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The Effect of Flaxseed (*Linum usitatissimum*) Ethanol Extract on LDL Cholesterol Levels and Foam Cell Number in the Aorta of Male Wistar Rats (*Rattus norvegicus*) Fed A High-Fat and High-Fructose Diet

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Abstract

A diet high in saturated fat and fructose leads to dyslipidemia, increasing atherosclerosis risk. Although lipid-lowering medications are available, they have certain limitations. Flaxseed, rich in ALA, lignans, and phytosterols, may improve lipid profiles and act as an antioxidant. This study evaluated the effectiveness of flaxseed in preventing LDL cholesterol elevation and aortic foam cell formation in male Wistar rats fed a high-fat, high-fructose (HFHF) diet. This actual experimental study used a post-test-only control group design for 35 days. Twenty-five male Wistar rats were randomly assigned to five groups: N (normal control, standard diet), K (negative control, HFHF diet), and three treatment groups (P1, P2, P3, HFHF diet**)** receiving flaxseed ethanol extract at doses of 200, 400, and 800 mg/kgBW/day. LDL cholesterol levels (mg/dL) and aortic foam cell counts (cells/HPF) were measured. Data were analyzed using Shapiro-Wilk and Levene tests for normality and homogeneity, followed by one-way ANOVA, Kruskal-Wallis, and Tukey-HSD post hoc tests. LDL cholesterol levels in groups N, P1, P2, and P3 were significantly lower than in group K ($p = 0.007$), with P2 showing the most significant inhibition ($p = 0.035$). Foam cell counts were lower in treatment groups than in group K ($p = 0.257$), but no significant differences were found among them. Flaxseed (*Linum usitatissimum*) ethanol extract at 400 mg/kgBW/day was the most effective in preventing an increase in LDL cholesterol. However, the extract at all three doses was ineffective in preventing an increase in aortic foam cells in male Wistar rats (*Rattus norvegicus*) fed an HFHF diet.

Keywords: Atherosclerosis; Dyslipidemia; Flaxseed; Foam cell; LDL

1. INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death worldwide, killing 17.9 million people, or 32% of all deaths. According to the WHO (2021), 85% of these deaths were caused by heart attacks and strokes (World Health Organization, 2021). Coronary heart disease (CHD) is the most common cardiac disease in adults in Indonesia. The number of people with it has grown from 0.5% in 2013 to 1.5% in 2018. Dyslipidemia, hypertension, diabetes, an unhealthy diet, a lack of exercise, an abnormal body mass index, smoking, and drinking too much alcohol are some of the things that can lead to CHD and atherosclerosis (Ministry of Health, Republic of Indonesia, 2018).

Atherosclerosis, which is when cholesterol, lipids, and/or calcium build up and harden along the walls of arteries, is a primary cause of coronary heart disease. The heart may not work as well because it does not get enough blood when this builds up. Atherosclerosis begins when small Apo-B particles, such as LDL cholesterol, clump together in the walls of arteries. This process causes foam cells to develop and plaque to accumulate through various cellular mechanisms (Juslim & Herawati, 2018; Li et al., 2022; Indonesian Heart Association, 2022). Foam cells, typically found in the tunica intima, are therefore considered an important indicator of worsening atherosclerosis (Maghfiroh et al., 2022). Dyslipidemia is a disorder characterized by abnormalities in lipid profiles, including aberrant levels of total cholesterol (TC), low-density lipoprotein (LDL), and triglycerides (TG), as well as reduced levels of high-density lipoprotein (HDL) (Ma'rufi & Rosita, 2014). Dyslipidemia causes one out of every three cases of cardiovascular disease and kills 2.6 million people. Throughout the world, LDL levels have increased, but the most significant rise has occurred in Indonesia. Because LDL contributes to atherosclerosis, this could lead to an increase in cases of the disease (Indonesian Endocrinology Association, 2021).

