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Effect of Chitosan-Coated *Centella asiatica* Nanoparticles on Kidney Histology Profile of Complicated Diabetic Mice

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Abstract. *Gotu kola (Centella asiatica) is a medicinal plant that has potential as an antioxidant and antidiabetic. Its compound is able to protect against diabetic nephropathy through the inhibition of oxidative stress. In terms of increasing drug potency and bioavailability of C. asiatica compounds, nanoparticle technology was used. This study aimed to determine the effect of C. asiatica nanoparticles coated with chitosan on the renal histology profile of complicated diabetic mice using a completely randomized design (CRD) with five treatment groups and five replications. The treatment groups were divided into K- (the experimental animals were not given any treatment), K+ (the experimental animals were induced by STZ), P1 (STZ + C. asiatica nanoparticles 120 mg/kgBW), P2 (STZ + C. asiatica nanoparticles 180 mg/kgBW), P3 (STZ + C. asiatica nanoparticles 240 mg/kgBW). The experimental animal model of complicated diabetes was induced by intraperitoneal STZ at a dose of 40 mg/kgBW for two days and STZ at a dose of 60 mg/kgBW for three days and then left for nine days. The C. asiatica nanoparticle therapy was given for 28 days. The parameters in this study were the results of scoring cell damage in the glomerular and tubular tissues (proximal and distal) of the kidney. The data obtained were tested for normality and homogeneity, then the normal and homogeneous data were tested with one-way ANOVA and further tested with Duncan's test. The statistical analysis results showed that C. asiatica coated with chitosan nanoparticles could reduce the damage to the histological profile of the glomerulus and tubules (proximal and distal) of the kidney of diabetic mice. C. asiatica-coated nanoparticles at a dose of 240 mg/kgBW showed the most optimal reduction in damage to the glomerular and tubular histology profiles.*

Keywords: *Centella asiatica, chitosan, diabetes nephropathy, histology, nanoparticles*

Citation

INTRODUCTION

Diabetes is a degenerative disease that poses a serious threat to global health. This disease is included in the top 10 diseases that cause death (Rana et al., 2021). Research

results from the International Diabetes Federation (IDF) showed that in 2021 were 537 million people in the world aged 20-79 years suffering from diabetes. The number of people with diabetes in Indonesia ranks fifth among the world's countries with 19.47 million sufferers (Sun et al., 2021).

Diabetes is a degenerative disease with a high concentration of sugar in the blood (hyperglycemia). This condition occurs because of the disruption of income and/or the work of the insulin hormone which is less effectively used by the body (Ugahari et al., 2016). Insulin is a hormone synthesized and secreted by cells in the islets of Langerhans in the pancreas. Insulin functions to help store glucose in the form of glycogen in the liver and muscles (Bilous & Donelly, 2015).

The development of complications in diabetes mellitus is associated with organ-specific microvascular disorders. The kidney organ as a blood filter is one of the organs that can be disrupted when chronic diabetes occurs. Complications of diabetes in the kidneys are also called diabetic nephropathy (Bilous & Donelly, 2015; Lathifah, 2017). About 20-40% of patients with type II diabetes mellitus and about 30% of patients with type I diabetes mellitus can develop complications in the kidneys (Stephens et al., 2020). Diabetic nephropathy has pathological characteristics including an increase in cell inflammation that causes degeneration of glomerular cells, proximal tubules, and distal tubules (Sancar-bas, et al. 2015). Another characteristic is characterized by the occurrence of tubular cell necrosis which can interfere with the reabsorption function (Esther & Manonmani, 2014). Changes in the glomerulus include the loss of podocyte epithelial cells with thinning of the meshwork (Alicic et al., 2017). In other studies, diabetes mellitus causes proteinuria, glomerulosclerosis, and significant reduction

of SOD1 and SOD3 in rat glomerular kidney (Setyaningsih et al., 2021).

