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Original Article

The Effect of Standardized Mangosteen Peel Extract, Nano-emulsion, Nano-chitosan and Treadmill Exercise on Atherogenic Rat Model

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Abstract

Background : Atherosclerosis, the most common cause of CVD, is associated with oxidative stress and cholesterol. Antioxidant and regular physical exercise have been considered as interventions to dampen the process. The mangosteen peel *(Garcinia mangostana Linn)* is known for containing a high amount of xanthones including α-mangostin with antioxidant effects. The current study investigates the anti-atherogenic potential of mangosteen peel extract in nano-emulsion and nano-chitosan formulations, singly and in combination with treadmill exercise, versus the statin, Atorvastatin.
Methods : A randomized controlled trial was conducted on 30 male Wistar rats, divided into six groups: normal diet control (C1), atherogenic diet control (C2), and four treatment groups receiving an atherogenic diet plus treadmill exercise combined with Atorvastatin (T1), standardized mangosteen extract (T2), mangosteen nano-emulsion (T3), or mangosteen nano-chitosan (T4). The aortic tunica intima and tunica intima-media thickness were measured histologically after 56 days.
Results : The aortic intimal thickness was noticeably higher in the atherogenic diet group (C2: 12.13±1.87 mm) compared to the normal diet group (C1: 4.27±0.75 mm). In the treatment groups, the intimal thickness ranged from 2.97±0.45 mm to 4.17±1.70 mm and showed no significant differences from the normal diet group. A similar pattern was seen in the intima-media thickness, with 145.63±17.12 mm recorded in the normal diet group and values ranging from 106.90±10.41 mm to 135.90±12.63 mm in the treatment groups, and 106.90±10.41 mm to 135.90±12.63 mm in the treatment groups suggesting no significant difference. Survival analysis using Kaplan-Meier curves showed obvious differences between groups ($p < 0.001$). The untreated atherogenic diet group (C2) had the poorest survival, with no rats survived until the end of the study. In contrast, survival improved in all treatment groups, with the Mangosteen Nano-chitosan (T4) group and the normal diet group (C1) achieving the best outcomes, as all rats in these groups survived.
Conclusion: Mangosteen peel extracts, whether in nano-emulsion or nano-chitosan forms, combined with treadmill exercise, showed significant differences in maintaining the survivability of rat with atherogenic-induced diet despite no significant differences in preventing atherogenesis compared to Atorvastatin or a normal diet. Further research is needed to confirm these potential therapeutic effects.

Keywords : Mangosteen skin, nano-emulsion, nano-chitosan, treadmill exercise, atherogenesis

INTRODUCTION

Atherogenesis is the pathobiological process underlying atherosclerosis in cardiovascular disease.[1] Atherosclerosis is a chronic immunoinflammatory, fibroproliferative disease of large and medium-sized arteries initiated by increasing lipids.[2] It is the most common cause of cardiovascular disease, which is the major cause of mortality and morbidity worldwide.[3] In 2016, the global prevalence of coronary artery disease (CAD) was 154 million, accounting for 32.7% of the global cardiovascular disease burden and 2.2% of the overall global disease burden.[4] Atherogenesis consists of three stages, initiation, progression, and complication.[5] The initiation stage is characterized by the recruitment of mononuclear leukocytes to the intimal layer of the vessel wall, induced by oxidized lipoprotein.[5] The progression stage is marked by the accumulation of smooth muscle cells that elaborate extracellular matrix macromolecules.[5] The complication stage is the progression of thrombosis.[5] This disease may progress when the atherothrombotic plaque ruptures, leading to coronary artery occlusion, reduced blood flow, and myocardial ischemia.[6] The main risk factor for atherosclerosis is high plasma cholesterol levels, particularly low-density lipoprotein (LDL), which plays a crucial role in atherogenesis, from plaque formation to plaque destabilization.[6]

