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ORIGINAL RESEARCH

The Effects of Red Palm Oil, Koja Bay Leaves, and Passion Fruit Seeds Formulation on Antioxidant Activity, Antihyperlipidemia, BDNF, and Lipase Enzyme Activity on Sprague-Dawley Rats

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Background: Local wisdom food ingredients in North Sumatra, Indonesia, are a source of phenolics which have antioxidant, antihyperlipidemia, neuronal survival, and growth. Administering products with antioxidant properties can provide a supporting effect in preventing inflammation and neurodegenerative process.

Objective: The main objective of this study was to analyze the formulation of red palm oil (*Elaeis guineensis* Jacq), koja bay leaves (*Murraya koenigii* L Spreng), and passion fruit seeds (*Passiflora edulis* Sims) to improve lipid profile, antioxidant activity, Brain-Derived Neurotrophic Factor (BDNF), and lipase enzyme activity of Sprague-Dawley rats.

Methods: This study was an in vivo and pre-post experimental study, starting with analyzing flavonoid of the three extract ingredients, then tested by giving it to rats for 14 days and ending with induction administration of lipopolysaccharide (LPS) for two days. This pre-post study on animals involved 36 rats divided into 6 groups. At the end of the study, termination and examination of malondialdehyde, lipid profile, glucose, BDNF, lipase enzyme activity and histopathological examination were carried out.

Results: The study results showed that there were significant values in several parameters, which were body weight, LDL, LDL/HDL ratio, BDNF, and lipase enzyme activity especially in the group of rats given LPS and the group with high calories-fat-protein. This study showed that there were significant differences in body weight, LDL levels, and LDL/HDL ratio in each group of rats, especially in the group given the formulation of the three extract ingredients, the significant dose showed in 300mg/kg body weight ($p < 0.001$).

Conclusion: The formulation of red palm oil, koja bay leaves, and passion fruit seeds showed significant reduction in LDL levels, LDL/HDL ratio, BDNF, and lipase enzyme activity.

Keywords: atheromatic, leaves, plaque analysis, preventive, oxidized, LDL, nutrition, oil, seed

Introduction

Coronary artery disease (CAD) is a form of atherogenesis process with manifestations of atherosclerosis.[1,2] This disease process does not only occur during the aging process but begins at an early age.[1,3] Coronary artery disease is not only the third leading cause of death worldwide but also the leading cause in the United States.[4] Estimated 1 in 5 deaths and accounts for approximately with 17.8 million deaths annually.[4] Several risk factors for CAD include a high-calorie diet, diabetes mellitus, hypertension, smoking habits, apart from that the infection process can cause atherosclerosis or

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accelerate the atherogenesis process.[5–8] There are several influences of microorganism infection on the initiation and development of atherosclerosis.[9–11]

These pathogenic microorganisms develop and cause inflammation and prooxidant reactions in infected body cells.[1,12–15] The theory of bacterial infection as the cause of atherosclerosis is known to have a mechanism through microbial symbiosis and inflammatory stimuli, the direct mechanism is the infection of blood vessel cells originating from microorganisms that are sources of infection. Indirect mechanisms also occur through the activation of inflammatory cytokines.[12,13] The causes that can cause changes in lipid profiles are mainly due to an imbalance between energy output and input resulting from a lack of physical activity and unbalanced food intake. The meaning of unbalanced intake is an excessive amount of calories, high saturated fat intake, and consumption of simple sugars for a long time. This metabolic disorder process can cause dyslipidemia which is summarized in it, which were an increase in total cholesterol, low density lipoprotein (LDL), and triglycerides, and a decrease in high density lipoprotein (HDL) levels.[6,16,17] Other parameters that can indicate the risk of metabolic disorders are increased levels of malondialdehyde (MDA), this increase is caused by the inflammatory process. This process has a different mechanism from the inflammatory process, the atherosclerosis process caused by increased MDA levels is the result of the lipid peroxidation process that continues due to the accumulation of free radical reactions.[16,18]

Acute infections such as those instigated by gram-positive bacteria, gram-negative bacteria, viruses, tuberculosis, and parasites, lead to changes in lipid metabolism.[9,19,20] These changes actually cause a decrease in total cholesterol, LDL-C, and HDL-C levels, while plasma triglyceride levels may see an increase or display abnormal values, especially in instances of malnutrition due to underweight conditions.[1,2] LDL levels often decrease but the prevalence of small dense LDL, which is lipoprotein (a), increases due to the exchange of triglycerides from triglyceride-rich lipoproteins to LDL followed by the hydrolysis of triglycerides.[1,21,22] Beyond affecting serum lipid levels, inflammation also adversely influences the function of lipoproteins.[1,16] The susceptibility of LDL to oxidation increases as the capacity of HDL to inhibit LDL oxidation is compromised.[23,24]

The infection process consists of acute infection of the blood vessel walls, which can occur in the direct mechanism category, namely plaque rupture and thrombosis.[19,20] The indirect mechanism is related to inflammation in non-vascular sites, which can cause increased cytokine secretion.[20,21] Another form of infection model can be caused by lipopolysaccharide (LPS) compounds, this compound is a component of the cell wall of gram-negative bacteria that can precipitate an inflammatory response.[22,23] This compound triggers a systemic inflammatory response syndrome, characterized by a variety of symptoms of infection. These symptoms include fever due to infection, tachycardia, hypotension, intravascular coagulation, angioedema, and organ failure.[11,23]

