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Porang (*Amorphophallus muelleri*) Biscuits Fortified with Moringa (*Moringa oleifera, Lamk*) Leaf Flour on decrease Inflammation in kidney of Obese Wistar Rats (*Rattus norvegicus*)

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ABSTRACT

Introduction: The high incidence of obesity is due to the habit of eating snacks. The habit of eating snacks can be replaced with healthy snacks, one of which is porang biscuits fortified with moringa flour. This study was conducted to determine the effect of intake of porang biscuits fortified with moringa flour on glomerular area and interstitial inflammation of the kidneys of Wistar rats modeled obesity.
Methods: Laboratory experimental research with randomized postest control group design. 30 Wistar male rats were divided into 5 groups. The negative control group (KN) was given standard feed, the positive control group (KP) was given high-fat high-fructose feed, the treatment groups (P1, P2, and P3) were given porang biscuits fortified with moringa flour with varying doses of moringa leaf powder 150, 250, and 350 mg/200grBB after induction. Glomerular area was measured with raster image. The results of glomerular area and interstitial inflammation score were analyzed by oneway ANOVA and post hoc Tukey HSD. Correlation of glomerular area and interstitial inflammation score with increasing dose were analyzed by Pearson.
Results: There was no significant difference in glomerular area between all groups and no correlation between glomerular area and increasing dose of moringa powder. There were significant differences in interstitial inflammation scores between KN-KP, KN-P1, KN-P2, KN-P3, KP-P3, P1-P3 and P2-KP. The correlation between interstitial inflammation and increasing dose of moringa leaf powder was p=0.000 and r=-0.776
Conclusion: There is a difference in the interstitial inflammation score of the kidneys of obese Wistar rats after intake of porang biscuits fortified with Moringa leaf powder at a dose of 350 mg/200 grBB while there is no difference in glomerular area. There is a strong negative correlation between increasing doses of moringa leaf powder and interstitial inflammation.

Keywords : porang; moringa; biscuit; obesity; kidney

INTRODUCTION

Sedentary habits, reduced physical activity, and snacking habits can cause the incidence of obesity to increase[1]. Obesity increases the risk of several diseases, such as diabetes mellitus, cardiovascular disease, chronic kidney disease, etc[2].

The habit of eating snacks in obese people can be replaced with healthy snacks, one of which is porang biscuits. Porang biscuits are healthy biscuits that have lower carbohydrate levels and high fiber[3]. However, the nutritional content of these biscuits does not have enough nutrients so they are enriched or fortified with the content contained in Moringa leaf powder.

Obesity can increase inflammation and oxidative stress. Prolonged inflammation and oxidative stress can cause damage to kidney tissue and lead to chronic kidney disease. Chronic kidney disease is kidney disease due to structural or functional abnormalities of the kidney[4]. These structural abnormalities include changes in renal histology to histopathology such as glomerulomegaly, glomerucosclerosis, renal tubular hypertrophy and interstitial inflammation[5].

Damage due to increased oxidative stress can be prevented with food ingredients that have antioxidant properties, one of which is moringa. Moringa has high antioxidant properties with active compounds such as polyphenols, flavonoids, carotene, and ascorbic acid[6]**.** Moringa also has anti-obesity properties, namely by modifying the lipid profile with regard to reducing levels of total cholesterol, triglycerides, low-density lipoprotein, very low-density lipoproteins, and increased levels of high-density lipoproteins. The compounds in this role are phenolics and flavonoids[7]. In addition to moringa, porang also has anti-obesity content, namely glucomannan

Glucomannan in porang plant has a role in weight loss. Weight loss is related to the properties of glucomannan that can form a gel that blocks the interaction between food and the digestive tract wall. In addition, glucomannan also has the property of increasing viscosity, thus reducing food absorption[8]. Glucomannan when mixed with water will expand so that it is absorbed by the body longer, resulting in a longer feeling of fullness[9]. Research by Saputri et al[10] on obese adults showed that glucomannan can reduce body weight, BMI, and body fat.