The atherogenesis process involves oxidative stress, particularly the oxidation of low-density lipoprotein (LDL), so administering antioxidants[7] and engaging in physical training[8] may reduce oxidative stress and attenuate atherosclerosis formation. Therefore, the administration of anti-oxidants might reduce oxidative cardiovascular injury in atherosclerosis.[9] *Garcinia mangostana Linn* pericarp, usually known as the mangosteen skin, is a tropical fruit that is usually used as a medicinal plant among the people of Southeast Asia.[10] Mangosteen is rich in phenol components, such as xanthone, tannins, and anthocyanins.[11] Xanthone has an antioxidant property to overcome the oxidative stress.[11] The xanthone component comprises α-mangostin, β-mangostin, and γ-mangostin.[12] Predominantly, the α-mangostin, and γ-mangostin, has anti-inflammatory properties.[13] It can decrease the thickness of aortic perivascular adipose tissue, therefore reducing the thickening of the intima media layer, increasing the HDL C levels, and lowering the LDL-C levels, along with the Triglyceride and Total Cholesterol.[14] It was proven to inhibit atherosclerosis by inhibiting LDL oxidation and decreasing ROS in endothelial cells.[15] Drug bioavailability is an important parameter to determine how successful drug molecules pass through pharmacological phases and give the expected effect.[16] Drug bioavailability is primarily influenced by drug solubility, and nanoparticle drug delivery systems, such as nano-emulsions and nano-chitosan, enhance solubility due to their large surface areas.[16] Besides antioxidants, physical exercise is one alternative treatment to reduce the risk factor of atherosclerosis and has the advantage of being cost-effective.[17] The protective effects of physical exercise against coronary disease may involve mechanisms such as improved endothelial function, reduced plaque progression, enhanced collateral formation, and decreased release of inflammatory mediators.[18] Physical exercise can induce cardiac preconditioning, providing sustainable protection against cardiac injury.[19] Regular aerobic exercise can reduce the progression of coronary lesions.[20] Regular aerobic exercise, such as treadmill workouts, improves endothelial function and helps prevent atherosclerosis progression.[21] The primary outcome of this study is to compare the effectiveness of several mangosteen skin extract preparations, which are standardized, nano-emulsion, and nano-chitosan preparations and treadmill exercise for preventing atherogenesis in atherogenic rats. Meanwhile, the secondary outcome of this study is to compare the effectiveness of various preparations of mangosteen peel extract with Atorvastatin, the commonly used drug of choice, in combination with treadmill exercise for inhibiting atherogenesis in rats.

METHODS

A true experimental with randomized control trial was conducted. This study was conducted at the Biomolecular Laboratory of Universitas Islam Sultan Agung Semarang from February to May 2022 for sample maintenance, treatment, and examination. Paraffin block preparation and HE (Hematoxylin-Eosin) staining of histopathologic samples were performed at the Anatomical Pathology Laboratory of Diponegoro University. Ethical clearance was granted by the Ethical Commission for Health Research, Faculty of Medicine, Diponegoro University (No. 51/EC/H/FK-UNDIP/VI/2022).

This study used 30 male Wistar *(Rattus norvegicus)* rats as subjects. The inclusion criteria were healthy male rats, aged 6–8 weeks with active movement. The exclusion criteria were rats that have defects, appeared sick (standing fur, mushy feces), and were dead before the study period. All subjects were simply randomized into six groups and given an adaptation period of wheel and treadmill running at a speed of 12 m/min for 56 days (8 weeks). Five rats were selected randomly and received a standard diet (standard AD-2 pellets and drinking water in bottles orally) for 56 days as the control group 1 (C1). The rest 25 rats were induced with purified atherogenic diet patented product (Envigo®) with high-fat diet formulation (20–23 % BW; 40–45 % kcal from fat), saturated fatty acids (SFA > 60% of total fatty acids), milkfat or butterfat, sucrose (34% by weight), cholesterol (0.2% total), and tap drinking water for 56 days (24–30). Control group 2 (C2) received no additional treatment.

Treatment group 1 (T1) received atorvastatin at a human-adjusted dose of 80 mg/day (1.44 mg/0.5 mL), once daily for 58 days. Treatment group 2 (T2) received mangosteen peel extract (Mastin®) at 800 mg/kgBW/day (0.2 mL), three times daily for 56 days. Treatment group 3 (T3) was given MG-loaded self-nanoemulsion (MNE) at 50 mg/kgBW (4 mL), once daily for 56 days. Treatment group 4 (T4) received MG-loaded self-nanochitosan (MNC) at 50 mg/kgBW (4 mL), once daily for 56 days. Treatment was done individually.