Cardiovascular disease pathogenesis is not only influenced by free radicals or infections but there is another factor that influences it, which is Brain-Derived Neurotrophic Factor (BDNF), found in large amounts in brain tissue and peripheral nerves, this neurotrophic factor provides a protective effect against ischemic brain injury.[24] Apart from that, BDNF plays an important role in cardiovascular function, with the angiogenesis reconstruction pathway.[24,25] The cellular mechanism when damage occurs is myocardial cells through the efferent nerve feedback pathway to the central nervous system causing cardiac ischemic damage conditions, myocardial cells through the efferent nerve feedback mechanism to increase BDNF expression in nerve cells, as well as an increase in BDNF levels in the central nervous system. This process will cause an increase in BDNF levels in the central nervous system which is reflected in the nerve cells. Furthermore, increased BDNF levels in the peripheral blood will activate molecular myocardial reconstruction related to angiogenesis and ischemic myocardial repair.[25,26]

Local food plants, especially from North Sumatra (Indonesia), has various abilities to prevent inflammatory processes, including antioxidant and anti-inflammatory formulations to overcome this problem, which originate from local wisdom, namely of red palm oil (*Elaeis guineensis* Jacq), koja bay leaves (*Murraya koenigii* L Spreng), and passion fruit seeds (*Passiflora edulis* Sims).[27–29] Phenolics found in red palm oil are important antioxidants because of their elevated redox potential, possible actions of phenolics are to function as chelating ions agents, captures free radicals, especially superoxide, peroxyl, and hydroxyl radicals thereby inhibiting DNA damage and lipid peroxidation, which can cause membrane damage, it helps the body fight excess free radicals that can potentially cause oxidative stress.[30,31] Potential

options for preventing and treating cellular problems caused by oxidative stress are antioxidants that accumulate in cellular tissues, including nerve cells in the body.[28]

The formula of red palm oil, koja bay leaves, and passion fruit seeds was high sources of water-soluble phenolics, which have antioxidant and anti-inflammatory properties, which is characterized by several features, including a decrease in the levels of inflammatory parameters and the activation of antioxidant activity, but the effect is not yet known.[32] Curry leaves, namely koja bay leaves are leaves distributed in Indonesia, which contain antioxidants.[32–34] The presence of several important phytochemicals, minerals, and minerals in this substance confers several beneficial properties, including antidiabetic, antioxidant, antibacterial, anti-inflammatory, and anti-carcinogenic effects.[32–34]

Passion fruit seeds are rarely used in North Sumatra and various surrounding areas, generally passion fruit seeds are discarded because some industries only use passion fruit flesh to be processed into syrup. There is no local industry that processes passion fruit seeds; however, passion fruit seeds are strong antioxidants that are very easy to find on local plantations and are part of the local wisdom of the mountains of North Sumatra.[32,33] Piceatannol (PIC, 3,3′,4′,5-trans-tetrahydroxystilbene) is a major bioactive chemical found in *P. edulis* seeds. It is an analogue of resveratrol.[26,27] Resveratrol exhibits a preventative effect on cardiovascular disorders and certain cancers. It has antiinflammatory properties, offers protection against sunlight, improves skin moisture, and shows effectiveness for diabetes. The use of plant materials in traditional medicine as an important ingredient in synthesizing drugs in the form of potions plays an important role because of its therapeutic action.[32,34]

This study aims to identify whether the formulation of the three extract ingredients can improve lipid profiles, reduce glucose levels, and have antioxidant properties in experimental rats with LPS induction. This research will test the formulation of the three extract ingredients on experimental rats for 2 weeks given a high calorie-fat intake and then given LPS intervention for 2 days. The assessments carried out were an examination of the lipid profile, LDL/HDL ratio, glucose levels, MDA levels, and histopathological examination. By combining several antioxidant and antihyperlipidemic effects derived from three plants with various types of antioxidants, it is hoped that it can provide a better synergistic effect.

Materials and Methods

This study is an in vivo and pre-post experimental study using a post-test only control group design. This design was chosen because both experimental animals and interventions are more controlled, measurable and reliable. In this study, randomization was carried out where the experimental animals were randomized with the same chance of receiving the test procedure.

Plant Material, Extraction, and Formulation

Plant determination was carried out by Herbarium Medanense (MEDA), Herbarium Universitas Sumatera Utara (USU), botanist Prof. Dr. Etti Sartina Siregar, SSi, MSi. Determination number 2668/MEDA/2024, stating Red Palm Seeds, Kingdom: Plantae, Division: Spermatophyta, Class: Monocotyledonae, Ordo: Arecales, Family: Arecaceae, Genus: Elaeis, Species: Elaeis guineensis Jacq.

Determination number 2669/MEDA/2024, stating Koja Bay Leaves, Kingdom: Plantae, Division: Spermatophyta, Class: Dicotyledonae, Ordo: Sapindales, Family: Rutaceae, Genus: Murraya, Species: *Murraya koenigii* (L) Spreng.

Determination number 2670/MEDA/2024, stating Passion Fruit Seeds, Kingdom: Plantae, Division: Spermatophyta, Class: Dicotyledonae, Ordo: Malpighiales, Family: Passifloraceae, Genus: Passiflora, Species: *Passiflora edulis* Sims.