Previous research conducted on obese Wistar rats treated with porang biscuits fortified with moringa flour showed that the biscuits can reduce the levels of low density lipoprotein (LDL), triglycerides, and the Lee obesity index[11]. However, research on the effect of porang biscuits

biscuits fortified with moringa flour on glomerular area and interstitial inflammation in the kidneys has not been widely done.

Therefore, this study was conducted to determine the effect of intake of porang biscuits fortified with moringa flour on glomerular area and interstitial inflammation of the kidneys of obese Wistar rats.

METHOD

This study used a laboratory experimental method with a randomized postest control group design and was conducted at the Anatomical Pathology Laboratory, Faculty of Medicine, Sebelas Maret University. The inclusion criteria for this study were male wistar strain rats aged 2 months with a body weight of 150-200 while the exclusion criteria consisted of rats that experienced signs such as: hair loss and dullness, drastic weight loss, sunken eyes and abnormal secretions, asymmetrical eyelids, soft and liquid feces, decreased appetite, limp appearance, and death before the study was completed.

30 Wistar male rats were taken by purposive sampling and divided into 5 groups by simple random sampling, namely the negative control group (KN) given standard feed in the form of BR-2 pellets and distilled water for 56 days, the positive control group (KP) given standard feed for 56 days and high fat high fructose (duck egg yolk 1ml/100grBB, beef fat 1 ml/100grBB, oxidized oil 1 ml/100grBB, fructose 66%) for 28 days without being given porang biscuits fortified with moringa leaf powder, treatment group 1 (P1) was given standard feed for 56 days and high-fat high-fructose feed for 28 days then continued with the feeding of porang biscuits fortified with moringa leaf powder at a dose of 150 mg/200grBB for 28 days from day 29 to day 56. treatment group 2 (P2) was given standard feed for 56 days and high fat high fructose feed for 28 days then continued with the feeding of porang biscuits fortified with moringa leaf powder dosage of moringa leaf powder 250mg/200grBB for 28 days from day 29 to day 56. Treatment group 3 (P3) was given standard feed for 56 days and high fat high fructose feed for 28 days then continued with the feeding of porang biscuits fortified with moringa leaf powder dosage of moringa leaf powder 350 mg/200grBB for 28 days from day 29 to day 56.

Moringa leaf powder fortified porang biscuits are made from porang tuber flour which is removed from oxalic acid and then fortified with moringa leaf powder. Moringa leaf powder is made by drying moringa leaves then blending and sieving.

Glomerular area in Hematoxylin-Eosin stain (H&E stain) microscopic preparation of kidney tissue was measured using image raster and then calculated from the average of nine field of view with 400x magnification.

Interstitial inflammation was observed in nine field of view with 100x magnification and then counted based on the number of mononuclear cells using a scoring that has been modified from the scoring system for inflammatory cell infiltration in white rat incision wound healing by Gunawan *et al*[12]. The scoring used can be seen in table 1.

Table 1. Interstitial Inflammation Scoring

The Number of Cells	Score
≤ 3	0
4 - 10	1
11 - 20	2
21 - 30	3
31 - 40	4
≥ 41	5

Glomerular area and interstitial inflammation score were analyzed by oneway ANOVA and posthoc Tukey HSD. Correlation between glomerular area and interstitial inflammation score with variation of Moringa leaf powder dosage was analyzed using Pearson's test.

RESULT

Glomerular Area

The average glomerular area in KN was 5045.60 µm2 ± 817.35, KP was 5517.64 µm2 ± 903.60, P1 was 4953.14 µm2 ± 677.53, P2 was 4554.77 µm2 ± 892.60, and P3 was 4679.82 µm2 ± 841.52. The results of the average glomerular area can be seen in Figure 1.

The results of the oneway ANOVA test of glomerular area showed a p value of 0.328. Tukey HSD post hoc test results showed p>0.05 between all groups. The histopathological picture of glomerular area can be seen in Figure 2.

