

ALPHA-MANGOSTIN POTENCY TO IMPROVE RENAL FUNCTION AND HISTOPATHOLOGICAL IMAGES IN HIGH-FAT DIET RATS

Laela Kinghua Liana¹, Gusti Azri Naufal², Susy Tjahjani^{3*}

¹Bagian Patologi, Fakultas Kedokteran, Universitas Kristen Maranatha, Bandung, Indonesia

²Program Sarjana Kedokteran, Fakultas Kedokteran, Universitas Kristen Maranatha, Bandung, Indonesia

³Bagian Parasitologi, Fakultas Kedokteran, Universitas Kristen Maranatha, Bandung, Indonesia

ARTICLE INFO

*Corresponding author :

Susy Tjahjani
Universitas Kristen
Maranatha,
Bandung, Indonesia
Email:
stjahjani@gmail.com

Kata kunci:

Alfa-mangostin
Kadar kreatinin
Histopatologi ginjal
In vivo

Keywords:

Alpha-mangostin
Creatinine level
Kidney histopathology
In vivo

Original submission:

July 7, 2024

Accepted:

August 21, 2024

Published:

September 30, 2024

ABSTRAK

Tujuan penelitian ini adalah untuk mengetahui apakah alfa-mangostin dapat melindungi ginjal pada tikus yang diberi pakan tinggi lemak dengan parameter: kadar kreatinin serum dan gambaran histopatologik ginjalnya. Uji eksperimental *in vivo* dilakukan pada 30 ekor tikus jantan Sprague Dawley dengan rancangan acak lengkap. Setelah adaptasi 7 hari, tikus dibagi dalam 6 kelompok perlakuan (masing-masing 5 replikasi) secara random. Uji selama 4 minggu: kelompok NC (pakan standar tanpa perlakuan 4 minggu); 5 kelompok lainnya :4 minggu pakan tinggi lemak dan pada 2 minggu terakhir ditambahkan perlakuan untuk masing-masing kelompok sebagai berikut: akuades, *corn oil*, alfamangostin dosis-1, alfamangostin dosis-2, simvastatin disebut kelompok PTL, CO, D1, D2, dan Sim. Kadar kreatinin diperiksa pada akhir minggu ke2 dan ke-4, selisih (%) dianalisis secara ANOVA/Kruskal Wallis yang dilanjutkan dengan Duncan/Mann Whitney dengan $\alpha = 0.05$. Hasil penelitian menunjukkan: penurunan kadar kreatinin D1/D2 = PTL ($p > 0,05$), gambaran histopatologik ginjal D1 lebih baik daripada PTL ($p < 0,05$). Disimpulkan: dampak perlindungan alfa-mangostin terhadap ginjal pada PTL belum terlihat dari penurunan kadar kreatinin tetapi sudah dapat dilihat pada perbaikan gambaran histopatologiknya, untuk ini diperlukan studi yang lebih lama.

ABSTRACT

Alpha-Mangostin Potency To Improve Renal Function And Histopathological Images In High-Fat Diet Rats. This study aimed to study whether alpha-mangostin could protect the kidneys of high-fat diet rats using the creatinine serum level and histopathological changes as parameters. The *in vivo* experimental study with a completely randomized design used 30 male Sprague Dawley rats. After 7 days of adaptation, they were randomly divided into six treatment groups, each with five replications. The study was done for four weeks: NC group (given standard pellet without treatment for four weeks), five other groups were given a high-fat diet for four weeks, and at the last two weeks were added with aquadest, corn oil, alpha-mangostin dose-1, alpha-mangostin dose-2, and simvastatin, namely PTL, CO, D1, D2, and Sim group. Creatinine level was examined at the end of week 2 and week 4; the difference percentage was analyzed using ANOVA/Kruskal Wallis ($\alpha = 0.05$) and continued with Duncan/Mann Whitney. It was shown that creatinine level decreased in D1/D2 = PTL mice ($p > 0,05$); kidney histopathological image in D1 was better than PTL ($p < 0,05$). It was concluded that the alpha-mangostin kidney protection effect was not yet visible according to creatinine level, but it was visible according to histopathological image; further studies are needed.