At the end of Day 56, surviving rats were terminated, and their aortic arch and aortic sinus were collected for histopathologic examination, including paraffin blocking, HE staining, and outcome interpretation by two independent blinded anatomic pathologists. For each sample, observations were done four times in four different quadrants. The thickness of the intima and media layers was measured using an oculo-micrometer, with a digital microscope at the Pathology Anatomy Laboratory, Faculty of Medicine, Diponegoro University. Statistical analysis was done with one-way ANOVA with post hoc Tukey Test using Graph pad prism 9.

RESULTS

The flow of study participants is depicted in Figure 1. A total of 30 male Wistar rats were recruited based on the inclusion and exclusion criteria, with all meeting the eligibility requirements. The rats were then randomly divided into six groups: two control groups (C1: normal diet control; C2: atherogenic diet control) and four treatment groups (T1: treadmill exercise with Atorvastatin; T2: treadmill exercise with Mangosteen Extract (ME); T3: treadmill exercise with Mangosteen Nano-emulsion Extract (MNE); T4: treadmill exercise

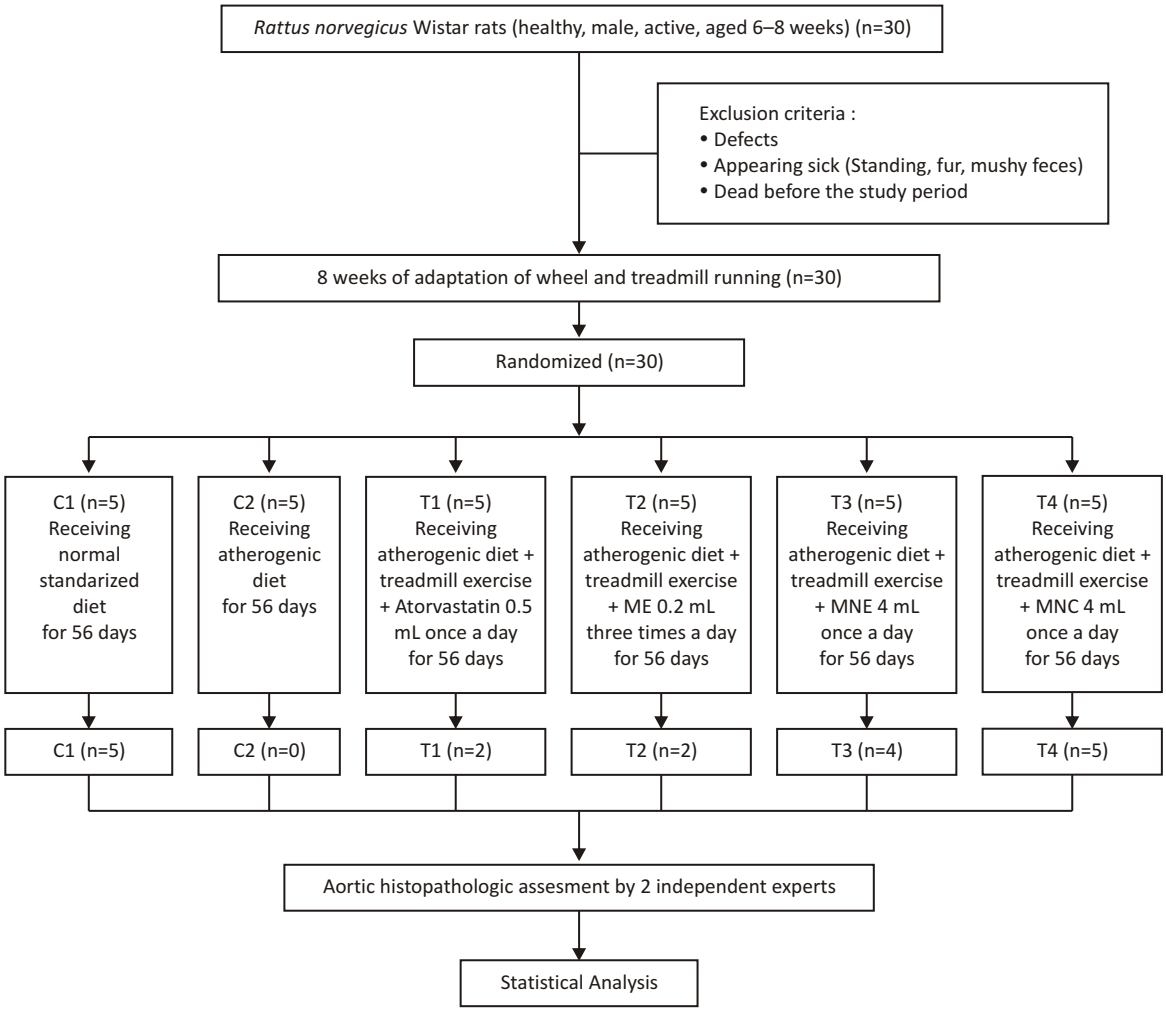

Figure 1. Consort flowchart depicting the progression of the study. Thirty Wistar rats were screened according to inclusion and exclusion criteria, and then randomly allocated into control and intervention groups. The rats were terminated on Day 56 and examined for aortic tunica intima and intima-media thickness through histopathological analysis.

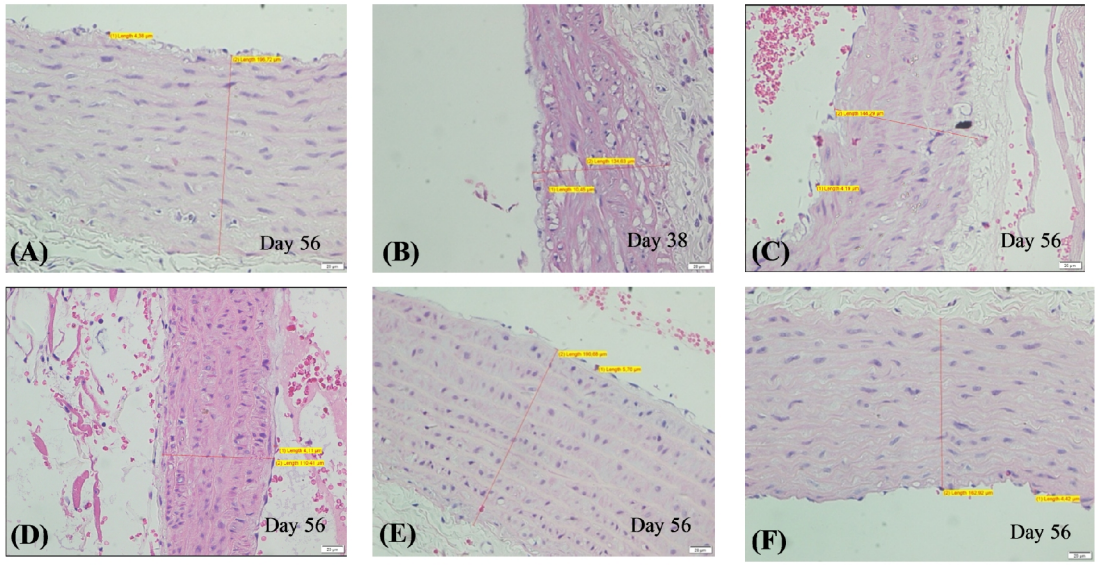

Figure 2. Histopathologic examination of aortic section with HE staining in 400x magnification. Male wistar rats treated with (A) Normal diet, (B) Atherogenic diet, (C) Atherogenic diet + treadmill exercise + Atorvastatin, (D) Atherogenic diet + treadmill exercise + ME, (E) Atherogenic diet + treadmill exercise + MNE, and (F) Atherogenic diet + treadmill exercise + MNC.