The results of the Pearson correlation test between variations in the dose of Moringa leaf powder and glomerular area showed a p value = 0.053 and a r value = -0.400.

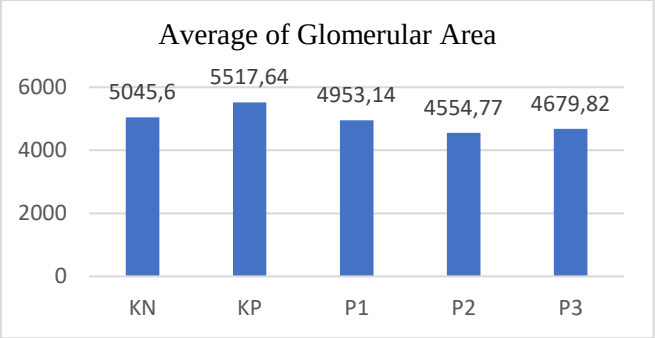
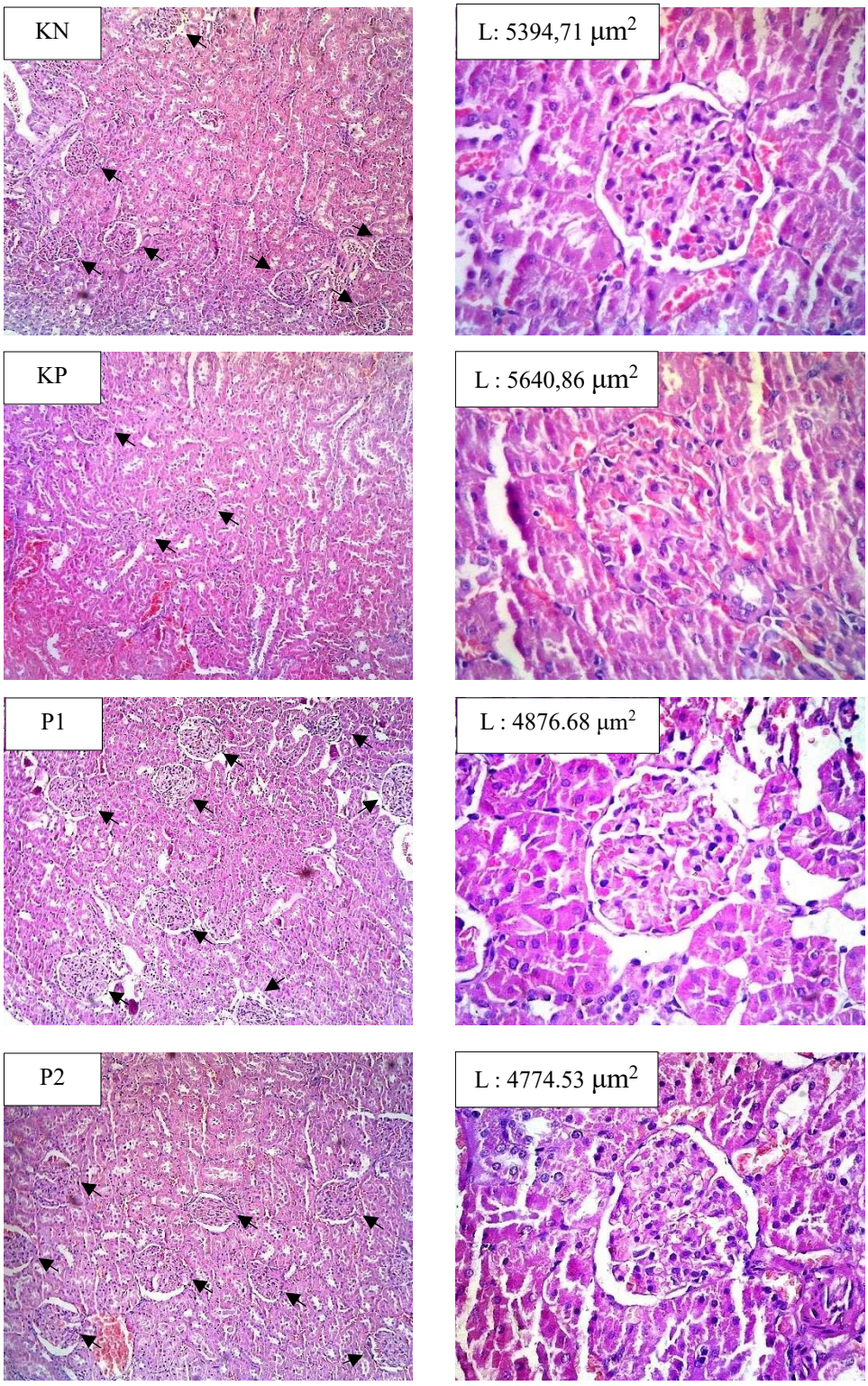