Chronic hyperglycemia in diabetics can increase the formation of AGEs (advanced glycation end products). AGE is the result of the reaction of glucose and other glycated compounds. AGE can cause oxidative stress when interacting with its receptor (RAGE) (Bilous & Donelly, 2015). RAGE is found in many types of cells such as renal mesangial cells, glomerular podocytes, endothelial cells, and many epithelial cells. The binding of AGE-RAGE leads to the formation of reactive oxygen species (ROS) via the NADPH oxidase pathway (Shen et al., 2020). Increased ROS through activation of various cellular responses causes cell and tissue damage effects (Mahmoodnia et al., 2017). AGE-RAGE interaction on endothelial cells also causes the formation of growth factor VCAM-1 (Vascular cell adhesion molecule 1) which causes inflammatory cell adhesion and increased vascular permeability (Bilous & Donelly, 2015).

Damage to cells and tissues due to oxidative stress from the glucose autoxidation process can be prevented by a balance of antioxidants in the body. Antioxidants are compounds that ward off free radicals (Nimse & Pal, 2015). Antioxidants are distinguished between endogenous antioxidants and exogenous antioxidants. Endogenous antioxidants are compounds produced in the body as enzymatic or non-enzymatic. Exogenous antioxidants are usually obtained from outside the body through food. The content in plants that function as medicine is also a source of exogenous antioxidants (Serang & Febrianto, 2018).

Exogenous antioxidants can be taken from active compounds from natural ingredients, such as one of the gotu kola plants (*Centella asiatica*). *C. asiatica* is a medicinal plant that grows naturally in tropical climates. *C.*

asiatica belongs to the Apiaceae family. This plant comes from Asian countries including India, China, Sri Lanka, Malaysia, and Indonesia (Orhan, 2012). *C. asiatica* has antioxidant activity of 84% (Hashim et al., 2011). The antioxidant properties are obtained because *C. asiatica* contains the main active compounds in triterpenes, namely asiatic acid, asiaticoside, and madecassoside (Razali et al., 2019). Other components of *C. asiatica* include volatile oils, phytosterols, flavonoids, tannins, and senteslosides (Hebbar et al., 2019).

Previous studies have stated that *C. asiatica* has potential as an antidiabetic and has antioxidant activity (Kabir et al., 2014; Muchtaromah et al., 2021; Tulung et al., 2021). Some *C. asiatica* preparations can reduce cell necrosis in pancreatic tissue. This shows that *C. asiatica* is able to regenerate necrotic cells (Muchtaromah et al., 2013). *C. asiatica* can also repair tubular injuries, and renal fibrosis due to renal ischemia (Arfian et al., 2020). The asiatic acid in *C. asiatica* is able to reduce oxidative stress and reduce excessive NO production in high carbohydrate-induced metabolic syndrome rats (Pakdeechote et al., 2014). Ethanolic extract of *C.asiatica* (CeA) has the potential to repair the kidney in diabetes mellitus rats. Treatment CeA significantly attenuated glomerular injury, increased ACE2 mRNA expression, and elevated SOD in the kidney (Setyaningsih et al., 2021). *C. asiatica* which contains asiaticoside can reduce the proteinuria ratio and improve renal pathology in the kidneys of diabetic rats (Zhu et al., 2020). It is known that there are many uses of *C. asiatica* in diabetic kidneys, mostly in the form of extracts. It is not well understood how the effect of *C. asiatica* nanoparticles on the diabetic kidneys. The nanoparticles can optimize the solubility of active substances, improve their bioavailability, increase the stability of

active substances, increase the absorption of macromolecular compounds, reduce irritation to the gastrointestinal tract due to active substances, and improve the pharmacological and therapeutic properties of drugs (Abdassah, 2017; Fahmi, 2020). Therefore, nanosystems are very effective for use in the delivery of bioactive compounds orally (Ganesan et al., 2017). Muchtaromah et al. (2021) stated that chitosan-coated *C. asiatica* nanoparticles have better antioxidant activity compared to *C. asiatica* extract. Singh et al. (2019) explained that chitosan-alginate nanoparticles of *C. asiatica* extract made using the principle of ionic gelation provide better physical stability than without nanoparticles. With the advantages of these nanoparticles, this study aimed to determine the effect of *C. asiatica* nanoparticles coated with chitosan on the histological profile of the kidney of complicated diabetes mice.