INTRODUCTION

Obesity is closely related to various pathological conditions and potentially induces various organ dysfunctions, even causing histopathologic abnormalities such as against the liver.^{1,2} Besides its correlation with NAFLD (non-alcoholic fatty liver disease), obesity can also cause kidney dysfunction and chronic kidney disease because of the imbalance between the nephron quantity and body weight so that as compensation, the glomerulus becomes enlarged. However, this enlargement happens without balancing of the podocytes and it also causes further reduction of nephron quantity,^{4,5} and it becomes a *circulus vicius*, which needs to be cut for it gradually potentially induces irreversible chronic kidney disease manifested as decreasing of nephron quantity, increasing of glomerular sclerosis, and enlargement as well as hyperfiltration of the remaining glomerulus.⁴

Alpha-mangostin is a dominant xanthone contained in mangosteen rind⁶ and potentially reduces body weight (BW) through several mechanisms^{7,8}, and it is hoped that this compound could improve kidney function and histopathology. Alpha-mangostin also has antioxidant activity, so it can scavenge free radicals. Free radical overproduction also happens in inflammatory conditions such as obesity. It is hoped that it might protect against further nephron damage.⁹⁻¹¹

This study aimed to explore the potential activity of alpha-mangostin to improve kidney function by examining plasma creatinine level and kidney degeneration manifested by decreased nephron quantity and glomerulus dilatation in high-fat diet Sprague Dawley mice.

METHODS

Materials: Simvastatin, propylthiouracyl (PTU) 0,01 % in aquadest, alpha-mangostin from Biopurify Phytochemical, Chengdu, China, standard pellet, and high fat diet.

Subjects: Thirty male mice, *Rattus norvegicus* Sprague Dawley strain, 8-10 weeks age, 190-200 g body weight (BW) were obtained from *iRATco Veterinary Laboratory Service*.

Ethical animal handling: The study has been approved by Komite Etik Penelitian FK Universitas Kristen Maranatha with ethical approval certificate No 059/KEP/VI/2022. The rats were put in a room with 12 hours of light and 12 hours of dark in an air-conditioned room with a temperature of 20-23 C, relative humidity of 40-60 %, enough food and drink, and 1 cage for each group. The cages were cleaned every day. Blood sampling was taken aseptically. The cervical dislocation was done under anesthesia for animal termination.

Procedure: This study was done in the Biomedical Laboratory, Faculty of Medicine, Maranatha Christian University, using a true experimental study with a completely randomized design. Six treatments, each with 5 replications, were done per-oral once a day for 4 weeks after 1 week adaptation period by consuming standard pellets and plain water *ad libitum*. The treatments were: standard pellet and plain water *ad libitum* only until the end of the experiment (NC group), 5 other treatment groups were fed with high fat diet (PTL) + aquadest containing 0,01 % PTU for 4 weeks experiment and at the third and fourth week (for 2 weeks) were treated with various treatments i.e: without any treatment (PTL group), corn oil (CO group), alpha-mangostin dose 7 mg/kgBW (D1 group), dose 35 mg/kgBW (D2 group), and simvastatin 3,6 mg/kgBW (Sim group). All of these treatments were done in 2 mL volume. Alpha-mangostin was diluted in corn oil, and simvastatin was diluted in aquadest.

Data collection: Blood samples for serum creatinine level examination were taken at the end of the second and fourth week of the experiment. The percentage of decrease in

creatinine serum level was calculated. Animal termination was done at the end of the experiment ethically using cervical dislocation after intra-peritoneal injection of ketamine 75 mg/kgBW and xylazine 10 mg/kgBW for anesthesia procedure.

Histopathological analysis: The kidneys were taken for histopathological examination with hematoxylin and eosin staining. The procedure was: organ fixation with formalin 10%, dehydration using alcohol with serial concentrations, clearing using xylol, and infiltration with paraffin liquid. After specimen planting, it was cut using a microtome, put in a warm water bath, and laid on the slides. After staining with hematoxylin and eosin, the slides were covered with cover glasses and examined using a light microscope at 400x magnification. Scoring was used to evaluate the images: score 0, score 1, score 2, and score 3, which showed <25 %, 25-50%, 50-75 %, and 75-100 % kidney degeneration consecutively. This degeneration manifested as glomerulus enlargement and decreased number of nephrons.

