Original Article

Effects of α-Mangostin-Loaded Self-Nanoemulsion (MG-SNE) and Physical Exercise on The Reduction of Waist Circumference in Wistar Rats

Andreas Arie Setiawan[1,2], Sugiri[2], Awal Prasetyo[3],
Eirin Yovita Kurniawan[4], Dominikus Evano Putra[4]

[1]Doctoral Study Program of Medical and Health Science, Faculty of Medicine Diponnegoro University Semarang, Indonesia
[2]Internal Medicine Division, Faculty of Medicine Diponegoro University/ Kariadi Hospital Semarang, Indonesia
[3]Anatomic Pathology Division, Faculty of Medicine Diponegoro University/ Kariadi Hospital Semarang, Indonesia
[4]Medical Professional Education Study Program, Faculty of Medicine Diponegoro University Semarang, Indonesia

Abstract

Background : Waist circumference (WC) is a marker of intra-abdominal adipose tissue and a risk factor for cardiometabolic disease. A higher risk of coronary heart disease was associated with an increased WC. Garcinia mangostana Linn's anti-inflammatory activity would reduce abdominal fat deposition and WC. Additionally, Garcinia mangostana Linn's potential would increase in nanotechnology. This study was aimed to demonstrate that WC in Wistar rats induced by an atherogenic diet can be decreased by α-Mangostin-loaded self-nanoemulsion (MG-SNE) treatment combined with physical activity.

Methods : Experimental research with Randomized Control Trial design using a total sample of 15 male white rats *(Rattus novergicus strain Wistar)* weighing 300 grams and aged between 6 and 8 weeks, split into 3 groups given physical exercise for 8 weeks along with 3 different doses of medication (group K received Atorvastatin 1.44 mg once; group P1 received Garcinia mangostana Linn pericarp extract, at a dose of 800 mg/kg, divided into 3 administrations; and group P2 received MG-SNE 50 mg/kg once). Waist circumference was measured using a metline, before and after treatment.

Results : WC decreased in Groups K, P1, and P2, with deltas of -5.00±21.21 mm, -12.50±24.75 mm, and -17.50±12.58 mm. The greatest decrease in WC was P2. There was no significant difference, according to the paired test between the pre-test and posttest in all groups. The *p* value >0.05 was determined to indicate that there were no significant differences between the groups.

Conclusion : WC in Wistar rats induced by an atherogenic diet can be decreased by MG-SNE treatment combined with physical exercise.

Keywords : Garcinia mangostana Linn, α-Mangostin self-nanoemulsion, Physical exercise, Waist Circumference

INTRODUCTION

Waist circumference (WC) can be more reliable as a general indicator of body size compared to body mass index (BMI). A risk factor for metabolic syndrome is obesity, with a waist circumference of at least 102 cm (40 inches) for males and at least 88 cm (35 inches) for females. The measurement of waist circumference raises the risk of cardiometabolic disease and can be used as an indicator of intra-abdominal adipose tissue. The primary cause of death worldwide, coronary heart disease (CHD), is one of the cardiovascular diseases that is more likely to occur in people with metabolic syndrome. According to WHO data, the number of deaths from CHD rises annually. The CHD death rate increased from 8.9 million in 2019 to 11.1 million in 2020, or over two million deaths. CHD is the cause of up to 30% of deaths worldwide, with developing nations accounting for 80% of these cases. Even though the death rate from CHD has decreased in developed countries, it still causes one-third of deaths in the over-35 age range.[1–3]

The effects of mangosteen peel *(Garcinia mangostana Linn)* on obesity, atherosclerosis, and lowering blood sugar levels have been the subject of numerous studies on the use of herbs in medicine in Indonesia.[2,3] Long-term consumption of Garcinia mangostana Linn does not adversely affect immunity, liver, or renal functions. Instead, it has a significant anti-inflammatory and antioxidant effects.[4] Garcinia mangostana Linn has a wide safety index and does not cause death.[5]