An amount of 1-kg of fresh koja bay leaves and passion fruit seeds were collected in June 2023 from plantations in North Sumatra and Aceh, Indonesia. The collected leaves and seeds were cleaned of impurities, washed under running water, and dried. The drying process comprised an initial open-air stage shielded from direct sunlight and a secondary stage in an oven at 50°C. The dried seeds were then processed into a fine powder using a blender and sifted through a 20-mesh sieve. The resulting powder was stored in a clean, tightly sealed container to maintain its quality. Both powders will be mixed with red palm oil, which was collected and produced from red palm oil plantations in North Sumatra, Indonesia. The formula which containing koja bay leaves and passion fruit seeds powder was prepared by diluting the

powder into 100 mL red palm oil (ratio 1:1:1), which was indirectly orally administered to rats. The solution was given every day at a predetermined dose, no oral toxicity was report.[35]

Method for Determining Total Flavonoid Levels

The total flavonoid content of the extract determined using the colorimetric method with aluminum chloride (AlCl3), quercetin was used as a standard compound. A standard curve was created using a series of concentrations of 4.6, 8,10,12 PPM. The equation y = 0.0707x – 0.0064 (R2 = 0.9998) was obtained where y is the absorbance at 423.5 nm and x is the quercetin concentration in µg/mL. The total flavonoid content of the extract is presented as quercetin equivalent per gram of extract (mg/g extract). Standard curve was generated by utilizing a range of concentrations consisting of three extract ingredients with a series of concentrations.

Experimental Animals

Healthy adult Sprague-Dawley rats purchased from The Pharmacology Laboratory Faculty of Medicine, Padjadjaran University, Bandung, West Java, Indonesia, that were nulliparous, weighing 150–160 g, and aged 3–4 months were used. The animals were used in stainless steel cages. In a standard tropical environment, treatment is made like daily life with alternating exposure to light and darkness for 12 hours, temperature conditions are set at 25–27°C, and relative humidity is 40–60%.

The acclimatization process was carried out in the laboratory for 7 days, the aim of which was to adapt the laboratory environment to the natural environment of Wistar rats. Wistar rats were given standard feed containing high calories and high protein-fat and were then divided according to the predetermined intervention.

This study used 36 healthy Wistar rats that exhibited signs of vitality, such as active feeding and drinking. Additionally, the rats were assessed for any physical abnormalities, such as wounds, bodily flaws, or hair loss, and none were observed based on their morphological appearance. Wistar rats that exhibit any form of disease, whether caused by infection or not, within the first 7 days of acclimatisation are ineligible for participation in the research.

All Wistar rats are marked with a number on their fur, which will be used in a simple randomization process. The next step is to make folded paper containing the number of each mouse, then carry out a drawing process to divide the rats into 6 intervention groups, each intervention group consisting of six rats. The criterion for dropping out of the test is if the mouse experiences illness or death so that it cannot fulfill the research procedures.

Intervention Group Division

Rats were divided randomly (randomization) and then grouped as follows, namely group 1: negative control (rats without any intervention), group 2: positive control (rats were only given LPS induction), group 3: high protein-fat control (rats were only given high calorie- fat-protein intervention), group 4: LPS induction and formula administration of 100 mg/kg body weight, group 5: LPS induction and formula administration of 300 mg/kg body weight, and group 6: LPS induction and formula administration of 500 mg/kg body weight.

Lipopolysaccharide Injection

Lipopolysaccharide (LPS) injection was carried out intrapulmonally at a dose of 1 µg/mL into the lower end of the left lung of the rat. Escherichia coli Lipopolysaccharide (LPS) (O111:B4), hexadecyl trimethylammonium bromide (HTAB), KH2PO4, K2HPO4, O-dianisidine, sterile distilled water, phosphoric acid, and Tween 20 were purchased from Sigma Aldrich, which function as pulmonary infection agents and modulate immune response. Intrapulmonary LPS injection was carried out on day 15 and 16, respectively (for 2 days). The increased response of the body's immune system to exposure to lipopolysaccharide causes the activation of mast cells, macrophages and neutrophils to release proteases. Protease is an enzyme that is proteolytic as a defense response to the respiratory tract. Increased protease production leads to exfoliation of respiratory tract epithelial cells.

Statistical Analysis

The data will be analyzed using the Statistical Package for Social Science (SPSS version 11.5) program. The statistical tests used are to determine whether the data has a normal distribution or not analytically, the Kolmogorov Smirnov test is

used. The p value <0.05, then the distribution is not normal, if the distribution is not normal, the median and range (minimum-maximum) are used. To analyze data between the two groups, the independent *t*-test is used. The test that will be carried out is the repeated ANOVA test to analyze comparative numerical variables of more than two paired groups. The further test that will be carried out is Post Hoc LSD which is used to determine whether a group has a significant difference from other groups.

Ethical Approval

All procedures were performed adhered to the European Community Guidelines (EEC Directive 1986; 86/609 / EEC) and were authorised by The official Ethics Committee under The Universitas Sumatera Utara organization. This ethics committee is located at The Universitas Sumatera Utara education center, Medan, Indonesia, with the decision letter number: 1035/KEPK/USU/2023.