Modern diet habits often involve frequent consumption of food and drinks that are rich in sugar, fat, and calories. A diet high in saturated fat significantly increases LDL cholesterol levels. On the other hand, consuming a diet high in fructose disrupts the body's ability to use glucose and increases fat production through lipogenesis (Malik & Hu, 2015). Lipogenesis can lead to dyslipidemia, atherosclerosis, fatty liver, insulin resistance, and obesity (Handayani et al., 2021). Hardimarta et al. (2021) and Gileva et al. (2022) found that a diet high in saturated fat and fructose accelerates the rise in LDL cholesterol levels and foam cell formation in the aorta(Gileva et al., 2022; Hardimarta et al., 2020). A previous study by Veonika et al. (2024) reported that micronutrients could reduce lipid content (Veonika et al., 2024). Nevertheless, lipid-lowering medications are widely used despite their known limitations (Farida & Putri, 2016). The use of herbal therapy is an option due to its relatively lower cost, good efficacy, and low side effects. Flaxseed, known as linseed or by its scientific name *Linum usitatissimum*, is rich in polyunsaturated fatty acids (PUFA), phytosterols, and lignans. Flaxseed contains 41% fat (73% PUFA and 8% MUFA), 21% protein, and 28-40% fiber. It also contains vitamins, minerals, phytosterols, and lignans. The primary PUFA in flaxseed is alpha-linolenic acid (ALA) at 56.93%, followed by linoleic acid (LA) at 15.82%. Inside the body, ALA can be

converted into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have anti-inflammatory properties, improve lipid metabolism, and support the myelination of neurons. The lignans, phytosterols, and fibers in flaxseed also contribute to better lipid profiles and help prevent atherogenic processes. Furthermore, the high levels of phytosterols and lignans in flaxseed act as antioxidants (Kanikowska et al., 2020; Shahidi et al., 2022).

Some studies have shown that flaxseed oil is effective in reducing small dense LDL (sd-LDL) levels while also significantly reducing LDL and total cholesterol (Kawakami et al., 2015). Flaxseed itself has also been linked to a reduction in the atherosclerotic process by reducing atheroma plaque formation and improving its contractility (Bujok et al., 2021). The research on the effects of commonly found flaxseed extracts on LDL cholesterol levels and the progression of atherosclerosis, particularly in Indonesia, is limited. There is a lack of specific studies investigating the effects of flaxseed extracts on LDL cholesterol levels and changes in the aortic lumen. This study aimed to investigate the effects of ethanolic flaxseed extract on LDL cholesterol levels and aortic foam cell counts in male Wistar rats fed a high-fat, high-fructose diet.

2. MATERIAL AND METHODS

2.1 Ethical clearance

This research received ethical approval from the Faculty of Medicine, University of Udayana Research Ethics Commission, with the number 2558 /UN 14.2.2.VII. 14/LT/2024.

2.2 Research design

This research is an actual experimental study with a randomized post-test-only control group design. Flaxseed extraction was conducted at the Integrated Biomedical Laboratory, Pharmacology Division, Faculty of Medicine, University of Udayana. The treatment and maintenance of the rats were carried out at the Integrated Biomedical Laboratory, Histology Division, Faculty of Medicine, University of Udayana. The phytochemical testing of flaxseed extract was conducted at the Integrated Research and Testing Laboratory of the University of Gadjah Mada. LDL cholesterol levels were measured at the Integrated Biomedical Laboratory, Biochemistry Division, Faculty of Medicine, Universitas Udayana. The histological specimens were prepared at the Anatomical Pathology Laboratory of the University of Udayana Hospital.

2.3 Flaxseed extraction and preparation of a high-fat, high-fructose diet

The flaxseed used in this study was registered with the Indonesian Ministry of Agriculture under the number KEMTAN RI PD 31.72-A.III.000-01-00634-10/21. The flaxseed extraction process began by grinding 9 kg of dried flaxseeds (Club Sehat; Jakarta, Indonesia) using a blender (Fomac; Jakarta, Indonesia). The flaxseed powder was placed in a maceration jar, and 18 L of 70% ethanol (Onemed; Sidoarjo, Indonesia) solvent was added until thoroughly soaked. The mixture was then stirred and left in a dark room for three days, with stirring performed twice daily, in the morning and evening. The residue and the maceration result were then separated using filter paper. Maceration was repeated three times, and all the maceration results

obtained were combined and then evaporated using a rotatory evaporator (Hahnvapor; Gyeonggi-do, South Korea) until a thick extract was obtained (Putri, 2018).

A high-fat diet was formulated by mixing 300 grams of melted lard with 200 grams of duck egg yolk, then combining with 100 ml of distilled water to form a homogenous suspension. This suspension was administered to rats at a dose of 1 ml per day in the morning via oral gavage. The daily intake of the standard diet was monitored by weighing the food before and after feeding, allowing for the calculation of individual daily food consumption. A high-fructose diet was induced by providing a 30% fructose (Rose Brand; Bandar Lampung, Indonesia) solution in the drinking water. This high-fat, high-fructose diet was given to rats for 35 days (Harsa, 2014; Susanti et al., 2019).