From research conducted on mice with diet-induced metabolic syndrome, which increases sugar and saturated fat levels and causes obesity, hypertension, left ventricular stiffness, dyslipidemia, and a fatty liver, it was determined that Garcinia mangostana Linn has anti-inflammatory effects. Garcinia mangostana Linn pericarp supplementation with 5% of diet-induced metabolic syndrome rats' diet, or 168 mg/kg/day α-mangostin, 355 mg/kg/day procyanidins, 3.9 mg/kg/day thocyanin, and 11, 8 mg/kg/day of hydroxycitric acid, for 8 weeks, can reduce body weight, alter mice's physiology and metabolism, and decrease the amount of waist fat deposition, waist circumference, and total body fat mass.. According to the study's findings, chronic Garcinia mangostana pericarp consumption can lessen inflammatory cell infiltration in mice with diet-induced metabolic syndrome, which in turn can lessen the symptoms of metabolic syndrome, liver problems, and cardiovascular disease.[5,6]

A recent advancement in nanotechnology allows for the transportation of drugs, proteins, peptides, and particles in structures as small as 0.1 to 100 nm.[7] The pericarp extract (MPE) of Garcinia mangostana Linn was successfully synthesized using polyvinylpyrrolidone (PVP) nanofiber mats, which increased the activity of the antioxidant release rate. However, further in vivo research is required to confirm the MPE's potential for anti-inflammatory and antioxidant effects.[8]

Another study was similarly successful in determining that the therapeutic efficacy of α-Mangostin (MG) microemulsion can be increased. The Alpha (α)-Mangostin-loaded self-microemulsion (MG-SME) has the potential to be a drug loading system characterized by encapsulation efficiency, size distribution, and morphology, resulting in high performance in terms of pharmacokinetics and tissue distribution.[9]

As a nano-sized delivery system, aqueous MG-SNE's spherical particles with an average diameter of 24.6 nm and an encapsulation efficiency of 87.26% effectively promote absorption in the digestive tract and alter its distribution in tissues. Specifically, the alteration of nanomicelles enhanced the solubility of α-mangostin by a factor of more than 10,000. Furthermore, polymeric nanoparticles allowed for targeted delivery and greatly improved the bioavailability of MG-SNE.[9] The purpose of this study is to demonstrate that waist circumference in Wistar rats with atherogenic diets can be decreased by MG-loaded self-nanoemulsion therapy in addition to physical activity.

METHODS

This research is an experimental study with a randomized pre- and post-test control trial (RCT) design. The research was conducted for 9 weeks, consisting of 1 week of acclimatization and 8 weeks of treatment. Male *Rattus norvegicus Wistar* strain mice, aged 6 to 8 weeks, who were active, met the inclusion criteria for this study. Meanwhile, mice who appeared unwell or crippled met the exclusion criteria for this study. This study reports changes in waist circumference in male Wistar rats given an atherogenic diet for eight weeks that included high fat (2023% by weight; 40–45% kcal from fat), saturated fatty acids (>60% of total fatty acids), milk fat or butterfat, sucrose (34% by weight), and cholesterol (0.2% total) in order to induce atherosclerosis. The animal model was treated with physical exercise combined with the administration of statins, *Garcinia mangostana Linn pericarp extract*, and MG-loaded self-nanoemulsion. Physical training was carried out by running 60 minutes at a speed of 12 m/minute, 5 days per week, for 8 weeks on a special rodent treadmill set at an incline of 0.

In total, 15 samples were used in this study, which were divided into 3 groups: 5 samples were used as the control group, which received treatment for 8 weeks consisting of parenteral administration of Atorvastatin 1.44 mg and physical exercise; 5 samples were used as treatment group 1 (P1), which received parenteral administration of Garcinia mangostana Linn pericarp extract at a dose of 800 mg/kg, divided into

3 administrations, along with physical exercise; and 5 samples were used as treatment group 2 (P2), which received parenteral administration of MG-loaded self-nanoemulsion 50 mg/kg once, along with physical exercise. Then, both before and after therapy, the research subjects' waist circumference was measured using a metline. The research was conducted at the Biomolecular Laboratory of Sultan Agung Islamic University The study adhered to national criteria for the care and use of laboratory animals and was authorized by the Faculty of Medicine, Diponegoro University's Health Research Ethics Committee (approval number: 51/EC/H/FK-UNDIP/VI/2022).

RESULTS

The average (mean) waist circumference of the mice was 201.43 ± 7.27 mm prior to treatment, according to the data collected. Following the course of treatment, there was a decrease in waist circumference, with a mean measurement of 186.88 ± 11.00 mm (see Table 1, Figure 1).