Figure 1. Bar Diagram of Average Glomerular Area in the KN, KP, P1, P2, and P3


KN
L: 5394,71 µm2
KP
L : 5640,86 µm2
P1
L : 4876.68 µm2
P2
L : 4774.53 µm2

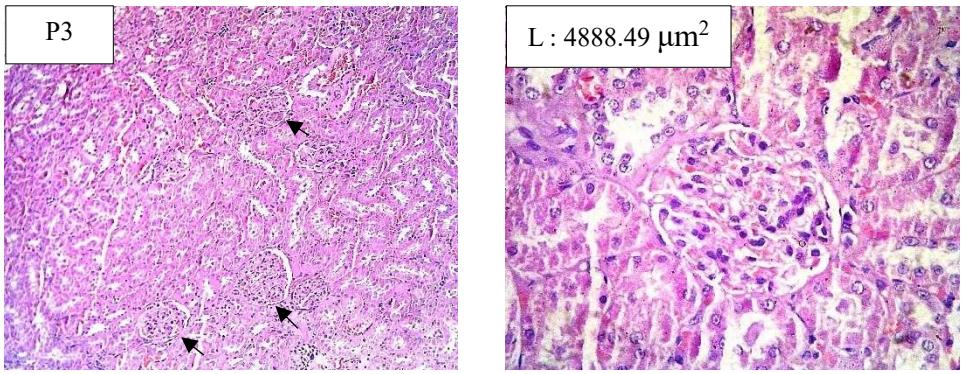


Figure 2. Figure of Histopathologic of Wistar Rats Kidney. Arrows (➘): glomerular. KN: negative control group. KP: positive control group. P1: treatment group 1. P2: treatment group 2. P3: treatment group 3. L: glomerular area (HE, 100x and 400x).

Interstitial Inflammation

The average score of interstitial inflammation in KN was 0.11 ± 0.983, KP was 1.76 ± 0.612, P1 was 1.44 ± 0.538, P2 was 1.35 ± 0.555, and P3 was 0.78 ± 0.538The results of the average interstitial inflammation score can be seen in Figure 3.

The results of the oneway ANOVA test showed a p value =0.000. Tukey HSD post hoc test results showed a value of <0.05 in the KN-KP, KN-P1, KN-P2, KN-P3, KP-P3, P1-P3, and P2-P3 groups. The histopathological picture of interstitial inflammation of kidney tissue can be seen in Figure 4. The results of the Pearson correlation test between variations in the dose of moringa leaf powder and interstitial inflammation showed a p value = 0.000 and an r value = -0.776.