2.4 Experimental animals

This study employed the Resource Equation Approach Formula to determine the minimum and maximum sample size. The minimal required sample was 3 per group (15 total), while the maximum was 5 per group (25 total). This study utilizes the maximum sample size, selecting 25 male Wistar rats bred at Laboratorium Biomedik Terpadu, Universitas Udayana, aged 2-3 months, weighing 180-200 grams, and in good health. Rats that were already ill, had died, or were not eating were excluded from the study (Kinanti et al., 2023). Samples were acclimated for one week in groups of 4-5 in a 40 x 30 x 20 cm cage with controlled ventilation, lighting, and temperature. A standard diet (feed 594) was given *ad libitum* throughout the experiment. After acclimatization, samples were divided into five groups: N (normal control) received a standard diet, K (negative control) was given a high-fat, high-fructose diet, while P1, P2, and P3 were administered a high-fat, high-fructose diet supplemented with 200, 400, and 800 mg/kg BW/day flaxseed ethanol extract for 35 days, respectively (Harsa, 2014; Rosmala et al., 2018). On the 36^{th} day, all rats were anesthetized with a mixture of 10% ketamine and 2% xylazine. Around 2 mL of blood was collected from the medial canthus of the orbital sinus for LDL cholesterol analysis. Euthanasia was performed with 2-3 times the anesthetic dose, followed by cervical dislocation. The thoracic aorta was then extracted, and the carcasses were buried.

2.5 Assessment of LDL cholesterol levels and histopathology

Blood samples of 2 ml were centrifuged using a Hettich EBA 3S (Tuttlingen, Germany) for 10-20 minutes at 3,000 rpm. Total cholesterol, HDL, and triglycerides were measured using a DiaSys Diagnostic System GmbH (Holzheim, Germany) CHOD-PAP enzymatic photometric test. LDL cholesterol levels were calculated using the Friedewald formula (LDL cholesterol (mg/dL) = Total cholesterol (mg/dL) – HDL cholesterol (mg/dL) – (Triglycerides (mg/dL)/5)). However, it has limitations and cannot be used for triglyceride levels greater than 400 mg/dL (Kumar et al., 2021).

Using a tissue processor (DEKO PATH Dk-TSM1; Shandong, China), the thoracic aorta was fixed in 10% neutral buffer formalin (Leica; Virginia, USA), dehydrated in 96% alcohol (JK Care; Jakarta, Indonesia), and cleared in xylene (Bratachem; Tangerang, Banten).

Subsequently, it was embedded in paraffin (Pro Histo; Jiangsu, China), sectioned into 4-5μm thick sections with a Leica microtome 820 (Bonn, Germany), and stained with hematoxylin and eosin (Kurniawan et al., 2022; ScyTek; Utah, USA). The prepared tissues were then examined under a microscope using the Olympus CX33 microscope with an EP50 camera (Guangzhou, China). Foam cell counts were conducted by observing under a microscope at 40x magnification to identify areas rich in foam cells and counting cells in five high-power fields (HPF) using 400x magnification (Han et al., 2015).

2.8 Statistical analysis

Data from this study were analyzed using SPSS software version 29.0.0. Descriptive analysis was presented as the mean, standard deviation (SD), minimum value (MIN), and maximum value (MAX). Data normality was assessed using the Shapiro-Wilk test, while variance homogeneity was evaluated using Levene's test. Since LDL cholesterol levels were normally distributed ($p \geq 0.05$) with homogenous variances, a one-way ANOVA was used to compare the means of three or more groups, followed by a Tukey-HSD post hoc test. The Kruskal-Wallis test was selected because the data on the number of aortic foam cells were not normally distributed ($p < 0.05$).

3. RESULTS AND DISCUSSION

A descriptive and comparative analysis of the lipid profiles (total cholesterol, triglycerides, HDL, and LDL) of rats was conducted after 35 days of a high-fat, high-fructose (HFHF) diet and flaxseed extract treatment (Table 1). Due to the prevalence of dropouts, the final analysis in this study only included 19 rats, with two rats from each treatment group (P1, P2, and P3) excluded. A descriptive analysis of LDL cholesterol levels (mean ± SD, mg/dL) across various experiments (Table 1) revealed that the normal control group (N) displayed a mean of 91.779 ± 29.780. In contrast, the negative control group (K) showed significantly elevated levels at 251.953 ± 91.111. The P1 group (200 mg/kgBW/day) exhibited a mean LDL cholesterol level of 145.788 ± 36.744, while the P2 (400 mg/kgBW/day) and P3 (800 mg/kgBW/day) groups exhibited mean levels of 112.209 ± 24.615 and 127.686 ± 56.043 mg/dL, respectively. The LDL cholesterol levels in all groups were normally distributed ($p \geq 0.05$) and showed homogeneity of variances ($p \geq 0.05$). The one-way ANOVA test yielded a p-value of 0.007 ($p < 0.05$), indicating a statistically significant difference between at least one group and all other groups compared.