MATERIALS AND METHODS

The material used in this study was *Centella asiatica* simplicia powder obtained from UPT Materia Medika Batu. Male mice (*Mus musculus*) of the Balb/C strain, aged 2-3 months and weighing 25- 30 grams, were taken from the Experimental Animal Development Unit, Malang City. Chitosan-coated *C. asiatica* nanoparticle produced in March 2021. The administration of *C. asiatica* nanoparticle therapy in mice and data collection was carried out from November 2021-April 2022. The research procedure has been approved by the Research Ethics Committee (KEP) of the Faculty of Science and Technology, State Islamic University of Maulana Malik Ibrahim Malang with the approval reference number: 021/EC/KEP-FST/2020.

Centella asiatica Nanoparticle

The manufacture of *C. asiatica* extract began with soaking 200 grams of smooth *C. asiatica* with 70% ethanol solvent in a ratio of 1:5. The solution was then homogenized for 24 hours, and then filtered. The maceration process was repeated. The filtrate was concentrated with a rotary evaporator at a temperature of 50°C.

The manufacture of *C. asiatica* coated with chitosan was carried out according to Pakki et al. (2016). It begins with dissolving 3 ml of 0.5% AAG in 600 ml of distilled water. Then 3 grams of commercial chitosan and 120 ml of 0.5% sTPP solution were added. The resulting mixture was homogenized at 1000 rpm for 10 minutes. 0.6 grams of *C. asiatica* extract was added to the solution and homogenized. Then 6 ml of tween 80 was added and homogenized for 90 minutes. Tween 80 was added as a stabilizer (Irianto & Mujianah, 2011). The homogeneous solution was sonicated with an amplitude of 80% and a frequency of 20 kHz, and a time span of 90 minutes. The sonicated solution was centrifuged. The resulting pellets were then put into a deep freezer. The frozen pellets were incubated for 24 hours at 50°C. The dried pellets were crushed (Pakki et al., 2016).

Animal Treatment

Male mice used as experimental animals were placed in cages, where each cage contained four mice. The mice were acclimatized for 14 days. The experimental animals were excluded if sick, died, or their body weight decreased by more than 10% during acclimatization. During acclimatization, mice were fed with BR1 pellets and drank ad libitum. After acclimatization, the mice were weighed to measure initial body weight..

The induction of complicated diabetes model mice was carried out by injecting Streptozotocin (STZ) with multiple low doses intraperitoneally following the Lukiati & Arifah (2019) procedure with modifications. Mice in the negative control (K-) were only given aquadest. For the first three days, 40 mg/kgBW was injected, and then for the next two days, 60 mg/kgBW was injected. After being injected with STZ for five days, the mice were left for nine days without any treatment. On day 14, the blood sugar levels of mice that had been fasted for 6 hours were checked. If fasting sugar levels were still below 126 mg/dL, then STZ injection was performed once with a dose of 40 mg/kg body weight. After confirming that the fasting blood sugar level of the mice was above 126 mg/dL, the mice were left until day 28 to get mice with complicated diabetes conditions.

The diabetic mice were then treated with *C. asiatica* nanoparticles for 28 days. The therapeutic solution was made by dissolving *C. asiatica* nanoparticles coated with dry chitosan with citrate buffer. The solution results were given orally to mice with three doses: the P1 group of 120 mg/kgBW, the P2 group of 180 mg/kgBW, and the P3 group 240 mg/kgBW.

Histological Preparations and Observations

On day 29, the sacrifice was performed under chloroform anesthesia. The mice were placed on a surgical board for surgery and their kidneys were taken for histological preparations. The removed kidney organs were washed with physiological NaCl (0.9%), then the organs were fixed by dipping them in 10% formalin. Histological preparations were made according to Utomo et al. (2012), using the paraffin histology sections method and HE staining (hematoxylin-eosin). The results of histology preparations were observed under a light microscope, scored and the histological

sections were documented. Histological profiles of mice (*Mus musculus*) were determined based on observations under a microscope. Scoring was carried out to determine the effect of giving *C. asiatica* nanoparticles coated with chitosan on the profile picture. Data analysis in this study was carried out using the SPSS 25 program.