Results

This research is an in vivo and pre-post experimental study of formulation of the three extract ingredients originating from local wisdom of the North Sumatra region, Indonesia, which were red palm oil, koja bay leaves, and passion fruit seeds in pre-post experimental study. This study began by examining the flavonoid content of each compound, then the study was carried out on experimental animals. The intervention study was carried out by giving the formulation of the three extract ingredients to the experimental animals for 14 days, followed by administering LPS for two days, after which the animals were terminated.

The flavonoid analysis was performed by comparing the samples to the antioxidant quercetin, which served as a standard, based on the equation y = 0.0707x – 0.0064 was obtained with a regression of 0.9998 in the standard solution. After carrying out calculations, a value was obtained for the total flavonoid content of the three samples extract. Table 1 shows significant differences in total flavonoid (TF) levels between red palm oil, koja bay leaf extract, and passion fruit seeds. The passion fruit seed extract has the highest TF content (517.11 ± 3.43 mg/g), followed by the koja bay leaf extract (200.83 ± 3.78 mg/g). The extract from red palm oil exhibited the lowest TF level (75.78 ± 10.76 mg/g, corresponding to 3.78 ug/L).

Table 2 showed the changes in mouse body weight before and after the study. The study showed significant changes in all groups before and after intervention. Based on the study results, it showed that the lowest body weight was found in the group treated with high calorie-fat-protein intake. The group with the highest average weight difference was group 6, with a mean of 65.83 ± 8.01 grams. Conversely, the group with the smallest rise in average weight difference was group 2, which received only LPS intervention. The mean differences for each group showed significant differences, especially in group 1 vs 2 (mean difference: 33.5; CI95%: 13.96 to 53.04, p = 0.001), group 2 vs 3 (mean difference: −24.67; CI95%: −44.2 to −5.13, p = 0.015), group 2 vs 4 (mean difference: −22.85; CI95%: -42.37 to −3.30, p = 0.023), and group 2 vs 6 (mean difference: −34.83; CI95%: −54.37 to −15.30, p = 0.001). The results of this study showed a significant increase in the difference in body weight pre and post intervention.

Table 3 shows the mean levels of lipid profile, MDA and glucose examinations, significant differences were seen in the intervention groups, especially in the results of LDL examinations and the LDL/HDL ratio. The results of this study showed that intervention for 14 days and exposure to LPS for 2 days showed a significant decrease in these two parameters. These results are presented more clearly in Table 4. Table 4 shows the post hoc LSD (least significant

Table 1 Total Flavonoid Content of Red Palm Oil, Koja Bay Leaf Extract, and Passion Fruit Seed Extract

Samples	Mean of Flavonoid Total (mg/g±SD)
Red palm oil	75.78±10.76
Koja bay leaf extract	200.83±3.78
Passion fruit seed extract	517.11±3.43

Abbreviation: SD, standard deviation.

Table 2 The Effect of Red Palm Oil, Koja Bay Leaf, and Passion Fruit Seed Formulation in Body Weight Pre and Post Intervention in Each Group

Group	Intervention	Body Weight (gram)		*p*-value
		Pre Intervention Mean ± SD	Post Intervention Mean ± SD	
1	Rats without any intervention	197.17±4.02	261.67±35.16	0.009*
2	Rats were only given LPS induction	193.17±5.85	224.17±5.56	0.001*
3	Rats were only given high calorie-fat-protein intervention	192.33±6.28	248.0±6.42	0.001*
4	LPS induction and formula administration of 100 g/kg body weight	193.33±6.12	247.17±4.26	0.001*
5	LPS induction and formula administration of 300 g /kg body weight	193.17±5.19	242.67±4.23	0.001*
6	LPS induction and formula administration of 500 g/kg body weight	196.83±3.71	262.67±7.53	0.001*

Notes: *significance= $p < 0.05$. Statistic test: T-dependent analysis.
Abbreviations: SD, standard deviation; LPS, lipopolysaccharide.

Table 3 The Effect of Red Palm Oil, Koja Bay Leaf, and Passion Fruit Seed Formulation on All Parameters

Parameters \ Group	1	2	3	4	5	6	*p*-value
Total cholesterol (mg/dL)	61.83±10.68	73.5±5.24	70.33±9.48	51.83±7.08	52.17±10.63	41.17±2.19	0.212
LDL (mg/dL)	7.67±1.21	12.17±6.96	13.83±2.99	9.0±3.16	5.83±2.13	4.67±2.80	0.001*
HDL (mg/dL)	24.17±5.84	19.17±9.70	22.83±3.12	19.33±1.86	19.33±3.26	15.50±8.09	0.201
Ratio LDL/HDL	0.33±0.08	0.60±0.13	0.53±0.24	0.38±0.09	0.32±0.14	0.32±0.12	0.005*
Triglyceride (mg/dL)	82.0±18.25	89.0±67.34	130.67±69.26	117.33±93.16	77.67±21.35	67.17±36.99	0.388
Malondialdehyde (mg/L)	8.05±1.44	10.97±6.88	10.58±0.69	11.13±2.06	12.09±2.77	8.73±4.34	0.399
Glucose (mg/dL)	68.33±12.48	107.5±78.97	93.17±22.35	104.17±13.22	100.33±20.57	82.83±46.44	0.545
BDNF (ng/mL)	0.74±0.14	1.06±0.22	0.84±0.33	0.72±0.15	0.64±0.13	0.81±0.16	0.023*
Lipase enzyme activity (U/L)	0.09±0.2	0.08±0.04	0.12±0.02	0.06±0.03	0.06±0.02	0.08±0.04	0.027*