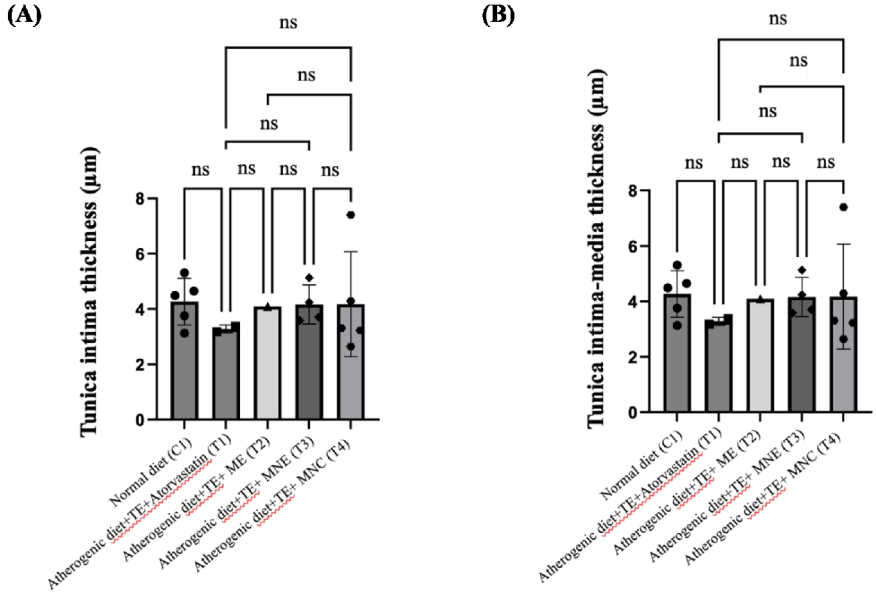

Figure 3. The effect of mangosteen peel extract (ME), mangosteen nano-emulsion extract (MNE), and mangosteen nano-chitosan extract (MNC) on the aortic wall thickness of Wistar rats. (A). Quantitative analysis of aortic tunica intima thickness. (B). Quantitative analysis of aortic tunica intima-media thickness. Wistar rats were given an atherogenic diet and the combination of treadmill exercise (TE) and atorvastatin (n=2) or mangosteen peel extract in ME (n=1), MNE (n=4), MNC (n=5). The thickness of each sample (A and B) was measured from aortic tissue histopathology examination, with averaged data from 4 different fields of view. Results are presented as Mean±SD; scale bar =100 µm.

with Mangosteen Nano-chitosan Extract (MNC)). Each group was monitored over a 56-day period, during which the intervention groups received their respective treatments as described in the methods section.

Survival outcomes varied by group. All rats in the normal diet control group (C1) survived till the end of the study (n=5), whereas none of the rats in the untreated atherogenic diet group (C2) survived (n=0) at the end of the study. Among the treatment groups, two rats survived in both the T1 (n=2) and T2 (n=2) groups, while

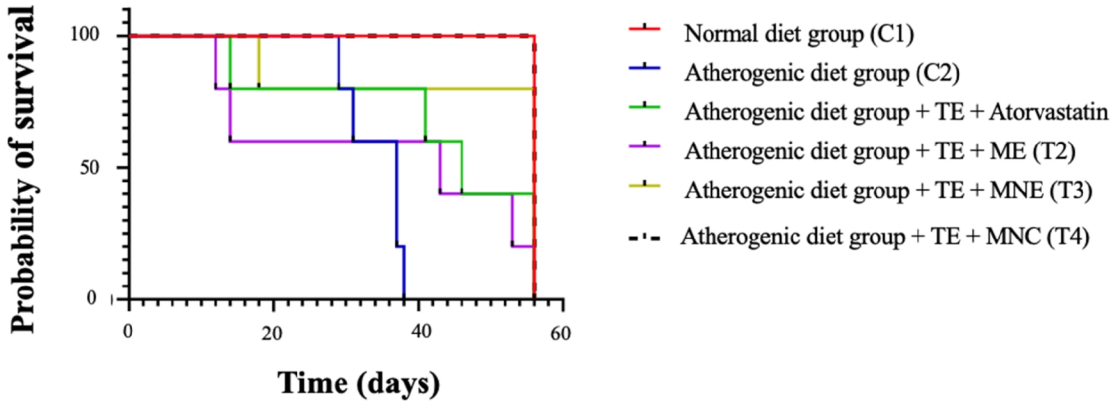

Figure 4. Kaplan-Meier Survival Curves for Rats Undergoing Different Diet Treatments. Kaplan-Meier survival curves were plotted to depict the survival of rats following various dietary interventions. Groups of rats were given either a standard diet or an atherogenic diet, in association with treadmill exercise and diverse therapeutic combinations, namely, atorvastatin, T1; mangosteen extract, ME, T2; mangosteen nano-emulsion extract, MNE, T3; mangosteen nano-chitosan extract, MNC, T4. These Kaplan-Meier survival curves give the probability of survival over time for each respective group, with observation from the lowest to the highest median survival are atherogenic diet grup (C2), followed by atherogenic + ME (T2), atherogenic + atorvastatin, atherogenic + MNE (T3), atherogenic + MNC (T4) and normal diet grup (C1). Log-rank test Mantel-Cox was performed to compare the survival distributions among the groups. *P*-value is less than 0.001, indicating a significantly different survival among the diet treatment groups.

four survived in the T3 group (n=4). The T4 group demonstrated the highest survival rate, with all rats completing the study period (n=5).