The results of the normality test of waist circumference data using Saphiro-Wilk showed a $p>0.05$ value in groups K and P1 before treatment. Meanwhile, in group P2, there was no normality test value because the waist circumference before treatment in group P2 was the same at 200 mm. Meanwhile, after treatment, groups K and P1 could not be tested for normality of data because,

at the end of the treatment, there were only 2 rats left. In group P2, after treatment, a $p>0.05$ was obtained, so it can be concluded that the data was normally distributed (Table 2).

Based on the results of the average waist circumference data before and after treatment obtained, it was found that the largest waist circumference difference was obtained in group P2, which received *MG-loaded self-nanoemulsion* 50 mg/kg once administered per round and physical exercise for 8 weeks (Figure 2).

Furthermore, a paired t-test was conducted using a paired T-Test for pre-test and post-test data. At the same time, a one-way ANOVA was used to compare treatment groups.

The results of the paired t-test between the Pre-test and the post-test Waist Circumference in groups K, P1, and P2 showed that the p-value was > 0.05, so there was no significant difference.

From the results of the unpaired t-test between groups K-P2 before treatment, no significant difference was found ($p>0.05$), with the Levene test $p<0.05$, so it can be concluded that the data obtained is not homogeneous. Meanwhile, waist circumference after treatment showed a value of $p>0.05$, and the Levene test $p>0.05$, so it can be concluded that there was no significant difference in waist circumference between groups, and the data obtained was homogeneous. Likewise, the delta WC obtained between groups showed a value of $p>0.05$ and

TABLE 1

Mean and Median Waist Circumference of the Rats

Variable	Mean ± SD (mm)	Median (min – max) (mm)
Pre-test	201.43 ± 7.27	200 (190 – 421)
Post-test	186.88 ± 11.00	185 (170 – 200)

Waist Circumference Comparisons
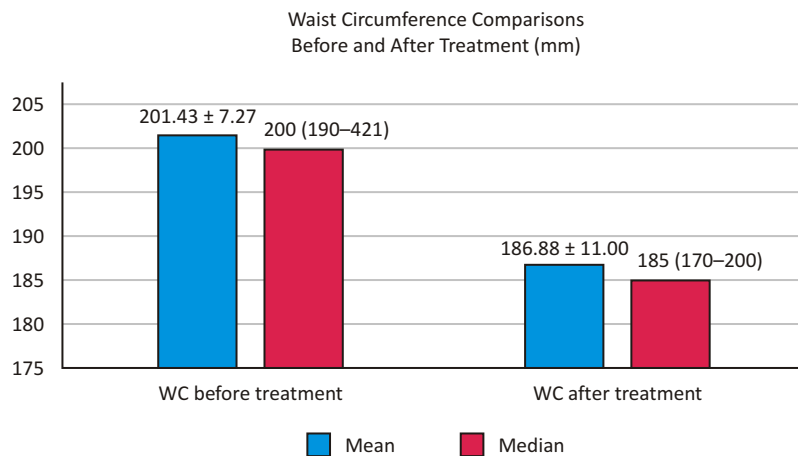Before and After Treatment (mm)

Figure 1. Difference between the mice's mean and median waist circumferences before and after treatment

TABLE 2
Descriptive and normality tests for waist circumference

WC	Groups	N	Mean ± SD (mm)	Median (min – max)	p
Pre-test	K	5	200.00 ± 10.00	200 (190 – 210)	0.119*
	P1	5	203.33 ± 10.33	200 (190 – 220)	0.473*
	P2	5	200.00	200,00	–
Post-test	K	2	190.00 ± 14.14	190 (180 – 200)	–
	P1	2	192.50 ± 3.54	192,5 (190 – 195)	–
	P2	4	182.50 ± 12.58	180 (170 – 200)	0.406*
Delta	K	2	-5.00 ± 21.21	-5 (-20 – 10)	–
	P1	2	-12.50 ± 24.75	-12,5 (-30 – 5)	–
	P2	4	-17.50 ± 12.58	-20 (-30 – 0)	0.406*