RESULTS AND DISCUSSION

Histological Profile of The Kidney Glomerulus

The histological profile of the kidney glomerulus of mice (*Mus musculus*) was assessed on the level of damage based on the stages of cell necrosis, namely, nucleus pyknosis, karyorexia, karyolysis, and widening of the distance between the glomerulus and Bowman's hoop (Figure 1). Histological profile of the renal glomerulus in the K- group showed that glomerular cells did not experience cell damage, characterized by a solid round purplish cell nucleus, and the distance between the glomerulus and Bowman's cap-

sule of 21.05 μm. In contrast, in the K+ group induced by streptozotocin, the distance between the glomerulus and Bowman's capsule widened to 142.10 μm. This is because the cells in the glomerular tissue begin to disappear due to many damaged cells. In this group also seen the stage of damage to the cell nucleus in the form of pyknosis nuclei, characterized by the shape of the nucleus that is not completely round due to the shrinkage of the nucleus.

In the P1 group the distance between the glomerulus and Bowman's capsule began to decrease to 61.77 μm, here was still a stage of damage to the karyorrhexis cell nucleus which was marked by the faded cell nucleus. In the P2 group, the widening of the distance between the glomerulus and Bowman's capsule decreased, which was 56.47 μm but the damage in the form of the pyknosis and karyorrhexis cell nuclei was still found. The P3 group had improved the distance between the glomerulus and Bowman's capsule, which was 38.47 m, and the least cell damage occurred compared to the P1 and P2 groups.
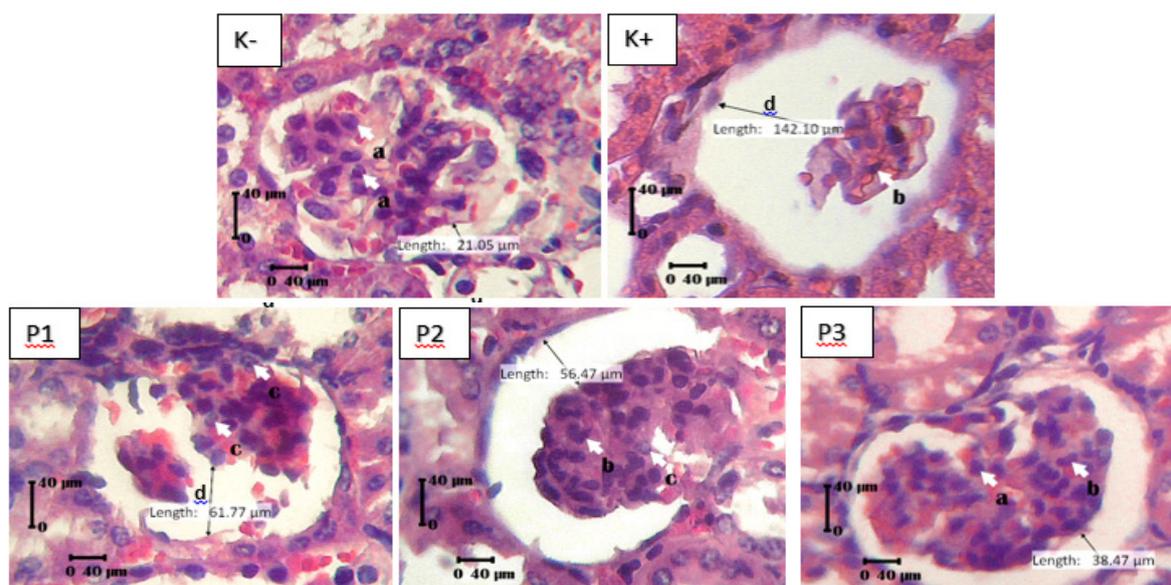

Figure 1. Histological profile of the kidney glomerulus of mice (*Mus musculus*) 400x. (a) normal cell, (b) nucleus pyknosis, (c) nucleus karyorrhexis, (d) The distance between the glomerulus and Bowman's capsule.
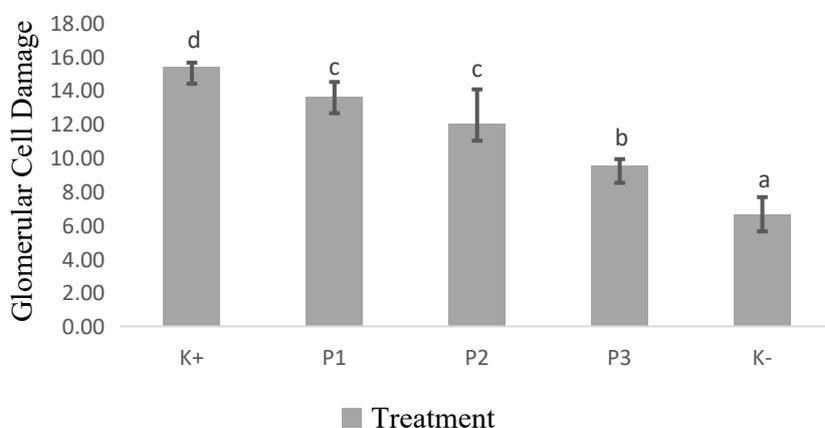
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Figure 2 The average score of glomerular cell damage