Statistical analysis: The decrease in the percentage of creatinine serum level was analyzed using ANOVA/Kruskal Wallis and Duncan/Mann Whitney, depending on its data homogeneity and distribution, with a 0.05 significance level. The scores of the level of kidney degeneration were analyzed using Kruskal Wallis and Mann Whitney, also with a 0.05 significance level.

RESULTS

The decreasing percentage of creatinine level and its result of ANOVA and Duncan analysis were shown in the graph image in Figure 1.

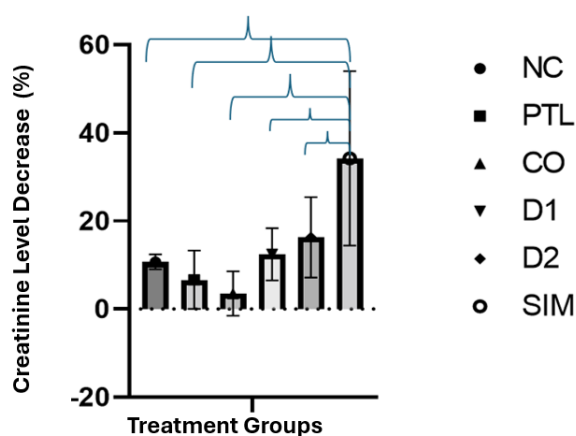


Figure 1. The bar chart of creatinine serum level bar chart decrease percentage because of various treatments: NC, PTL, CO, D1, D2, and Sim consecutively from left to right. The above curly brackets between 2 bars indicate significant differences (P<0.05).

As shown in Figure 1, only the Sim group reduced creatinine levels significantly (p<0.05). The decrease in creatinine levels in other treatments was not different (P>0.05). The impact of the treatments against kidney structure degeneration score is shown in the graph image in Figure 2.

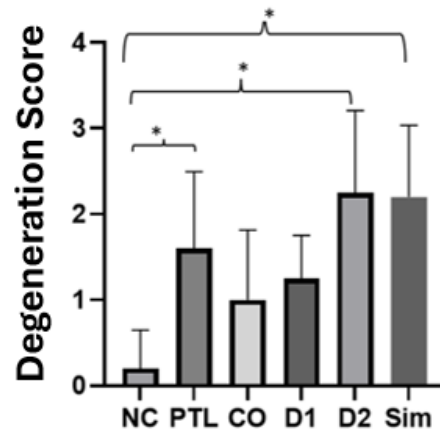


Figure 2. The graph shows kidney degeneration because of several treatments. The above curly brackets between 2 bars indicate significant differences ($P < 0.05$).

As shown in Figure 2, the degeneration score in the PTL group was more than NC, and the score in D2 and Sim was also more than NC ($p < 0.05$). However, the degeneration score in CO and D1 was not different from NC's ($p > 0.05$). It meant that CO and D1 could prevent kidney degeneration because of PTL. Kidney histopathological images according to various scores are shown in Figure 3.

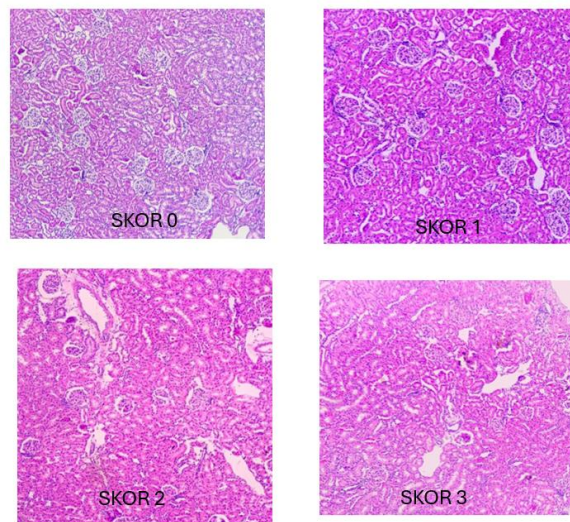


Figure 3. The kidney histopathological images with various degeneration scores.