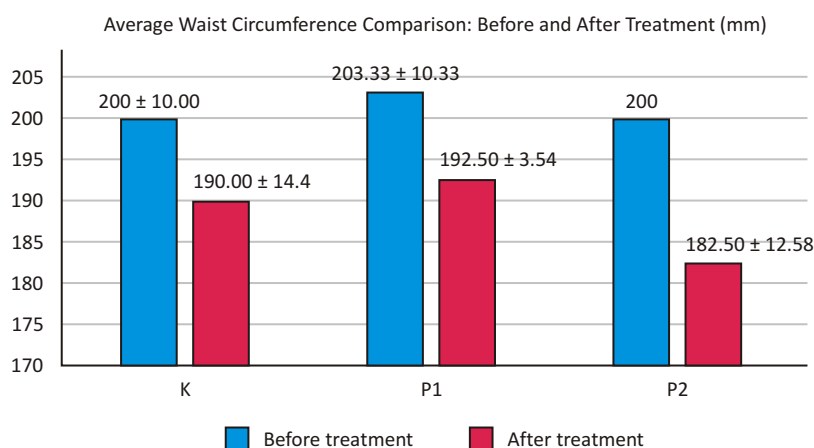Details: * Normal ($p > 0.05$)

Figure 2. Average waist circumference comparison graph pre- and post-treatment

the Levene test $p>0.05$, so it can be concluded that there was no significant difference between groups, and the data variance was homogeneous (Table 3).

DISCUSSION

Waist circumference indicates intra-abdominal adipose tissue and increases the risk of cardiometabolic disease.[10,11] Based on the Cross-Sectional Study by Badrooj *et al*. in 2022, the potential for inflammatory processes can also be seen from anthropometry, one of which is waist circumference. The study also mentioned that a Pro-inflammatory Diet will increase Waist Circumference, including poor anthropometric measurements. An increase in waist circumference will increase metabolic risk, so it is recommended to provide cardioprotective drug therapy.[12]

Previous studies have found that mangosteen contains xanthone, a substance with anti-inflammatory and antioxidant activity from mangosteen peel isolation. The compound α-mangosteen has been found to have a variety of biological activities, with anti-inflammatory, anti-tumor, cardioprotective, antidiabetic, antibacterial agents, antifungal, antiparasitic, antioxidant, and anti-obesity. This supports the results of research that α-mangosteen compounds have activity as cardioprotective and can reduce waist circumference.[12]

Based on a study by Ibrahim MY *et al*. in 2016, the compound α-mangostin induces apoptosis of 3T3-L1 preadipocytes by inhibiting fatty acid formation and can suppress intracellular lipid accumulation in differentiated adiposity and stimulation of lipolysis in mature adipocytes. The study further explained that the inhibition of fatty acid formation by α-mangosteen is due to a stronger effect on the ketoacyl formation domain and a weaker effect on the acetyl/malonyl transferase domain. So, based on these studies, the compound α-mangostin is useful in the therapy or prevention of
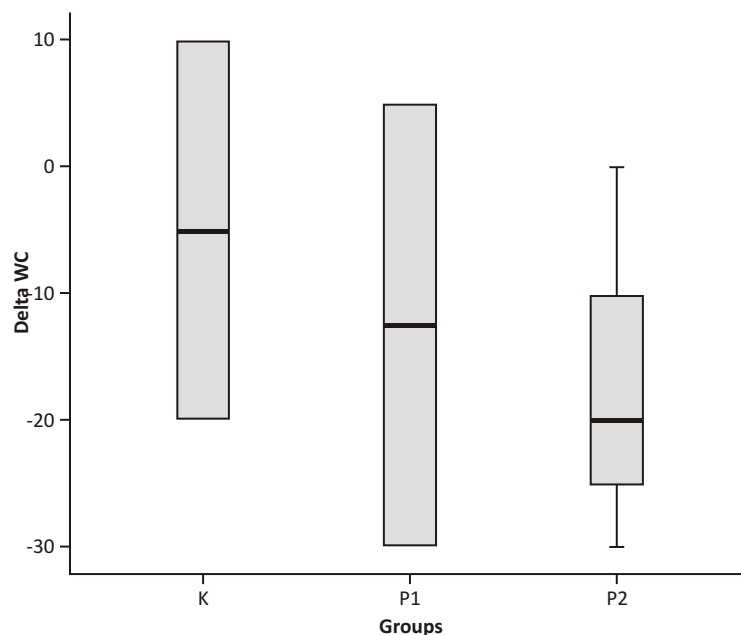
Figure 3. Waist circumference delta amongst groups K, P1, and P2.
There is no significant difference between treatment groups