The Tukey-HSD post hoc test (Table 2) revealed a significant decrease in LDL cholesterol levels between the normal control group (N) and the negative control group (K), as well as between the negative control group (K) and P2 at a dose of 400 mg/kg BW/day. There was no significant decrease in LDL cholesterol levels between the negative control and P1 ($p = 0.14$) or P3 ($p = 0.069$). A descriptive and comparative analysis of HDL levels and foam cell counts in control and treatment groups following 35 days of a high-fat, high-fructose (HFHF) diet and flaxseed extract treatment (Table 3) revealed a statistically significant difference in HDL levels ($p < 0.05$). In contrast, no significant difference was observed in foam cell counts ($p \geq 0.05$). A

quantitative analysis of the phytochemical composition of the flaxseed ethanol extract, measured by spectrophotometry and gas chromatography (Table 4).

Table 1. Descriptive and comparative analysis of the lipid profiles (total cholesterol, triglycerides, HDL, and LDL) in rats after 35 days of HFHF diet and flaxseed extract treatment. *Description*: n = sample size; SD = standard deviation; p value = significance; * = significant value; N = normal control group; K = negative control group; P1 = 200 mg/kg BW/day; P2 = 400 mg/kg BW/day; P3 = 800 mg/kg BW/day.

Variable	Group	n	Mean ± SD	Minimal	Maximal	p
Total cholesterol (mg/dL)	N	5	265.745 ± 34.418	231.92	313.83	0.006*
	K	5	425.745 ± 94.325	302.13	560.64	
	P1	3	301.773 ± 55.994	243.62	355.32	
	P2	3	261.347 ± 26.368	242.55	291.49	
	P3	3	301.418 ± 38.421	259.57	335.11	
Triglycerides (mg/dL)	N	5	343.575 ± 45.956	298.32	411.17	0.16
	K	5	385.251 ± 75.146	289.94	498.88	
	P1	3	380.447 ± 110.682	272.07	493.30	
	P2	3	277.467 ± 17.366	257.54	289.39	
	P3	3	321.787 ± 49.918	281.56	377.65	
LDL (mg/dL)	N	5	91.779 ± 29.780	57.64	117.31	0.007*
	K	5	251.953 ± 91.111	120.48	372.11	
	P1	3	145.788 ± 36.744	107.02	180.10	
	P2	3	112.209 ± 24.615	96.97	140.61	
	P3	3	127.686 ± 56.043	64.60	171.70	

There were significant differences in LDL cholesterol levels among the treatment groups. The normal control group (N) had lower levels compared to the negative control group (K). In comparison, the P2 (400 mg/kg BW/day) also showed significantly lower levels compared to the negative control group (K). Descriptive analysis of foam cell counts (mean ± SD, mg/dL) across different experiments found that the normal control group (N) exhibited 0.40 ± 0.89. In contrast, the negative control group (K) showed higher levels at 1.40 ± 1.52. Among the treatment groups, P1 had foam cell counts of 0.33 ± 0.58, P2 had 0,00 ± 0.00, and P3 had 0.00 ± 0.00. The foam cell counts in all groups were not normally distributed ($p < 0.05$) but exhibited homogeneity of variances ($p \geq 0.05$). The Kruskal-Wallis test yielded a p-value of 0.15, indicating no significant difference between any of the groups compared. The histopathological features of the thoracic aorta lumen are shown in Figure 1, while those of foam cells in the tunica intima of the thoracic aorta are shown in Figure 2.