DISCUSSIONS

According to the results shown in Figure 1, creatinine levels decrease in all of these treatments each other was not different ($p>0.05$) except against Sim ($P<0.05$). This might be related to the short duration of a high-fat diet and alpha-mangosteen treatment, so it could not yet reduce the creatinine level significantly. Mild kidney pathology increases the glomerular filtration rate, which might also become the other cause.¹² Significant difference against other treatments was only shown in the Sim group included against NC as well as against PTL ($p<0.05$). Simvastatin could improve kidney function and reduce the creatinine level even in experimental animals with chronic kidney failure.^{13,14} The Renal protection effect of simvastatin might be faster and stronger than others, and it is needed to be studied further. Figure 2 showed that alpha-mangostin could protect against kidney degeneration according to histopathological images. A similar study showed that mangosteen rind extract could protect kidney glomerulosclerosis.¹⁵ Reported also that mangosteen rind extract could protect against renal proximal tubule damages of DM mice model because of reducing inflammation process and oxidative stress.¹⁶ Alpha-mangostin could also improve experimental animals' lipid profile and reduce their body weight⁸. That is why it could reduce free radicals, which could have impact against kidney damage.^{16,17} It is also known that the dominant xanthone contained in the mangosteen rind extract is alpha-mangostin.¹⁸ In this study, the potency of alpha-mangostin to protect the kidney was at D1 ($p<0.05$), while there was no protection effect in the D2 group. The former study showed that high antioxidant concentration could act as a pro-oxidant, especially for phenolic antioxidants.¹⁹ That study could clarify the effect of the D2 group.

Alpha-mangostin D1 group could improve kidney degeneration according to histopathology, while against creatinine level, there was no significant difference ($p>0.05$). As mentioned before, it might even raise the glomerular filtration rate in not severe kidney degeneration.¹² Sim group could reduce the creatinine level significantly. The anti-inflammation and antioxidant activity of the statin might cause it.¹⁴ Histopathologically, the Sim group did not improve the degeneration significantly. This might be due to the histopathological improvement of the kidney occurring outside the area of the kidney that was visible on histopathological examination. It needed to be confirmed.

CO treatment could also improve kidney histopathology, and there was no significant difference against NC ($p>0.05$). The high content of phytosterol²⁰ might cause it, and this phytosterol could improve the lipid profile;²¹ and it also could act as an anti-inflammatory and antioxidant.²² This finding also becomes an opportunity to be studied further. This study needs to be improved in further studies with longer duration of high-fat diet and longer duration of alpha-mangostin and other treatments to understand the treatment effects better. The toxicity study of alpha-mangostin is also needed to be studied further.

CONCLUSIONS

Alpha-mangostin might potentially improve the kidney degeneration of high-fat diet rats, as shown in kidney histopathological images in D1 treatment, although there was still no effect against creatinine level. It is needed to be studied further to get the more real effects. In the future, after longer and various more extended studies, including a toxicity study, alpha-mangostin might be considered for improving kidney pathology, especially in a high-fat diet.

ACKNOWLEDGEMENTS

We would like to thank Maranatha Christian University for funding this study.