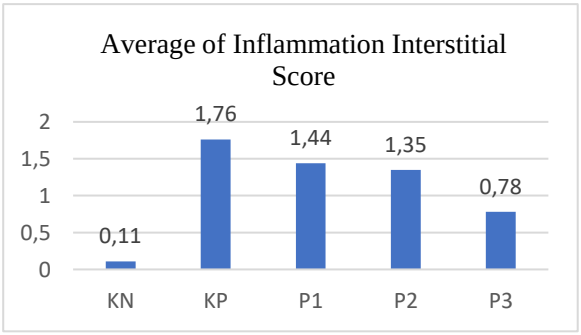
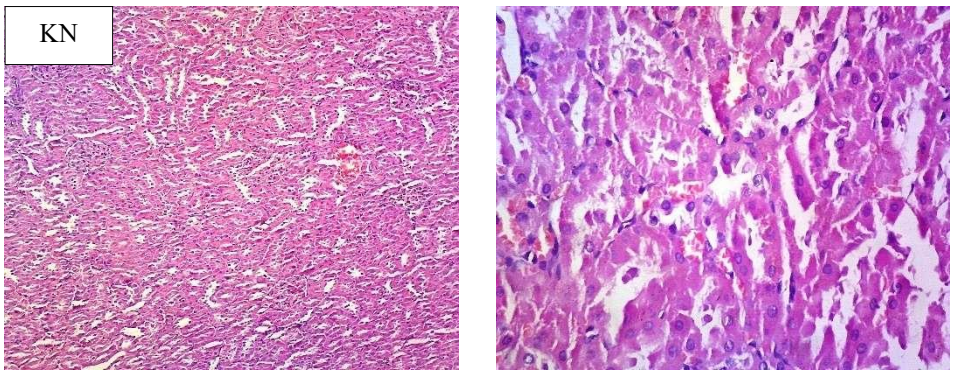


Figure 3. Bar Diagram of Average Inflammation Interstitial Score in the KN, KP, P1, P2, and P3