TABLE 3
Waist Circumference (WC) Difference Test Results

Groups	WC		p	Delta WC
	Pre test	**Post test**		
K	200.00 ± 10.00	190.00 ± 14.14	0.795[¶]	-5.00 ± 21.21
P1	203.33 ± 10.33	192.50 ± 3.54	0.605[¶]	-12.50 ± 24.75
P2	200.00	182.50 ± 12.58	0.069[¶]	-17.50 ± 12.58
p	0.753[§]	0.594[§]	–	0.727[§]
Lavene	0.018	0.426**	–	0.264**

Details : * Significant ($p < 0.05$); ** Homogen ($p > 0.05$); ¶ Paired t; § One Way Anova

obesity.[13]

A significant relationship exists between increased waist circumference and the prevalence of abdominal obesity. Where an increase in waist circumference can also increase the risk and prevalence of abdominal obesity. Based on the research by Robert Ross in 2020, an increase in waist circumference is also a major cause of increased risk factors for cardiometabolic events. Thus, decreasing waist circumference will also reduce the risk of cardiometabolic events.[14]

Based on the results of the study, it was found that there was a decrease in waist circumference in the control group who received treatment with Atorvastatin 1.44 mg once administered per round and physical exercise for 8 weeks; treatment group 1 (P1) which was given *Garcinia mangostana Linn pericarp extract*, at a dose of 800 mg / kgbb, divided by 3 times administration, per round, and

physical exercise for 8 weeks; and treatment group 2 (P2) which was given MG-loaded self-nanoemulsion 50 mg/kg once administered, per round, and physical exercise for 8 weeks. This is based on the research of Oliver *et al.*, where it was found that supplementation of *Garcinia mangostana Linn pericarp* as much as 5% of the diet of rats with diet-induced metabolic syndrome, or a dose of 168 mg/kg/day α-mangosteen, 355 mg/kg/day procyanidins, 3.9 mg/kg/day thocyanin and 11.8 mg/kg/day hydroxy citric acid, for 8 weeks, can reduce body weight, change the physiology and metabolism of rats, reduce waist fat deposition, reduce waist circumference and whole body fat mass.[6]

A crucial factor in determining how well drug molecules move through pharmacological phases including biopharmaceutics, pharmacokinetics, and pharmacodynamics is drug bioavailability. One of the
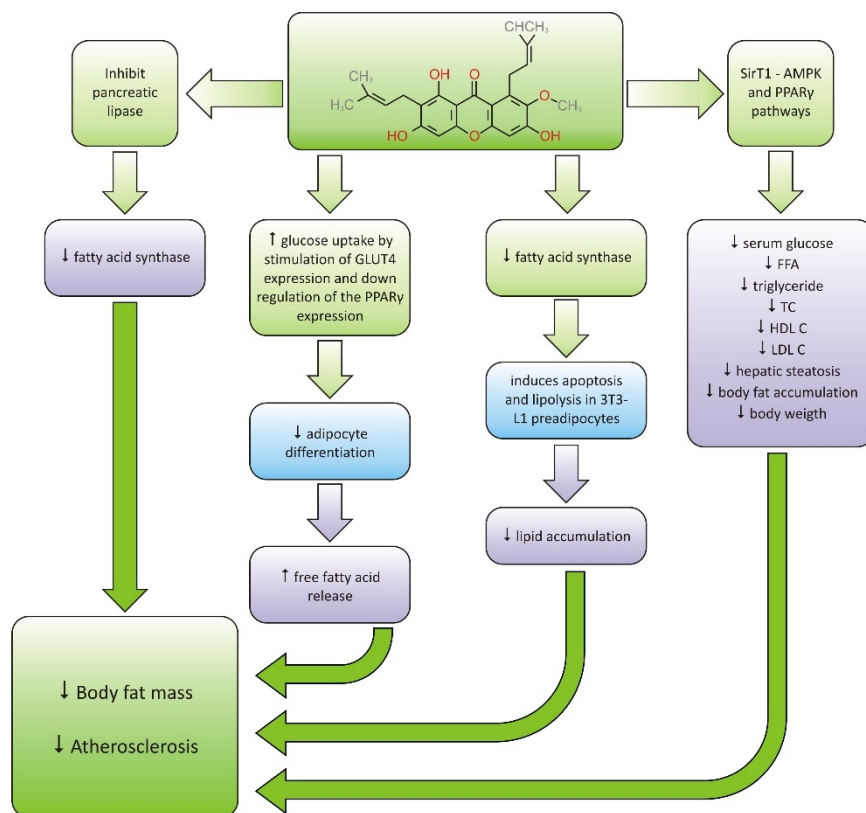
Figure 4. Diagram illustrating the method by which α-mangostin reduces body fat mass.
GLUT4: Glucose transporter type 4; PPARγ: peroxisome proliferator-activated receptor-γ; SirT1: Sirtuin 1;
AMPK: 50 AMP-activated protein kinase; FFA: free fatty acids; TC: total cholesterol;
HDL: high density lipoprotein; LDL: low density lipoprotein.[1]