REFERENCES

1. Abuzaid AS, Iskandar EY, Kurniati NF, Adnyana IK. Preventive effect on obesity of mangosteen (*Garcinia mangostana* L.) pericarp ethanolic extract by reduction of fatty acid synthase level in monosodium glutamate and high-calorie diet-induced male wistar rats. *Asian J Pharm Clin Res.* 2016;9(3):3–6.
2. Ansori ANM, Susilo RJK, Hayaza S, Winarni D, Husen SA. Renoprotection by *Garcinia mangostana* L. pericarp extract in streptozotocin-induced diabetic mice. *Iraqi J Vet Sci.* 2019;33(1):13–9.
3. Cahyawati PN, Lestari DPO, Siskayani AS, Ariawan IMT. Simvastatin improves renal function and glomerulosclerosis in ischemic-reperfusion injury. *Indones Biomed J.* 2020;12(2):143–8.
4. Chae HS, Kim YM, Bae JK, Sorchhann S, Yim S, Han L, et al. Mangosteen Extract Attenuates the Metabolic Disorders of High-Fat-Fed Mice by Activating AMPK. *J Med Food.* 2016;19(2):148–54.
5. Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y. Obesity & inflammation: The linking mechanism & the complications. *Arch Med Sci.* 2017;13(4):851–63.
6. Fatima S, Shahid M, Ahmed MN, Shafi S, Sabir S, Shah NUA, et al. Effect of B - Sitosterol Supplement Along With Fat Modified Diet for the Management of Dyslipidemia. *J Popul Ther Clin Pharmacol.* 2024;31(04):1744–62.
7. Gounden V, Bhatt H, Jialal I. Renal Function Test [Internet]. 2023. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK507821/>
8. Liu QY, Wang YT, Lin LG. New insights into the anti-obesity activity of xanthenes from *Garcinia mangostana*. *Food Funct.* 2015;6(2):383–93.
9. Manna P, Jain SK. Obesity, Oxidative Stress, Adipose Tissue Dysfunction, and the Associated Health Risks: Causes and Therapeutic Strategies. *Metab Syndr Relat Disord.* 2015;13(10):423–44.
10. Mo S, Dong L, Hurst WJ, Van Breemen RB. Quantitative analysis of phytosterols in edible oils using APCI liquid chromatography-tandem mass spectrometry. *Lipids.* 2013;48(9):949–56.
11. Prasad R, Jha RK, Keerti A. Chronic Kidney Disease: Its Relationship With Obesity. *Cureus.* 2022;14(10).
12. Puelles VG, Douglas-Denton RN, Cullen-McEwen LA, Li J, Hughson MD, Hoy WE, et al. Podocyte number in children and adults: Associations with glomerular size and numbers of other glomerular resident cells. *J Am Soc Nephrol.* 2015;26(9):2277–88.
13. Setyawati LU, Nurhidayah W, Khairul Ikram NK, Mohd Fuad WE, Muchtaridi M. General toxicity studies of alpha mangostin from *Garcinia mangostana*: A systematic review. *Heliyon [Internet].* 2023;9(5):e16045.
14. Shen Q, Chitchumroonchokchai C, Thomas JL, Gushchina L V, DiSilvestro D, Failla ML, et al. Adipocyte reporter assays: Application for identification of anti-inflammatory and antioxidant properties of mangostin xanthenes. *Bone [Internet].* 2008;23(1):1–7.
15. Sreekumar R, Unnikrishnan J, Fu A, Nygren J, Short KR, Schimke J, et al. Impact of high-fat diet and antioxidant supplement on mitochondrial functions and gene transcripts in rat muscle. *Am J Physiol - Endocrinol Metab.* 2002;282(5 45-5):1055–61.
16. Suaniti NM, Manurung M, Utari NMM. Efek penambahan antioksidan ekstrak metanol kulit buah manggis (*Garcinia mangostana* L.) terhadap perubahan kadar FFA, bilangan asam, dan bilangan peroksida biodiesel. *J Kim.* 2017;11(1):49–55.

17. Tjahjani S, Widowati W. Potensi Beberapa Senyawa Xanthone sebagai Antioksidan dan Anti-malaria serta Sinergisme dengan Artemisinin in Vitro. *J Indon Med Assoc.* 2013;63(3):95–9.
18. Tsuboi N, Okabayashi Y, Shimizu A, Yokoo T. The Renal Pathology of Obesity. *Kidney Int Reports [Internet].* 2017;2(2):251–60.
19. Walker EB. HPLC analysis of selected xanthenes in mangosteen fruit. *J Sep Sci.* 2007;30(9):1229–34.
20. Wibawa IDGAP, Sumadewi KT, Cahyawati PN. Simvastatin Memperbaiki Degerasi Hidropis dan Nekrosis Sel Hepatosit Mencit Subtotal Nefrektomi. *JBN (Jurnal Bedah Nasional).* 2022;6(1):22.
21. Widowati W, Laksmiawati DR, Wargasetia TL, Afifah E, Amalia A, Arinta Y, et al. Mangosteen peel extract (*Garcinia mangostana* L.) as protective agent in glucose-induced mesangial cell as in vitro model of diabetic glomerulosclerosis. *Iran J Basic Med Sci.* 2018;21(9):972–7.
22. Yang R, Xue L, Zhang L, Wang X, Qi X, Jiang J, et al. Phytosterol contents of edible oils and their contributions to estimated phytosterol intake in the Chinese diet. *Foods.* 2019;8(8).