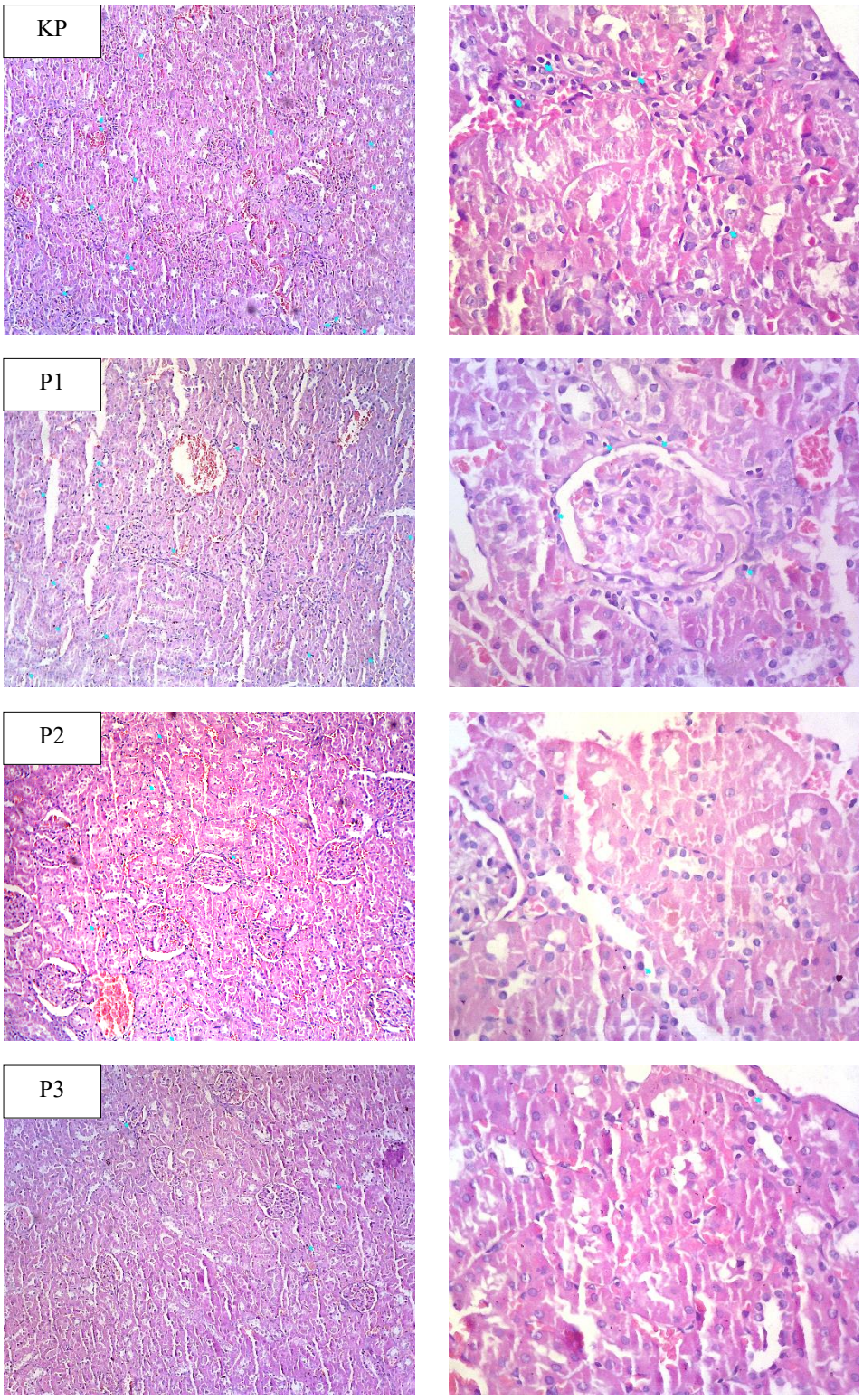


Figure 4. Figure of Interstitial Inflammation of Wistar Rats Kidney. Blue arrows (➘): interstitial inflammation. KN: negative control group. KP: positive control group. P1: treatment group 1. P2: treatment group 2. P3: treatment group 3. (HE, 100x and 400x).

DISCUSSION

Glomerular Area

Obesity can cause chronic inflammation. Prolonged inflammation can cause structural changes in the kidney in the form of histopathological changes. Histopathology that occurs is glomerulomegaly and inflammation in the tubulo-interstitial[5].

In this study, the results of K2 glomerular area increased when compared to the K1 group. The increase occurred due to fat accumulation. Fat that accumulates can cause RAAS to be induced which, if the induction is excessive, can cause the kidneys to work harder in reabsorbing sodium so that glomerulomegaly occurs[5]. However, the increase is not considered significant because the main organ responsible for lipid metabolism is the liver. The kidney is not considered an important organ in lipid metabolism as glomerular filtration prevents large molecules such as lipoproteins from reaching the proximal tubules[13]. In addition, the high-fat diet in this study was carried out with limited time. Renal histology abnormalities such as bowman's space narrowing, glomerular and tubular injury occur during high fat induction for 8-10 weeks. These abnormalities become worse if the high-fat diet is prolonged for >20 weeks which can cause renal injury in the form of hypertrophy, glomerulosclerosis, and renal fibrosis[14]**.**

The results of glomerular area in K3, K4, and K5 decreased when compared to the K2 group. K2-K3, K2-K4, and K2-K5 but the decrease did not show a significant difference. Increasing the dose of moringa leaf powder also showed no correlation with glomerular area. Glomerular area in this study is at the level of tissue structure. The impact of obesity and the feeding of porang biscuits fortified with moringa flour will be more visible when viewed at the DNA, protein, and cellular levels. In pathology research, there are several parameters that can be assessed starting from the organ level to the DNA level. These parameters are assessed macroscopically, then continued by looking under a microscope with HE or IHC painting, then using in situ hybridization and finally using PCR[15].

Interstitial Inflammation

Obesity can cause fat accumulation so that adipocytes will hypertrophy. Hypertrophied adipocytes can cause hypoxia resulting in cell death. The dead cells will then be lysed. The lysed adipocytes will release free fatty acid (FFA). Excessive FFA will go to the liver through portal vein drainage which causes the release of pro-inflammatory cytokines resulting in an inflammatory process[16]. In addition to removing FFA, lipolysis increases adipokines and decreases adiponectin. Decreased adiponectin can reduce nitric oxide in the blood vessel wall and increase leukocyte adhesion, causing inflammation[17].