Notes: *significance= $p < 0.05$. Statistic test: ANOVA analysis. Group: (1) Rats without any intervention. (2) Rats were only given LPS induction. (3) Rats were only given high calorie-fat-protein intervention. (4) LPS induction and formula administration of 100 mg/kg body weight. (5) LPS induction and formula administration of 300 mg/kg body weight. (6) LPS induction and formula administration of 500 mg/kg body weight.
Abbreviations: BDNF, brain-derived neurotrophic factor; HDL, high density lipoprotein; LPS, lipopolysaccharide; LDL, low density lipoprotein; MDA, malondialdehyde; SD, standard deviation.

Table 4 Post-Hoc LSD Analysis in Body Weight, LDL Levels, LDL/HDL Ratio, BDNF, and Lipase Enzyme Activity Post Intervention

	Mean Difference	*p*-value	CI 95%	
			Minimum	Maximum
Post intervention body weight (gram)				
Group 1 vs 2	37.50	0.001	19.48	55.52
Group 1 vs 5	19.0	0.039	0.98	37.02
Group 2 vs 3	–23.83	0.001	–55.52	–19.48
Group 2 vs 4	–23.0	0.011	–41.85	–5.81
Group 2 vs 5	–18.50	0.045	–36.52	–0.48
Group 2 vs 6	–38.50	0.001	–56.52	–20.48
Group 5 vs 6	–20.0	0.031	–38.02	–1.98
LDL (mg/dL)				
Group 1 vs 2	–4.5	0.043	–8.84	–0.16
Group 1 vs 3	–6.17	0.007	–10.51	–1.82
Group 2 vs 5	6.33	0.006	1.99	10.68
Group 2 vs 6	7.50	0.001	3.16	11.84
Group 3 vs 4	4.83	0.03	0.49	9.18
Group 3 vs 5	8.00	0.001	3.66	12.34
Group 3 vs 6	9.17	0.001	4.82	13.51

(*Continued*)

Table 4 (Continued).

	Mean Difference	*p*-value	CI 95%	
			Minimum	Maximum
LDL/HDL ratio				
Group 1 vs 2	–0.27	0.003	–0.44	–0.09
Group 1 vs 3	–0.20	0.023	–0.37	–0.03
Group 2 vs 4	0.22	0.015	0.05	0.39
Group 2 vs 5	0.28	0.002	0.11	0.45
Group 2 vs 6	0.28	0.023	0.11	0.45
Group 3 vs 5	0.22	0.015	0.05	0.39
Group 3 vs 6	0.22	0.015	0.05	0.39
BDNF (ng/mL)				
Group 1 vs 2	–0.32	0.01	–0.56	–0.08
Group 2 vs 4	0.34	0.007	0.097	0.573
Group 2 vs 5	0.42	0.001	0.183	0.66
Group 2 vs 6	0.25	0.04	0.012	0.488
Lipase enzyme activity (U/L)				
Group 2 vs 3	–0.33	0.046	–0.066	–0.006
Group 3 vs 4	–0.052	0.003	0.019	0.084
Group 3 vs 5	–0.052	0.003	0.019	0.084
Group 3 vs 6	0.038	0.023	0.006	0.071

Notes: Statistic test: Post hoc LSD analysis.
Abbreviations: BDNF, Brain-derived Neurofactor; CI, confidence interval; LSD, least significant difference; LDL, low density lipoprotein; HDL, high density lipoprotein; vs, versus.

difference) analysis of all groups for significant results, namely post-intervention body weight, LDL levels, and LDL/HDL ratio. These results show a significant difference in post-intervention body weight, especially in group 2 vs (versus) 6.

Meanwhile, the largest significant reduction in LDL levels was shown in group 3 vs 6, this shows that the group treated with high calorie-fat-protein intake showed a difference in reduction compared to the group treated with LPS induction and administration of 500 g/kg body weight of the formulation of the three extract ingredients. The smallest difference in reduction was in group 3 vs 4, which showed the smallest difference, this shows that changes were already visible in the LPS induction and administration of 100 g/kg body weight group.

The LDL/HDL ratio also showed significant differences between groups, namely in groups 2 vs 4 and 3 vs 6, which showed differences in the administration of the formulation solution for the three ingredients. The smallest doses showed a significant difference, namely in the group with the minimum difference, namely the LPS induction and administration of 100 g/kg body weight group.

Figures 1–3 showed the histopathological picture of the aortic blood vessels. It can be observed that the histopathological picture of the mouse aorta in normal conditions shows that the layers of the aortic wall are neatly arranged, the vascular smooth muscle cells and endothelial cells are still neatly arranged Rats models of hyperlipidemia and atherosclerosis that have been fed a high-calorie-protein-fat diet show a disorganized layer of endothelial cells. Smooth muscle cells in the tunica intima begin to become irregular, and vacuoles (vacuolization) occur. In atherogenesis, the formation of atheroma plaque on the aortic wall which almost fills the aortic lumen will narrow the diameter of the aortic lumen. The increasingly narrow diameter of the aortic lumen causes blood flow to become blocked.

