flour and organic quail eggs on the size of the distal tubule cells of pre-diabetic white rats had a significant effect (p<0.05). Based on Table 1, it can be seen that the average thickness of distal tubule cells in all treatment groups is: D0 (6.818b ± 0.435), D1 (7.633c ± 0.575), D2 (6.233b ± 0.462), D3 (6.783b ± 0.564), dan D4 (5.486a ± 0.165). The treatment group D0 showed significantly different results than groups D1 and D4. The results of group D0 were not significantly different from the treatment group D1. However, the results were significantly different from those of groups D2 and D3. The treatment group D1 showed significantly different results than D2, D3, and D4. The D2 group's results were not significantly different from the D3 group's, but showed significantly different results than D4. The D3 group showed significantly different results from the D4 group. Based on this evidence, it is possible to conclude that turmeric flour and organic quail eggs provide a preventive mechanism against pre-diabetic state in distal tubules.

Based on the results of the research, it can be proven that the injection of human insulin at 1.8 IU/head/day in male white rats affects the size of the distal tubule cells. The normal distal tubular cell size ranged from 5.080 ± 0.42 m to 6.83 ± 0.34 μm (Walton et al., 2016). When exogenous insulin at a rate of 160 mg/head injected to white rats, it caused an increase in blood glucose levels up to 300 mg/dl and changes the structure of the distal tubule with a value of 26.40 ± 0.18 (Widiastuti, 2021).

Distal tubular damage is caused by high blood glucose levels. Damage occurs in the form of inflammation on the distal tubular epithelial cells. Swelling of the distal tubular epithelium results from injury of cell nucleus that causes the tubule to be narrowed. The necrosis that occurs is caused by impaired exposure to toxic compounds that occur continuously. Cells that undergo necrosis will release various kinds of mediators that become manifestations of inflammation.

Based on the observations in Figure 4, it can be seen that there are thickening in distal tubular cells. The distal tubular epithelial cells of group D1 were thicker in size than the other groups. In groups D2, D3, and D4, the distal tubular cell thickness was close to that of the control group. In relation to this, it can be proven that there is a protective response of pre-diabetic distal tubule cells that is treated with turmeric powder and organic quail eggs.

Research conducted by Muliani (2010) proved curcumin that is contained in turmeric affects the performance of the distal tubule cells in exchanging Na+ and Cl ions outside the cells, as well as K+ ions inside the cells. Curcumin has the ability to affect the ionic balance inside a cell. Cells need to carry out active transport of Na+ and K+ ions so that the ion conditions are balanced. Excess Na+ ions will be bound to curcumin to prevent cells from swelling.

Research done by Dharmaputra et al. (2020) proved that curcumin has the ability to prevent Alum, R837, and MSU that plays a role in inflammation reaction activation by pressing down K+ efflux as a potassium pump machine. Syam et al. (2015) stated that patient that suffer from kidney damage needs adequate nutrition to help the recovery process.
Yimagou et al. (2016) stated that organic quail egg is rich in antioxidant, mineral, and vitamin that can be used as a nutrition to improve the body immune system. Research done by Teuşan et al. (2008) stated that organic quail egg consumption has synergetic effect with bioactive compound such as curcumin in order to treat pre-diabetic condition. Aba et al. (2015) also stated that egg from a quail that is fed with organic feed contains important nutrition such as amino acids (leucine, valine, and alanine), vitamin E and mineral such as zinc.

Leucine is known for its potential to decrease blood glucose level of pre-diabetic patient. Leucine is the strongest agent to increase insulin performance compared to other amino acids. Alanine is the type of amino acid that plays a vital role in balancing blood glucose level and helps to transform glucose into energy. Vitamin
E supplementation can alter insulin receptor of cell by increasing membrane motility.

CONCLUSION

The conclusion of this research is that turmeric powder and organic quail egg have the potential to fix the damaged kidney tissues of pre-diabetic male white rat (Rattus norvegicus L.). Further research is needed to understand the benchmarks of kidney tissue damage such as filtration rate, absorption volume of proximal tubule, and glomerular collagen deposition. The study of urea and creatinine content examination is also needed to detect the physiological abnormalities of kidney.

ACKNOWLEDGEMENTS

This paper is a part of the first author's thesis supervised by Dr. Tyas Rini Saraswati, M.Kes.. The author would like to present this to the supervisor for being an inspiring and supporting figure throughout the process.

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