The histopathologic examination of male Wistar rats with various interventions is detailed in Figure 2. This analysis was conducted by two independents, blinded anatomical pathologists, ensuring an unbiased assessment. This examination specifically focused on measuring the aortic thickness of the tunica intima and the tunica intima-media across study groups. It was done after 56 days using HE staining, but in rats who died before the study period, the aortic thickness of tunica intima and tunica intima-media was counted before 56 days.

The mean aortic tunica intima thickness in Wistar rats on a normal diet (C1) is 4.27±0.84 µm (Figure 3A) and on an atherogenic diet (C2) is 12.13±2.10 µm (data is not shown in Figure 3 because it was taken before the study period ended). For the groups given an atherogenic diet, all subjects were treated with treadmill exercise with different interventions regarding the group. The rats were terminated and examined by histopathologist after the study endpoint (56 days), showing the tunica intima thickness was as follows: 3.28±0.14 µm in the group treated with Atorvastatin (T1) 4.10 µm in the group treated with ME (T2), 4.16±0.71 µm in the group treated with MNE (T3), and 4.17±1.90 µm in the group treated with MNC (T4). At the study endpoint, the number of study subjects differs, but as compared to the results, statistical analysis showed no significant difference between the normal diet control group (C1) and the atherogenic diet intervention groups treated with treadmill exercise and Atorvastatin (T1), ME (T2), MNE (T3), and MNC (T4) as shown in Figure 3A.

Our observation on the same group as previously mentioned showed that aortic tunica intima-media thickness in Wistar rats with a normal diet (C1) is 145.63±19.14 µm. In the group treated with Atorvastatin (T1) is 107.03±4.66 µm, in the group treated with ME (T2) is 115.56 µm, in the group treated with MNE (T3) is 147.67±13.03 µm, and in the group treated with MNC (T4) is 135.9±14.12 µm (Figure 3B). Statistical analysis showed no significant difference between the normal diet control group (C1) and the atherogenic diet intervention groups treated with treadmill exercise and Atorvastatin (T1) (p=0.0660), ME (T2) (p=0.4171), MNE (T3) (p=0.9996), and MNC (T4) (p=0.8469).

Several study subjects died before the study ended. All rats died in the atherogenic diet group (C2) died with a median survival of 37 days. In a group receiving an atherogenic diet, combined with treadmill exercise and Atorvastatin (T1), three rats died, with a median survival of 46 days. Additionally, in the group receiving an atherogenic diet, treadmill exercise and ME (T2), four rats died, with a median survival of 43 days. In contrast, no deaths were observed in the normal diet group (C1), or in the atherogenic diet groups receiving treadmill exercise combined with MNE (T3) or MNC (T4). These findings are illustrated in Figure 4, which represents the Kaplan-Meier Survival Curves for the study groups.

DISCUSSION

Our study aims to evaluate the effectiveness of mangosteen peel *(Garcinia mangostana linn pericarp)* extract in nano-emulsion and nano-chitosan formulations for preventing atherogenesis, which is measured from the thickness of aortic tunica intima and tunica intima-media thickness. In this study, there is no significant difference in both aortic tunica intima and tunica intima-media thickness between rats receiving a normal diet (C1) and rats receiving an atherogenic diet in combination with treadmill exercise and Atorvastatin (T1), mangosteen peel extract (T2), mangosteen nano-emulsion extract (T3), and mangosteen nano-chitosan extract (T4).

Our study shows a direct relationship between mangosteen peel extracts administration and atherogenesis by examining tunica intima and media of rat aorta. We found that rats receiving an atherogenic diet, treadmill exercise, and various preparations of mangosteen peel extracts (standardized mangosteen extract 800 mg/kgBW/day; Mangosteen Nano-emulsion Extract 50 mg/kgBW; Mangosteen Nano-chitosan Extract 50 mg/kgBW) didn't show statistically significant differences in aortic tunica intima and tunica intima-media thickness compared to the normal diet group. Additionally, we compared the effects of Atorvastatin, a widely used lipid-lowering agent,[22] with various mangosteen skin extract preparations, including standardized herbal medicine (Mastin®), nano-emulsion extract (MNE) and nano-chitosan extract (MNC). We observed that the administration of Atorvastatin didn't result in significant differences in tunica intima and tunica intima-media thickness compared to the various preparations of mangosteen peel extracts. Our findings suggest that these mangosteen preparations do not significantly differ from Atorvastatin in their impact on atherogenesis, highlighting its potential as an adjuvant therapy for patients at risk of atherosclerosis. However, this result had to be interpreted carefully and further evidences are needed to confirm this effect.