In all figures in this study, no vacuoles or vacuolization occurred. Rats induced by high calorie-fat-protein diets also did not show endothelial dysfunction and the formation of atheroma plaques. The results of analysis of the wall thickness and lumen diameter of the rat aorta after administering formulation of the three ingredients with varying doses for 14 days did not show significant differences between interventions.

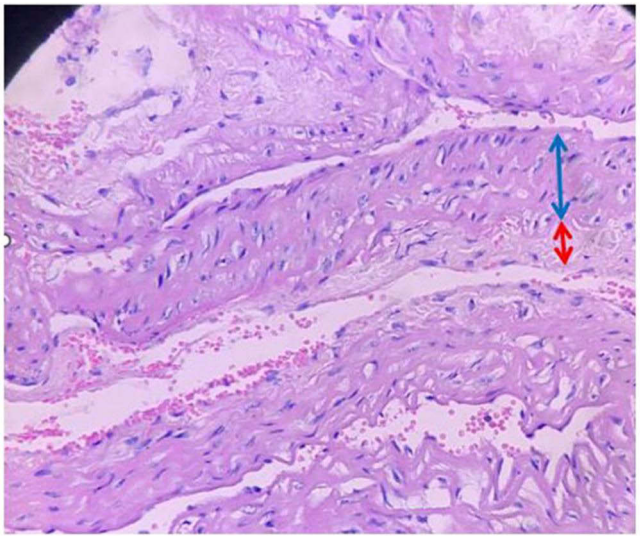

Figure 1 HIstopatology of the rat aorta in high calorie-fat-protein diet (red arrow: atheromatous plaque, blue arrow: tunica media).

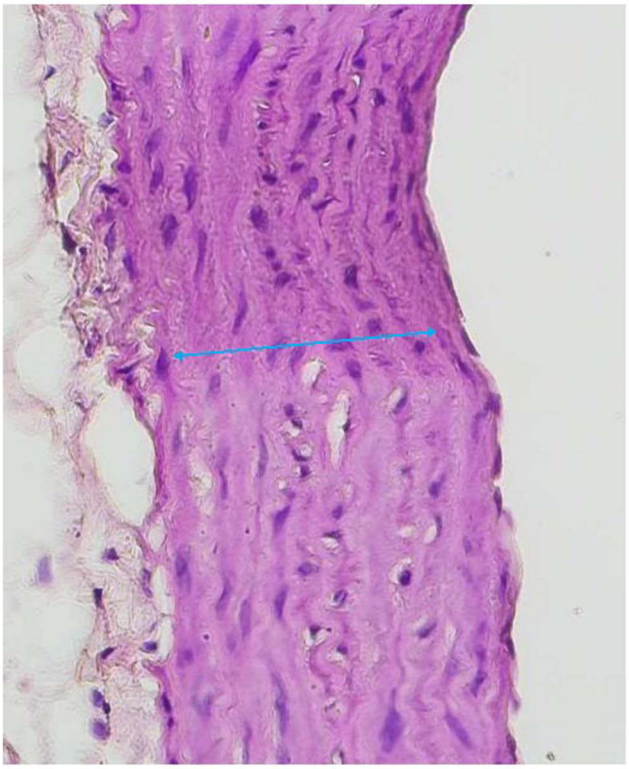

Figure 2 Histopatology of the rat aorta in group 3 (blue arrow: tunica media).

Discussion

This study aims to analyze the formulation of red palm oil, koja bay leaves, and passion fruit seeds to improve lipid profile, antioxidant activity, Brain-Derived Neurotrophic Factor (BDNF), and lipase enzyme activity of Sprague-Dawley rats. Initial study on the flavonoid content of the three ingredients shows that the highest content is passion fruit seeds. This level is among the highest levels compared to previous studies. Passion fruit seed containing piceatannol, has

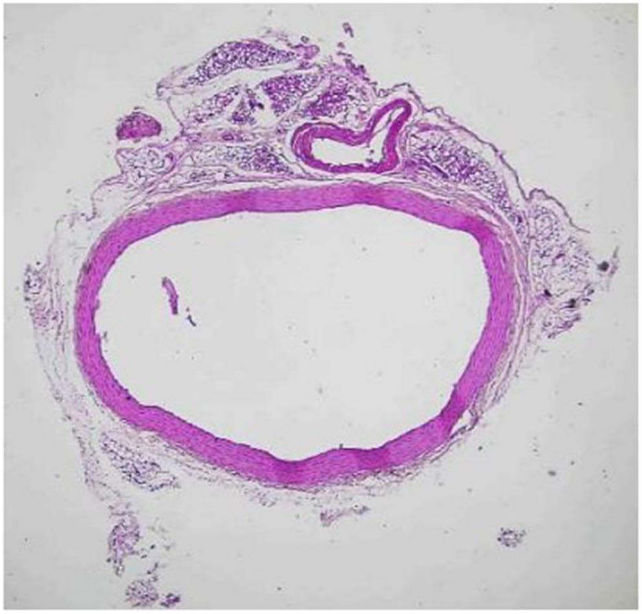

Figure 3 Histopatology of the rat aorta in group 6.

antioxidant effects based on piceatannol activity, containing powerful antioxidants, and they act through radical scavenging or metal chelating activities.[36–38]

Even though it has the lowest total flavonoid content, the red palm oil extract in this study had higher flavonoid levels compared to previous research, which were 4.80 ± 0.80 ug/L.[39] Total flavonoid levels in koja bay leaves have been previously evaluated from two different plant origins, located in Malaysia and Sri Lanka. Other research found that M. koenigii methanol extract from Sri Lanka had total flavonoid levels of 9.75 ± 0.05 mg/g.[40,41] The koja bay leaves used in this study came from the North Sumatra region, and had higher flavonoid levels (200.8392 ± 3.7874 mg/g) compared to the flavonoid levels from previous studies.