main characteristics that can boost a drug's bioavailability to the maximum is its solubility in water. At the moment, the most widely utilized method for treating diseases with nanomedicine is the utilization of drug delivery systems based on nanoparticles. Based on a study by Tri Rizki in 2023, nanoemulsion is an emulsion system with a particle size between 10 and 100 nm, with very small droplet size characteristics. The principle of nanoemulsion synthesis uses a high-energy method by dispersing the material using high energy to produce a small-sized suspense so that it will shorten the homogenization cycle by providing an attraction that expands the surface of the material that interacts with the homogenizer.[15]

According to the study results, rats in group P2 who received MG-loaded self-nanoemulsion 50 mg/kg once administered per round and physical exercise for 8 weeks experienced the greatest decrease in waist circumference. This is possible because nanoemulsions can increase the potential for clinical efficacy due to improved pharmacokinetic performance of the drug loading system, encapsulation efficiency, size distribution, morphology, and tissue distribution. Furthermore, the nano-sized delivery system will efficiently improve gastrointestinal absorption.

A study by Belalcazar *et al*. 2013 found that research subjects given statins only experienced weight loss but no decrease in waist circumference. Meanwhile, this study found a decrease in waist circumference in the P1 group who received Atorvastatin 1.44 mg once administered per round and physical exercise for 8 weeks. This is not show by previous studies. The decrease in waist circumference in group P1 may be due to physical exercise performed by rats.[16]

Based on the research results by Sinha *et al*. in 2020, it was stated that treadmill exercise for 12 weeks can reduce central obesity.[17] Physical exercise reduces body weight, waist circumference, fat mass (especially visceral fat), blood pressure, and inflammation. Physical exercise can also improve insulin sensitivity.[18–20] In addition, based on research by Pons *et al*. in 2013, physical exercise has a cardioprotective effect, especially in cases of obesity.[21]

CONCLUSION

According to the results of this study, waist circumference in Wistar rats that have been given an atherogenic diet can be decreased by administering an MG-loaded self-nanoemulsion along with physical activity.

REFERENCES

1. Tousian Shandiz H, Razavi BM, Hosseinzadeh H. Review of Garcinia mangostana and its Xanthones in Metabolic Syndrome and Related Complications. Phyther Res. 2017;31(8):1173–82.

2. Zhang L, Tong Z, Han R, Guo R, Zang S, Zhang X, et al. Global, Regional, and National Burdens of Ischemic Heart Disease Attributable to Smoking From 1990 to 2019. J Am Heart Assoc. 2023;12(3).

3. Organization WHO. The Top 10 Causes of Death. Natl Vital Stat Reports [Internet]. 2020; Available from: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death

4. Disease CA. Inflammation, Atherosclerosis, and Coronary Artery Disease. 2005;1685–95.

5. Ades PA, Savage PD. Obesity in coronary heart disease: An unaddressed behavioral risk factor. Prev Med (Baltim). 2017;104:117–9.

6. Xie Z, Sintara M, Chang T, Ou B. Daily consumption of a mangosteen-based drink improves in vivo antioxidant and anti-inflammatory biomarkers in healthy adults: A randomized, double-blind, placebo-controlled clinical trial. Food Sci Nutr. 2015;3(4):342–8.

7. Candra AA, Nani D, Jurusan I, Politeknik P, Lampung N, Soekarno J, et al. Uji Toksisitas Akut Kulit Manggis (Garcinia mangostana L.) Kajian Histopatologis Hati. Jur Peternak Politek Negeri Lampung. 2016;(September):214–8.