In this study, the interstitial inflammation score in K2 increased when compared to K1. This result is consistent with a study conducted by Salim et al[18] which showed that obesity can increase inflammatory cells in the kidney tissue.

Significant differences in the results of interstitial inflammation occurred in the K2-K5 group, which decreased the inflammatory score from 1.76 to 0.78. This indicates that the optimum level to reduce interstitial inflammation score is 350 mg/200grBB. Increasing the dose of Moringa leaf powder correlated negatively and strongly with interstitial inflammation scores.

The decrease in interstitial inflammation scores occurs through several therapeutic mechanisms from moringa plants, namely antioxidant and anti-inflammatory effects. Active substances in moringa leaves that act as antioxidants include tannins, saponins, and flavonoids that neutralize free radicals and prevent the breakdown of hydroperoxides into free radicals. While the active substances that act as anti-inflammatory include quercetin and isothiocyanate which play a role in reducing the inflammatory process by inhibiting the action of NF-kβ[19].

Porang plants contain glucomannan and calcium oxalate. Calcium oxalate is a compound in the form of needle-shaped crystals, which when consumed will cause itching and heat. High levels of calcium oxalate in the body can erode the lining of the gastrointestinal tract and kidney tubules[20]. Therefore, the calcium oxalate contained in porang tubers must be removed by washing with water or boiling continuously[21].

In this study, the picture of histological abnormalities in the kidneys due to obesity was not too severe, so the researcher gave advice for future researchers to conduct further research with a longer duration of obesity induction to see worse kidney histology abnormalities.

CONCLUSION

Porang biscuits fortified with Moringa leaf powder at a dose of 350 mg/200grBB made a difference in the interstitial inflammation score of the kidneys of obese Wistar rats, while the glomerular area did not make a difference.

There is a negative and strong correlation between increasing the dose of moringa powder and interstitial inflammation.

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REFERENCES

1. Kiess W, Kirstein AS, Stein R, Vogel M. Obesity After the Covid-19 Pandemic and Beyond. 2022;35(2):135–8. https://doi.org/10.1515/jpem-2022-2135
2. Chooi YC, Ding C, Magkos F. The Epidemiology of Obesity. Metabolism. 2019;92:6–10. https://doi.org/10.1016/j.metabol.2018.09.005
3. Mahirdini S, Afifah DN. Pengaruh substitusi tepung terigu dengan tepung porang (amorphophallus oncopphyllus) terhadap kadar protein, serat pangan, lemak, dan tingkat penerimaan biskuit. J Gizi Indones. 2016;5(1):42–9. https://doi.org/10.14710/jgi.5.1.42-49
4. Lakkis JI, Weir MR. Obesity and Kidney Disease. Prog Cardiovasc Dis. 2018;61(2):157–67. https://doi.org/10.1016/j.pcad.2018.07.005
5. Wang M, Wang Z, Chen Y, Dong Y. Kidney Damage Caused by Obesity and Its Feasible Treatment Drugs. Int J Mol Sci. 2022;23(2). https://doi.org/10.3390/ijms23020747
6. Tjong A, Assa YA, Purwanto DS. Kandungan Antioksidan Pada Daun Kelor (Moringa Oleifera) dan Potensi Sebagai Penurun Kadar Kolesterol Darah. J e-Biomedik.
7. Ali Redha A, Perna S, Riva A, Petrangolini G, Peroni G, Nichetti M, et al. Novel Insights on Anti-Obesity Potential of the Miracle Tree, Moringa oleifera: A Systematic Review. J Funct Foods. 2021;84. https://doi.org/10.1016/j.jff.2021.104600
8. Nissa C, Madjid IJ. Potensi Glukomanan pada Tepung Porang sebagai Agen Anti-obesitas pada Tikus dengan Induksi Diet Tinggi Lemak. J Gizi Klin Indones. 2016;13(1):1. https://doi.org/10.22146/ijcn.22751
9. Laksmitawati DR, Marwati U, Sumiyati Y, Pratami DK, Sari IP. The Effect of Amorphophallus muelleri blume and Moringa oleifera Leaves on Body Weight, Feed Intake, and Hepatic