This study showed a decrease in LDL levels and the LDL/HDL ratio compared to other parameters, and occurred in the group with the lowest solution levels. This proves that the ingredients contained in passion fruit seeds, red palm oil, and koja bay leaves provide a synergistic effect in reducing LDL levels and the LDL/HDL ratio. This effect was seen when administering the solution of the three ingredients at the lowest dose but did not show any changes to the aortic wall due to the short time required. This decrease may be caused by the role of antioxidants in the three supplementation ingredients in rats.

In this study showed that natural antioxidants can be obtained from fruit, leaves, roots and seeds. Metabolic compounds that act as antioxidants include coumarins, flavonoids, saponins, tannins, alkaloids and triterpenoids.[42–44]Flavonoids are able to inhibit the development of heart disease, through their potential as antioxidants.[3,40,45] The action of flavonoids is similar to estrogen, namely as cardioprotective.[45] It works through the mechanism of improving the lipid profile, which were reducing total cholesterol, LDL and triglycerides and increasing HDL. Reducing cholesterol by flavonoids can also be done through other mechanisms, namely increasing bile acid secretion and reducing cholesterol metabolism.[16,46,47] Flavonoids are the most effective antioxidants for inactivating hydroxyl and peroxyl lipid radicals, as well as forming complex bonds with metal ions, thereby preventing the formation of reactive oxygen species (ROS).[39,47,48] ROS compounds have a damaging effect if the balance between oxygen and antioxidants is disturbed.[5,6] Apart from flavonoids, saponins play a role in reducing the risk of heart disease.[5,6] Saponins play a role in inhibiting cholesterol absorption in the intestine.[5,6] The consequence of inhibiting cholesterol absorption is that cholesterol is excreted from the body with feces, which is the main pathway for excreting cholesterol.[16] It suggested that saponins will bind to bile acids and increase the excretion of bile acids in feces and neutral sterols (such as coprostanol and cholestanol).[49,50] This causes the conversion of cholesterol to bile acids to greatly increase in an effort to maintain the bile acid depot.[49,50]

The mechanism of action of antioxidants also inhibits the formation of intracellular ROS to combat oxidative stress and cell damage.[28,51] Previous studies have shown that antioxidant activity in polyphenols has weakened oxidative stress, reduced post-meal and fasting blood glucose levels, and increased insulin release and sensitivity, therefore, the antioxidant phytochemicals present in these three formulation ingredients provide antioxidant effects.[51,52]

In this study, the role of LPS in plaque formation could not be found due to the short intervention period of LPS administration, however the process of reducing LDL levels could be seen clearly by administering the three ingredients during the intervention period. Acute infection of the blood vessel wall can occur in the direct mechanism category, forming plaque rupture and thrombosis.[9,53] The indirect pathway is associated with inflammation in non-vascular locations, leading to heightened secretion of cytokines.[19] The occurrence of this type of infection can be attributed to the presence of lipopolysaccharide (LPS), a constituent of the cell wall of gram-negative bacteria. LPS is responsible for initiating a systemic inflammatory response syndrome, which is characterised by symptoms such as fever, elevated heart rate, low blood pressure, intravascular coagulation, angioedema, multiple organ failure, and, in severe instances, septic shock. The immune system recognises LPS as a pathogen-associated molecular pattern (PAMP), and when it is introduced into the body, it triggers inflammation in several organs.[12,13]

Cytokine release is critical in almost every stage of the immune response, and consequently, systemic dysregulation of inflammatory homeostasis may explain the potential higher risk for developing cardiovascular disease.[19,20] The possible mechanism is that increased levels of cytokines are overexpressed in the pathogenesis of cardiovascular disease.[20,54] The role of cytokines provides protective and provocative effects in the process of atherosclerosis, for example IL-13, a prototypical Th2 cytokine, has been shown to protect against experimental atherosclerosis through the induction of alternatively activated macrophages.[20,55] However, previous studies have suggested that Th2 cytokines may also play a detrimental role in cardiovascular disease.[56,57]

This study showed no changes in malondialdehyde levels in all groups, the inflammatory processes that cause high oxidative stress and produce levels of pro-oxidants. Oxidative stress is thought to be involved in the etiology of LPS-induced endotoxemia and organ damage.[10,58] Excessive production of COX-2 and iNOS leads to the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), including superoxide anion radicals (O2•−) and nitric oxide (NO). The highly reactive O2•− readily interacts with NO, creating peroxynitrite (ONOO−), a potent toxic mediator implicated in LPS-induced organ injury.[21,23] Lipid peroxidation, induced by the overproduction of ROS and RNS, is posited as a critical contributor to cellular damage. LPS further modifies the cellular redox state by augmenting malondialdehyde (MDA) levels, a recognized indicator of lipid peroxidation.[46] However, changes to a high calorie-fat-protein diet did not show changes in MDA levels, this is likely due to the lack of intervention time and lack of stimulation of the occurrence of free radicals.[46]