Other than involvement in preventing atherogenicity, the effect of mangosteen peel extract on lipid profiles and oxidative stress markers was reported. In one study investigating the effect of mangosteen peel ethanolic extract on hypercholesterol diet-fed Wistar rats found that mangosteen peel ethanolic extract 200 mg/kgBW didn't produce significant results, while at the dose of 400 mg/kgBW, mangosteen extract can lower total cholesterol level and raise HDL level. The most effective dose is 800 mg/kgBW, where at this dose, mangosteen extract improves lipid profile, decreasing H_2O_2 level, NF-KB and iNOS.[23]

The mean aortic tunica intima thickness in Wistar rats on an atherogenic diet (C2) was significantly higher than the normal diet group (C1), confirming the successful induction of atherogenicity. We expected that all rats would survive throughout the study period. However, all the rats in the C2 group died before the end of the study and were therefore excluded from the analysis. It suggests the death of the rats is due to the progression of the disease, which is a total blockage in the aorta following the atherogenesis induction with a high-fat diet, as illustrated in Figure 4, representing the Kaplan-Meier survival curve. This is supported by another study stating that a high-fat diet can induce remarkable cardiotoxicity by promoting cardiac injury.[24] Our study examines a relatively new and understudied drug packaging method which is in nanoparticle size expected to improve drug absorption. However, we acknowledge that our study's limitations include the small number of study subjects and the loss of subjects before the end of the study period. Therefore, the result of our study needs to be interpreted carefully.

CONCLUSION

Our study concludes that atherogenesis in rats receiving the combination of mangosteen peel extracts in any preparations (Mastin®, nano-emulsion, nano-chitosan) along with treadmill exercise didn't differ significantly from rats on a normal diet. Furthermore, when compared to Atorvastatin, a commonly prescribed medication for lowering lipid profiles, no significant atherogenesis changes were found between the Atorvastatin group and mangosteen peel extracts in any preparations (Mastin®, nano-emulsion, nano-chitosan), Suggesting potential usage of mangosteen peel extracts in the prevention of atherogenicity.

Our study warrants further study to explore the potential effect of mangosteen peel extracts in atherosclerotic disease.

REFERENCES

1. Herrmann J, Lerman LO, Mukhopadhyay D, Napoli C, Lerman A. Angiogenesis in atherogenesis. Arterioscler Thromb Vasc Biol. 2006 Sep 1;26(9):1948–57.
2. Falk E. Pathogenesis of atherosclerosis. J Am Coll Cardiol. 2006 Apr 18;47(8 Suppl).
3. Zhao Y, Qu H, Wang Y, Xiao W, Zhang Y, Shi D. Small rodent models of atherosclerosis. Biomed Pharmacother. 2020 Sep 1;129.
4. Bauersachs R, Zeymer U, Brière JB, Marre C, Bowrin K, Huelsebeck M. Burden of Coronary Artery Disease and Peripheral Artery Disease: A Literature Review. Cardiovasc Ther. 2019;2019.
5. Libby P. Changing concepts of atherogenesis. J Intern Med. 2000 Mar 1;247(3):349–58.
6. Severino P, D'amato A, Pucci M, Infusino F, Adamo F, Birtolo LI, *et al.* Ischemic Heart Disease Pathophysiology Paradigms Overview: From Plaque Activation to Microvascular Dysfunction. Int J Mol Sci. 2020 Nov 1;21(21):1–30.
7. Schwenke, D. C. (1998). *Antioxidants and atherogenesis*. https://doi.org/10.1016/S0955-2863(98)00046-1