Microscopic changes of necrotic cells can be observed in the cytoplasm and parts of cell organelles. It is distinguished by three stages; pyknosis, which is characterized by nucleus shrinking, increases of clumping and the chromatin density, irregular borders, and black color; karyorrhexis, which shows the torn membrane nucleus, and the scattered chromatin material as the cell nucleus has been destroyed; and Karyolysis, the cell nucleus has been digested so that it cannot be stained and cannot be seen (Wang & Zane, 2010).

Based on Figure 2, it is shown that the average score in the K+ group is higher than the K- group. This shows the occurrence of glomerular cell damage in mice treated with diabetes complications when compared to healthy mice. This damage occurs because hyperglycemia in diabetes induces an increase in AGE production. The binding of AGE to its RAGE receptor can trigger the production of reactive oxygen species (ROS). ROS causes oxidative stress and cellular damage in the kidney (Sanajou et al., 2018).

Chronic hyperglycemia in diabetes is the most direct factor that accelerates AGE formation from glucose autoxidation pathway (the Wolff pathway), the conversion of glucose to fructose (the sorbitol pathway), and the lipid oxidation pathway (the acetol pathway) that all generate AGE precursor compound. AGE precursor compounds such as glyoxal (GO), methylglyoxal (MGO) and 3-deoxyglucosone (3-DG) bind covalently to long-lived proteins and structural components of the connective tissue matrix or basement membrane, such as collagen, to form stable AGE compounds (Song et al., 2021). AGEs accumulate in the plasma and tissues of diabetic patients (Singh et al., 2014). Previous studies showed high consumption of fructose in animal experiment can lead to the accumulation of AGEs in various types of associated tissues with peripheral insulin resistance (Aragno & Mastrocola, 2017).

AGE-mediated damage cell occurs via the main signal transduction receptor AGE. Two main types of receptors AGEs include the scavenger receptor (SR) and the RAGE receptor. RAGE is transmembrane receptors that belong to the immunoglobulin superfamily and interact with various ligands (Nowotny et al., 2015; Shen et al., 2020). RAGE is expressed on several cells, such as vascular cells, cells immune system, retinal Muller cells, podocytes kidney, glomerular mesangium, neurons, microglial cells, as well as in

many types of epithelial cells. AGE binding with RAGE leads to the formation of reactive oxygen species (ROS), activation of second messengers such as protein kinase C (PKC), NF-κB transcription factor release (Phosphorylated NF-κB enters the nucleus to copy expression proinflammatory cytokine genes associated with stress and inflammation) and formation of the growth factor VCAM-1 which causes vessel adhesion inflammation (Bilous & Donelly, 2015). Vascular inflammation in large or small blood vessels over time will interfere with the surrounding cells. Lack of oxygen due to disconnection intake of blood vessels can cause cell necrosis (Yan et al., 2008).

Activation of RAGEs also causes endoplasmic reticulum (ER) stress, producing a stress response that leads to inflammation or apoptosis. The accumulation of AGEs in the ER interferes with normal protein folding through a crossed-binding effect. AGEs can also cause cross-linking of deep mitochondrial proteins respiratory chain, reducing ATP synthesis and increasing free oxidative radical production. The path described above can lead to a vicious circle leading to intracellular damage, impaired cellular function, and finally cell death (Song et al., 2021).