8. John OD, Mouatt P, Panchal SK, Brown L. Rind from purple mangosteen (Garcinia mangostana) attenuates diet induced physiological and metabolic changes in obese rats. Nutrients. 2021;13(2):1–19.

9. Nikalje AP. Nanotechnology and its Applications in Medicine. Med Chem (Los Angeles). 2015;5(2):81–9.

10. Sriyanti I, Edikresnha D, Rahma A, Munir MM, Rachmawati H, Khairurrijal K. Mangosteen pericarp extract embedded in electrospun PVP nanofiber mats: Physicochemical properties and release mechanism of α-mangostin. Int J Nanomedicine. 2018;13:4927–41.

11. Xu WK, Jiang H, Yang K, Wang YQ, Zhang Q, Zuo J. Development and in vivo evaluation of self-microemulsion as delivery system for α-mangostin. Kaohsiung J Med Sci. 2017;33(3):116–23.

12. Yoon Y-W, Lee H-M, Oh H-W. Correlation of Dyslipidemia with Waist Circumference and Waist-Height Ratio. Korean J Fam Pract. 2016;6(6):560–7.

13. Carroll S, Cooke CB, Butterly RJ, Moxon JWD, Moxon JWA, Dudfield M. Waist circumference in the assessment of obesity and associated risk factors in coronary artery disease patients. Coron Heal Care. 2000;4(4):179–86.

14. Badrooj N, Keshavarz SA, Curtis T, Yekaninejad MS, Pooyan S, Ghodoosi N, et al. The Relationship between Empirical Dietary Inflammatory Pattern with Anthropometric Measures in Women with Overweight and Obesity: A Cross-Sectional Study. Iran J Public Health. 2022;51(6):1348–54.

15. Ibrahim MY, Hashim NM, Mariod AA, Mohan S, Abdulla MA, Abdelwahab SI, et al. α-Mangostin from Garcinia mangostana Linn: An updated review of its pharmacological properties. Arab J Chem [Internet]. 2016;9(3):317–29. Available from: http://dx.doi.org/10.1016/j.arabjc.2014.02.011

16. Ross R, Neeland IJ, Yamashita S, Shai I, Seidell J, Magni P, et al. Waist circumference as a vital sign in clinical practice: a Consensus Statement from the IAS and ICCR Working Group on Visceral Obesity. Nat Rev Endocrinol [Internet]. 2020;16(3):177–89. Available from: http://dx.doi.org/10.1038/s41574-019-0310-7

17. Rizki T, Yasni S, Muhandri T, Yuliani S. Sintesis Nanoemulsi dari Ekstrak Kulit Manggis dengan Metode Energi Tinggi. J Teknol dan Ind Pangan. 2023;34(1):109–18.

18. Belalcazar LM, Haffner SM, Lang W, Hoogeveen RC, Rushing J, Schwenke DC, et al. Lifestyle intervention and/or statins for the reduction of C-reactive protein in type 2 diabetes: From the look AHEAD study. Obesity. 2013;21(5):944–50.

19. Kumar T. Comparative study between effects of treadmill walking and briskwalking on central obesity in obese and overweight men. J Med Sci Clin Res. 2020;08(02):737–43.

20. Zakharova AN, Milovanova KG, Orlova AA, Dyakova EY, Kalinnikova JG, Kollantay OV, et al. Effects of Treadmill Running at Different Light Cycles in Mice with Metabolic Disorders. Int J Mol Sci. 2023;24(20).

21. Kim KB, Kim K, Kim C, Kang SJ, Kim HJ, Yoon S, et al. Effects of exercise on the body composition and lipid profile of individuals with obesity: A systematic review and meta-analysis. J Obes Metab Syndr. 2019;28(4):278–94.

22. Pierard M, Conotte S, Tassin A, Boutry S, Uzureau P, Boudjeltia KZ, et al. Interactions of exercise training and high-fat diet on adiponectin forms and muscle receptors in mice. Nutr Metab [Internet]. 2016;13(1):1–13.

23. Pons S, Martin V, Portal L, Zini R, Morin D, Berdeaux A, et al. Regular treadmill exercise restores cardioprotective signaling pathways in obese mice independently from improvement in associated co-morbidities. J Mol Cell Cardiol [Internet]. 2013;54(1):82–9.