8. Peñín-Grandes S, Martín-Hernández J, Valenzuela PL, López-Ortiz S, Pinto-Fraga J, Solá L del R, *et al*. Exercise and the hallmarks of peripheral arterial disease. Atherosclerosis. 2022 Jun 1;350:41–50.
9. Boonprom P, Boonla O, Chayaburakul K, Welbat JU, Pannangpetch P, Kukongviriyapan U, *et al*. Garcinia mangostana pericarp extract protects against oxidative stress and cardiovascular remodeling via suppression of p47phox and iNOS in nitric oxide deficient rats. Ann Anat. 2017 Jul 1;212:27–36.
10. Sari N, Katanasaka Y, Sugiyama Y, Miyazaki Y, Sunagawa Y, Funamoto M, *et al*. Alpha Mangostin Derived from Garcinia magostana Linn Ameliorates Cardiomyocyte Hypertrophy and Fibroblast Phenotypes in Vitro. Biol Pharm Bull. 2021 Oct 1;44(10):1465–72.
11. Wihastuti TA, Aini FN, Tjahjono CT, Heriansyah T. Dietary Ethanolic Extract of Mangosteen pericarp Reduces VCAM-1, Perivascular Adipose Tissue and Aortic Intimal Medial Thickness in Hypercholesterolemic Rat Model. Open Access Maced J Med Sci. 2019 Oct 15;7(19):3158–63.
12. Alam M, Rashid S, Fatima K, Adnan M, Shafie A, Akhtar MS, *et al*. Biochemical features and therapeutic potential of α-Mangostin: Mechanism of action, medicinal values, and health benefits. Biomedicine & Pharmacotherapy. 2023 Jul 1;163:114710.
13. Setiawan AA, Budiman J, Prasetyo A. Anti-Inflammatory Potency of Mangosteen (Garcinia mangostana L.): A Systematic Review. Open Access Maced J Med Sci. 2023 Jan 2 [cited 2024 Aug 29];11(F):58–66.
14. Kumar V, Bhatt PC, Kaithwas G, Rashid M, Al-abbasi FA, Khan JAJ, *et al*. α-Mangostin Mediated Pharmacological Modulation of Hepatic Carbohydrate Metabolism in Diabetes Induced Wistar Rat. Beni Suef Univ J Basic Appl Sci. 2016 Sep;5(3):255–76.
15. Wihastuti TA, Sargowo D, Heriansyah T, Aziza YE, Puspitarini D, Iwana AN, *et al*. The reduction of aorta histopathological images through inhibition of reactive oxygen species formation in hypercholesterolemia rattus norvegicus treated with polysaccharide peptide of Ganoderma lucidum. Iran J Basic Med Sci. 2015;18(5):514.
16. Wathoni N, Rusdin A, Motoyama K, Joni IM, Lesmana R, Muchtaridi M. Nanoparticle Drug Delivery Systems for α-Mangostin. Nanotechnol Sci Appl. 2020;13:23.
17. Chacon D, Fiani B. A Review of Mechanisms on the Beneficial Effect of Exercise on Atherosclerosis. Cureus. 2020 Nov 23;12(11).
18. Bowles DK, Laughlin MH. Mechanism of Beneficial Effects of Physical Activity on Atherosclerosis and Coronary Heart Disease: Mechanism of beneficial effects of physical activity on atherosclerosis and coronary heart disease. J Appl Physiol. 2011 Jul;111(1):308.
19. Frasier CR, Moore RL, Brown DA. Exercise-induced cardiac preconditioning: how exercise protects your achy-breaky heart. J Appl Physiol (1985). 2011 Sep [cited 2024 Aug 29];111(3):905–15.
20. Niebauer J, Cooke JP. Cardiovascular effects of exercise: role of endothelial shear stress. J Am Coll Cardiol. 1996;28(7):1652–60.
21. Mika P, Konik A, Januszek R, Petriczek T, Mika A, Nowobilski R, *et al*. Comparison of two treadmill training programs on walking ability and endothelial function in intermittent claudication. Int J Cardiol. 2013 Sep 30;168(2):838–42.
22. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, *et al*. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. 2019 Jun 18;139(25):E1082–143.
23. Wihastuti TA, Widodo MA, Heriansyah T, Sari NAK. Study of the inhibition effect of ethanolic extract of mangosteen pericarp on atherogenesis in hypercholesterolemic rat. Asian Pac J Trop Dis. 2015 Oct 1;5(10):830–4.
24. Feriani A, Bizzarri M, Tir M, Aldawood N, Alobaid H, Allagui MS, *et al*. High-fat diet-induced aggravation of cardiovascular impairment in permethrin-treated Wistar rats. Ecotoxicol Environ Saf. 2021 Oct 1;222:112461.