The condition of hyperglycemia in diabetics is also related to the RAA (Renin-Angiotensin-Aldosterone) system thereby causing changes in hemodynamics such as hyperfiltration in the glomerulus. Novaes et al. (2020) stated that high glucose can stimulate the synthesis of Angiotensin II. Angiotensin II is a protein formed from Angiotensin I with the help of Angiotensin Converting Enzyme (ACE), Angiotensin I is formed from the protein angiotensinogen with the help of renin. Angiotensin II can stimulate vasoconstriction in arterioles glomerular efferent resulting in a decrease in the diameter of the

efferent arteriole and increases the glomerular capillary pressure which causes a higher rate of filtration inside the glomerulus. Hyperfiltration according to Chagnac et al. (2019) can increase shear stress in glomerular epithelial cells (podocytes). Mechanical pressure causes damage to the podocyte cells and limited ability of the podocytes to grow.

The P1 group showed an improvement in the glomerular profile which was significantly different from the K+ group while in the P3 group the average level of cell damage was greatly decreased. These results showed that the P3 group with a dose of 240 mg/kgBW of *Centella asiatica*-coated chitosan nanoparticles gave the best results in reducing kidney glomerular cell damage in mice (*Mus musculus*) with diabetes complications when compared to other dose groups.

In this study, it was shown that the larger the dose given, the better the histological profile of the mice's kidney glomerulus. This condition is indicated by the decrease in the observed cell damage. It is possible that the more active substance contained in *C. asiatica* causes more cells in the glomerular tissue to regenerate, so that fewer cells experience signs of necrosis. Asiatic acid is one of the main compounds in *C. asiatica* known to protect against diabetic nephropathy in rats by inhibiting oxidative stress (Chen et al., 2018). Previous studies showed that the administration of Asiatic acid can reduce ROS, AGE, and RAGE. In addition, it can reduce the interaction of AGE and RAGE so that the activation of NF-kB which triggers oxidative stress and cell damage can also be reduced (Hung et al., 2015). Docking studies show several compounds contained in *C. asiatica* such as asiaticoside, madasiic acid, and madecasis acid bind to the AGE binding site so that the compound is able to prevent AGE from directly binding to RAGE. Other compounds

on *C. asiatica* such as asiatic acid and iso-thankunic acid can bind to the binding RAGE site. The ability of the ligand to bind to the RAGE binding domain can block the ability of other ligands to bind to RAGE (Legiawati et al., 2020).

C. asiatica is known to reduce blood pressure in rats induced by intravenous Angiotensin II. The main compounds of *C. asiatica* include triterpenic acid, glycosides, asiatic acid, alkaloids and flavonoids (Mohebbati et al., 2020). Flavonoid compounds have ACE inhibitory ability resulting in decreased levels angiotensin II. ACE (Angiotensin Converting Enzyme) is a converting enzyme angiotensin I to angiotensin II (Widiasari, 2018). If the ACE enzyme is inhibited then the formation of angiotensin II can be suppressed, so that the hemodynamic rate on decreased glomerulus. This is reinforced by the research of Maneesai et al. (2016) which stated that asiatic acid compounds also have the ability to improve hemodynamics by reducing renin-angiotensin activity. Repair of hemodynamics in the glomerulus can reduce cell damage.

Nanoparticle technology in *C. asiatica* extract coated with chitosan has been tested to have higher antioxidant activity than *C. asiatica* extract (Muchtaromah et al., 2021). The higher antioxidant activity of *C. asiatica* nanoparticles might be due to the effect of the smaller particle size causing the larger surface area of a particle. The wider particle surface causes more contact between the *C. asiatica* compound particles and free radicals. Thus, an increase in the antioxidant activity of *C. asiatica* compounds causes an increase in the repair of damaged glomerular cells due to oxidative stress that occurs in complicated diabetic hyperglycemia conditions.