A high-fat, high-fructose (HFHF) diet consisting of duck egg yolk, lard, and 30% fructose was administered. Previous research demonstrates that these diets increase LDL-cholesterol, total cholesterol, and triglycerides, while reducing HDL-cholesterol (Harsa, 2014). This high-fat diet contains many saturated fats, which can increase LDL cholesterol levels and lead to the formation of foam cells in the aorta (Han et al., 2015). The high-fructose diet further exacerbates these effects by promoting de novo lipogenesis and impairing glucose metabolism. The diet can lead to oxidative stress, insulin resistance, and endothelial dysfunction, ultimately contributing to the development of atherosclerosis (Febrianingsih, 2019; Malik & Hu, 2015; Susanti et al.,

2019). Flaxseed (Linum usitatissimum) is a good source of lignans, omega-3 fatty acids, flavonoids, and fiber. It has been shown to work to lower LDL cholesterol levels. Omega-3 fatty acids, especially alpha-linolenic acid (ALA), help cholesterol get to the liver, increase the number of LDL receptors in the liver, and change how the body uses HDL. Alpha-linolenic acid (ALA), an omega-3 polyunsaturated fatty acid (PUFA), reduces foam cell formation by inhibiting the expression of CD36 and ACAT1 while facilitating cholesterol efflux through the PPARγ/LXRα/ABCA1 pathway (Moss et al., 2016; Pizzini et al., 2017; Poznyak et al., 2021). Lignans also act as antioxidants by preventing the oxidation and absorption of LDL. Flavonoids and isoflavones also help lower cholesterol by inhibiting the body's production of cholesterol and increasing the availability of LDL receptors. Flaxseed contains phenolic compounds that work as antioxidants by neutralizing ROS and making the endothelium work better by making nitric oxide (NO). Lignans and flavonoids alter the oxidative stress pathway and reduce the number of foam cells by decreasing oxidized LDL and inhibiting the secretion of adhesion molecules. Fiber in flaxseed also helps reduce cholesterol and prevents the body from producing more cholesterol.

Table 2. Post hoc test results for total cholesterol and LDL cholesterol levels among control and treatment groups after 35 days of flaxseed ethanol extract administration in rats fed a HFHF diet. *Description*: p value = significance; * = significant value; N = normal control group; K = negative control group; P1 = 200 mg/kg BW/day; P2 = 400 mg/kg BW/day; P3 = 800 mg/kg BW/day.

Variable	Group	p
Total Cholesterol	N vs K	0.007*
	N vs P1	0.92
	N vs P2	1.00
	N vs P3	0.923
	K vs P1	0.086
	K vs P2	0.016*
	K vs P3	0.084
	P1 vs P2	0.95
	P1 vs P3	0.99
	P2 vs P3	0.99
LDL	N vs K	0.005*
	N vs P1	0.71
	N vs P2	0.99
	N vs P3	0.91
	K vs P1	0.14
	K vs P2	0.035*
	K vs P3	0.069
	P1 vs P2	0.95
	P1 vs P3	0.99
	P2 vs P3	0.99

Phytochemicals in flaxseed ethanol extract were analyzed by spectrophotometry and gas chromatography (Fadli et al., 2024). The phytochemical analysis revealed that the extract

contained elevated concentrations of total phenols (4.57%) and flavonoids (0.36%). Previous studies found a high amount of omega-3, but this extract contained more omega-6 (linoleic acid, 27.53%) and less omega-3, as will be explained later. The low omega-3 concentration may be due to its susceptibility to oxidation, as it contains multiple double bonds. It can also be affected by the extraction method and the maturity of the seeds (Kauser et al., 2024; Mercola & D'Adamo, 2023). Omega-6 fatty acids in moderate amounts promote cholesterol metabolism by enhancing LDL receptor activity and affecting CYP7A1 activity through LXRα activation (Djuricic & Calder, 2021). Although omega-6 can reduce foam cells by downregulating ox-LDL uptake and cholesterol accumulation, excessive intake may induce pro-inflammatory effects and increase cardiovascular risk (Bruen et al., 2017).

Table 3. Descriptive and comparative analysis of HDL levels and foam cell counts in control and treatment groups following 35 days of flaxseed ethanol extract administration in rats fed a HFHF diet. *Description*: n = sample size; SD = standard deviation; p value = significance; N = normal control group; K = negative control group; P1 = 200 mg/kg BW/day; P2 = 400 mg/kg BW/day; P3 = 800 mg/kg BW/day.