Histopathology in Mice. Int J Appl Pharm. 2021;13(special issue 2). https://doi.org/10.22159/ijap.2021.v13s2.16

10. Saputri R, A'yun RQ, Huriyati E, Lestari LA, Rahayoe S, Yusmiati Y, et al. Pengaruh Pemberian Jelly Mengandung Glukomanan Porang (Amorphophalus oncophyllus) dan Inulin sebagai Makanan Selingan terhadap Berat Badan, IMT, Lemak Tubuh, Kadar Kolesterol Total, dan Trigliserida pada Orang Dewasa Obesitas. J Gizi Klin Indones. 2021;17(4):166. https://doi.org/10.22146/ijcn.58343
11. Budiani DR, Subandono J, Sarsono, Hermawan D. Mengatasi Obesitas dengan Asupan Nutrisi Berbahan Tepung Porang Terfortifikasi Tepung Daun Kelor. Deepublish; 2023.
12. Gunawan SA, Berata IK, Wirata IW. Histopatologi Kulit pada Kesembuhan Luka Insisi Tikus Putih Pasca Pemberian Extracellular Matrix (ECM) yang Berasal dari Vesica Urinaria Babi. Indones Med Veterinus. 2019;8(3):313–24.
13. Sudhakaran S, Bottiglieri T, Tecson KM, Kluger AY, McCullough PA. Alteration of lipid metabolism in chronic kidney disease, the role of novel antihyperlipidemic agents, and future directions. Rev Cardiovasc Med. 2018;19(3):77–88. https://doi.org/10.31083/j.rcm.2018.03.908
14. McPherson KC, Shields CA, Poudel B, Fizer B, Pennington A, Szabo-Johnson A, et al. Impact of obesity as an independent risk factor for the development of renal injury: implications from rat models of obesity. Am J Physiol Physiol. 2019;316(2):F316–27. https://doi.org/10.1152/ajprenal.00162.2018
15. Salto-Tellez M, James JA, Hamilton PW. Molecular pathology - The value of an integrative approach. Mol Oncol. 2014;8(7):1163–8. https://doi.org/10.1016/j.molonc.2014.07.021
16. Paleva Rheza. Mekanisme Resistensi Insulin Terkait Obesitas. Insul Resist Mech Relat to Obesity. 2019;10(2):354–8.
17. Rahmawati A. Mekanisme Terjadinya Inflamasi dan Stres Oksidatif pada Obesitas. 2014;5(1):1–8. https://doi.org/10.18860/elha.v5i1.3034
18. Salim HM, Kurnia LF, Bintarti TW, Handayani H. The Effects of High-fat Diet on Histological Changes of Kidneys in Rats. Biomol Heal Sci J. 2018;1(2):109. https://doi.org/10.20473/bhsj.v1i2.9675
19. Vergara-Jimenez M, Almatrafi MM, Fernandez ML. Bioactive components in Moringa oleifera leaves protect against chronic disease. Antioxidants. 2017;6(4):1–13. https://doi.org/10.3390/antiox6040091
20. Nasir S, St.A. . Rahayuningsih, Radjit BS, Ginting E, Harnowo D, Mejaya IMJ. Tanaman Porang : Pengenalan, Budidaya, dan Pemanfaatannya. Winarto A, editor. Pusat Penelitian dan Pengembangan Tanaman Pangan; 2015. 978-979–1159 p.
21. Handayani T, Aziz YS, Herlinasari D. Pembuatan dan Uji Mutu Tepung Umbi Porang (Amorphophallus Oncophyllus Prain) di Kecamatan Ngrayun. MEDFARM J Farm dan Kesehat. 2020;9(1):13–21. https://doi.org/10.48191/medfarm.v9i1.27