Histological Profile of The Renal Tubules

The histological profile of the renal tubules was assessed for the level of damage based on the stages of cell necrosis, namely, nuclear pyknosis, karyorrhexis, karyolysis, and widening of the distance between the tubules.
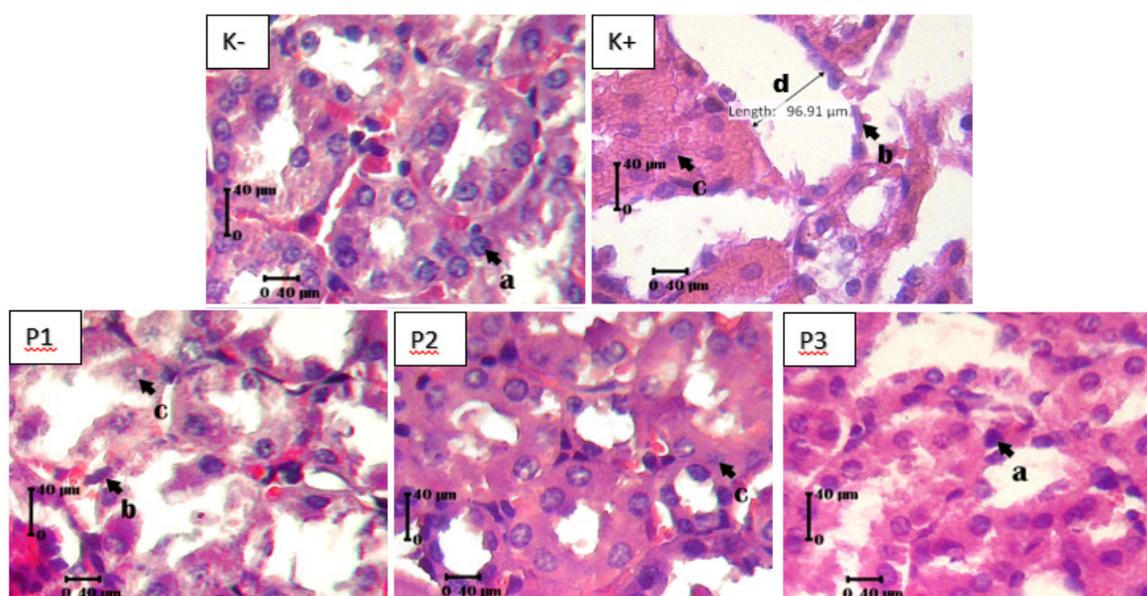

Figure 3. Histological profile of the renal tubules of mice (*Mus musculus*) 400x. (a) normal cell, (b) nucleus pyknosis, (c) nucleus karyorrhexis, (d) The distance between the glomerulus and Bowman's capsule

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The histological profile of the renal tubules is presented in Figure 3. The K- group showed a normal tubular cell profile with intact round nuclei, good tissue structure, and no widening of the distance between the tubules. In contrast, the histological profile of the tubular tissue in the K+ group showed a widening of the distance between the tubules of 96.91 µm. This was caused by the large number of cell damage, and several stages of cell necrosis were found, namely pyknosis and karyorrhexis nuclei. The P1 group showed cell damage in the form of pyknosis and karyorrhexis nuclei as well as some incomplete cell tissue, but in this group there was no widening of the distance between the tubules. The histological profile in the P2 group of tissues was getting better because the tissue looked intact and there was no widening of the distance between the tubules. However, there was still a stage of damage to the cell nucleus in the form of karyorrhexis nuclei. For the P3 group, the histological profile showed that there had been cell repair and no widening of the distance between the tubules.
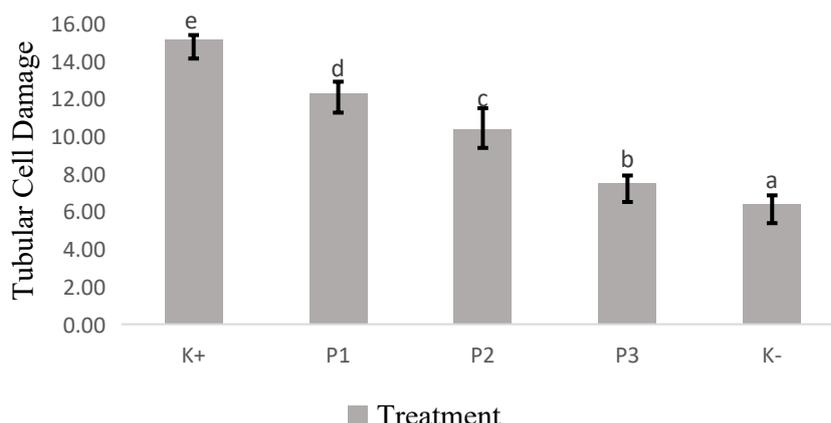