In this study, when the rats were given red palm oil (mixed with koja bay leaf and passion fruit seed), which contained high level of antioxidants (passion fruit seed, koja bay leaf, and red palm oil), in addtion to vitamins A and E in the palm oil.[30,31,59] Tocotrienols and tocopherols, respectively, make up 70% and 30% of the vitamin E present in red palm oil.[31,60,61] Tocotrienols have been suggested to inhibit the activity of the enzyme HMG CoA reductase and thereby regulate serum LDL levels.[62,63]

This study results report that passion fruit seeds have the highest content of flavonoid, which is piceatannol, this is a natural analogue of resveratrol, this form of polyphenol has strong activity and good antioxidant power in overcoming increased LDL levels, which will later cause cardiovascular disease. Piceatannol is found in passion fruit seeds, which functions as a specific inhibitor of splenic tyrosinase kinase.[28,29,64] Other biological activities of piceatannol include antioxidant, anticancer and anti-inflammatory effects.[37,38] Another theory is that the fiber content in passion fruit seeds may bind bile acids, preventing their absorption from the intestines, while increasing their excretion through feces, thereby increasing the conversion of cholesterol from blood serum into bile acids. Increasing fiber consumption can reduce energy absorption by depleting food energy availability while maintaining other important nutrients.[37,38]

This study did not show a difference in glucose levels. This is probably due to the short time and lack of a high-carbohydrate intervention, despite earlier studies showing a drop in blood glucose levels in Rats fed a high fat diet.[47] Previous studies also found that Piceatannol works on glucose absorption, so that the absorption process is better, in

addition, piceatannol increases AMPK activation, and functions as a translocation of glucose transporter 4 (GLUT4).[49] Furthermore, piceatannol reduces that rise in blood glucose levels in early stage and improves poor glucose tolerance.[32] Polyphenols, including resveratrol and piceatannol, have been shown to lower postprandial hyperglycemia in rats on a high protein-fat diet. The mechanism of action of piceatannol shows the inhibitory activity of intestinal α-glucosidase, which supports its effectiveness as a preventative for diabetes mellitus.[54]

This study showed that BDNF had significant differences between groups, it showed that higher serum BDNF was associated with reduced risk of cardiovascular diseases and death.[25,26] Apart from that, BDNF is a neurotrophic factor found in large amounts in brain tissue and peripheral nerves with protective effects against ischemic brain injury. BDNF also plays an important role in cardiovascular function, with the angiogenesis reconstruction pathway.[24–26]

This study showed that other antioxidant effect was from koja bay leaves, with antioxidant, antidiabetic, anti-inflammatory and antitumor activities through molecular pathways, including Bcl-2, Bax, NF-κB and TNFα.[32–34] The way this antioxidant works is by reducing free radical in the lumen membrane.[32] Reactive oxygen species attack almost all cell components including membrane lipids and produce lipid peroxidation (LPO).[32,34] Antioxidant activity works to capture existing free radicals.[32] Koja bay leaves are a source of carbazole alkaloids, essential oils, terpenoids and flavonoids, which produce good antioxidant biological activity and pharmacological effects.[32,34] The flavonoids contained are quercetin, apigenin, kaempferol, routine, catechin, and myricetin.[32–34] Meanwhile, the active ingredients are mahanine, mahanimbine, isomahanine, koenimbine, and girinimbine.[33,34]

The combination of these three ingredients provides a synergistic effect to reduce LDL levels and the LDL/HDL ratio. This formulation is expected to provide antioxidant, anti-inflammatory, and antihyperlipidemic effects. Cardiovascular complications result in the deposition of fatty plaques in small-diameter blood vessels and further hardening and narrowing (atherosclerosis) that can progress to cerebral stroke, acute or chronic kidney failure, loss of sensation and permanent blindness.[65] Plant-derived antioxidants prevent cell damage by inhibiting the peroxidation chain reaction, thereby reducing oxidative stress.[65,66]

The limitation of this study is that it did not examine inflammatory parameters and other effects on nerve cells, more examinations about nerve and longer time will produce better study results. The more parameters of cell function that this formula affects, the better the results will be. The proposed future study is on humans, to see the similarities in the effects of providing three local wisdom food ingredients from North Sumatra, Indonesia, so that they become a form of supplementation in preventing the process of atherosclerosis and reducing the risk of CAD.

Conclusion

This results showed that supplementation with formulation of the three extract ingredients which are red palm oil, koja bay leaves, and passion fruit seeds showed an increase in body weight, a decrease in LDL levels, and an LDL/ HDL in experimental animals. This is caused by the antioxidant activity of each compound. This property is synergistic of all formulation of the three extract ingredients. This study did not show changes in MDA and glucose levels. It is hoped that the importance of looking at the role of these three ingredients can be addressed to humans as a preventive measure against the occurrence of atherosclerosis and preventing CAD.

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Author Contributions

Each author has made substantial contributions to this study, encompassing various areas such as conceptualization, study design, implementation, data collection, analysis, and interpretation. All authors have been involved in drafting, revising, and critically reviewing the article. They have provided their final approval for the version to be published and have participated in the decision regarding the choice of journal for submission. Furthermore, all authors agree to take responsibility for every aspect of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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