Figure 4. The average score of tubular cell damage

Based on Figure 4, the score in the K+ group is significantly different from the K- group. This shows that in the treatment of complicated diabetic mice, tubular cell damage occurs when compared to healthy mice. This condition is caused by hyperglycemia which triggers free radicals and oxidative stress in tubular epithelial cells. The proximal and distal tubules are sites of glucose reabsorption and other minerals, making the cells susceptible to damage from hyperglycemia. High glucose levels can induce cell damage through the activation of Aldose Reductase (AR) causing the formation of AGEs through the polyol pathway (Barrera-chimal & Jaisser, 2020).

The tubular profile improved in the P1 group In the P3 group, the average level of tubular cell damage was significantly decreased compared to the P1 and P2 groups, although it was still significantly different from the K- group. These results showed that the P3 group with a dose of 240 mg/kgBW of *C. asiatica*-coated with chitosan gave the best results in reducing kidney tubular cell damage in mice (*M. musculus*) with diabetes complications when compared to other dose groups. The test results also showed that the higher the dose of *C. asiatica*-coated chitosan nanoparticles used, the lower the level of cell damage in the kidney tubular tissue of complicated diabetic mice.

Cell repair occurs due to the antioxi-

dant properties of *C. asiatica*. *C. asiatica* has the main pentacyclic triterpene compound, namely Asiatic acid. Asiatic acid has strong antioxidant activity that can inhibit or slow down the formation of glycation end products (AGE) (Lv et al., 2018). AGEs are produced by changes in the structure of proteins, lipids, or DNA caused by high glucose levels. AGE accumulation in the kidney was positively correlated with the development of diabetic kidney disease. So the inhibition of AGE is an essential factor in repairing kidney cell damage due to diabetes complications (Barrera-chimal & Jaisser, 2020).

According to Yin (2015), pentacyclic triterpenes can reduce AGE levels in the kidneys by inhibiting the expression activity of the enzymes aldose reductase (AR) and sorbitol dehydrogenase (SDH). AR and SDH play a role in the formation of AGEs through the polyol pathway. Previous studies showed that Asiatic acid in *C. asiatica* is also able to repair kidney injuries induced by cisplatin, it is associated with anti-inflammatory action and improves kidney function by reducing the number of cell apoptosis. Asiatic acid inhibits mRNA expression of proinflammatory cytokines including TNF-α and IL-1 β as well as suppressing NF-κB activation, which can reduce cell damage (Yang et al., 2018). *C. asiatica*-coated chitosan nanoparticle also contains flavonoids such as quercetin, kaempferol, luteolin and apigenin (Muchtaromah et al., 2021). Flavonoids reduce cell damage through modulating cytokines transcription factors and molecules adhesion involved in inflammation, by inhibiting phosphorylation and degradation of IκB, which acts as an inhibitor of NF-κB as inflammatory transcription factors that induce the production of pro-inflammatory cytokines (Chen et al., 2017).

Diabetes causes the accumulation of AGEs in the renal tubules, so the drug delivery system with a specific target organ is beneficial in optimizing the use of the compounds contained in the drug. It is known according to Chen et al. (2020) and Hauser et al. (2021), nanoparticle technology with the use of chitosan macromolecules as a carrier showed the accumulation and release of drugs specifically in the kidney. it has a high affinity for the proximal kidney tubule so the chitosan-coated *C. asiatica* nanoparticle technology is expected to optimize the delivery of the compounds contained in *C. asiatica* to inhibit free radicals contained in kidney complications of diabetes.

CONCLUSION

Centella asiatica nanoparticles coated with chitosan can be used as a treatment to treat diabetes complications in the kidneys by reducing the damage to the histological profile of the glomerulus and kidney tubules of complicated diabetic mice (*M. musculus*). This study showed that the dose of *C. asiatica* nanoparticles coated with chitosan 240 mg/kgBW gave the most optimal reduction in cell damage compared to other doses.

AUTHOR CONTRIBUTION

B.M, E.B.M, W.E.P. designed and supervised all of the research; A.M.K.F., P.D.F., M.R.D. wrote the manuscript and analyzed the data; A.M.K.F and M.R. collected samples and analyzed in the laboratorium.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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