Variable	Group	n	Median	Minimal	Maximal	p
HDL (mg/dL)	N	5	109.063	81.25	124.06	0.006*
	K	5	91.250	88.13	105.00	
	P1	3	80.938	75.56	82.19	
	P2	3	95.938	85.63	99.38	
	P3	3	112.813	81.56	133.75	
Aortic Foam Cell (cell/HPF)	N	5	0.000	0.00	2.00	0.15
	K	5	1.000	0.00	3.00	
	P1	3	0.000	0.00	1.00	
	P2	3	0.000	0.00	0.00	
	P3	3	0.000	0.00	0.00	

Our research showed that flaxseed ethanol extract significantly lowered LDL cholesterol levels ($p = 0.035$), especially at a dose of 400 mg/kg BW/day (P2) compared to the negative control (K) after 35 days. The phenolic compounds (lignans and flavonoids) and omega-6 in flaxseed extract prevented the elevation of LDL cholesterol levels. However, there were no significant differences between the negative control group (K) and the treatment groups (P1 and P3) because the dose in P1 was too low (200 mg/kg BW/day) and the dose in P3 was too high (800 mg/kg BW/day). This result aligns with that of Yang et al. (2021), who found that excessive flaxseed supplementation does not enhance its lipid-lowering effect (Yang et al., 2021). Group P2 had the lowest levels of LDL cholesterol among the treatment groups, but the differences were not statistically significant. This result suggests that increasing flaxseed extract does not offer additional benefit in lowering LDL cholesterol. The extract's higher omega-6 and lower omega-3 content may have reduced its effectiveness in lowering LDL (Yang et al., 2021). The LDL cholesterol levels in the negative control group (K) were still significantly higher ($p = 0.005$) than in the normal control group (N), indicating an adverse effect of consuming a diet high in fat and sugar. Moreover, the persistence of higher LDL cholesterol

levels in the treatment groups compared to the normal control group (N) suggests that doses of 200, 400, and 800 mg/kg body weight per day were insufficient to reduce LDL levels effectively. This outcome may be related to variations in bioactive compound content (influenced by extraction method, flaxseed variety, and seed maturity), individual variability in lipid metabolism, or the relatively short duration of treatment.

The negative control and treatment groups of rats were fed a high-fat diet in addition to a standard diet (type 594) containing at least 3% fat. This diet had 300 g of lard (43.95% saturated fat) and 200 g of duck egg yolks (31.85% saturated fat) (Polat et al., 2013; Xu et al., 2016). Similar formulations in previous studies have led to substantial elevations in total cholesterol, LDL cholesterol, and triglycerides, accompanied by diminished HDL cholesterol levels (Juslim & Herawati, 2018; Harsa, 2014). Saturated fatty acids stimulate hepatic triglyceride synthesis, which in turn increases LDL and VLDL levels, thereby contributing to the development of atherosclerosis (Chan et al., 2015). The high-fructose diet (30% fructose in drinking water) increased small dense LDL (sdLDL) levels even further in three weeks, which contributed to atherosclerosis (Sanches et al., 2015). Fructose disrupts glucose and lipid metabolism, promotes visceral fat accumulation, and induces insulin resistance, leading to increased hepatic triglycerides and an increased risk of cardiovascular disease (Malik & Hu, 2015; Sanches et al., 2015).

Table 4. Quantitative analysis of the phytochemical composition of flaxseed ethanol extract, measured by spectrophotometry and gas chromatography. *Description*: % (w/w) = percent weight by weight.

Type	Parameter	Result	Unit
Phenolic content	Total Phenol	4.57	% (w/w)
	Total Flavonoid	0.36	% (w/w)
Saturated fat	Methyl arachidate	25.99	%Relative
	Methyl heneicosanoate	29.68	%Relative
	Methyl tricosanoate	0.16	%Relative
	Methyl lignocerate	0.15	%Relative
Unsaturated fat	Methyl palmitoleate	12.25	%Relative
	Methyl cis-9-oleate	4.08	%Relative
	Methyl linolelaidate	27.53	%Relative
	Methyl cis-11-eicosenoate	0.16	%Relative

Descriptive analysis revealed variations in median foam cell counts among groups, although the comparative test showed no statistically significant differences ($p = 0.26$). The negative control group (K) had the highest foam cell count in the aorta (seven cells/HPF; median=1), followed by the normal control group (N), with two cells/HPF; median=0), and the treatment group (P1) (one cell/HPF; median=0). The treatment group (P2 and P3) showed no foam cells (median = 0). The high-fat, high-fructose (HFHF) diet in K likely contributed to foam cell formation, as saturated fats and fructose promote LDL cholesterol production and lipogenesis by bypassing the phosphofructokinase enzyme (Indonesian Endocrinology Association, 2021). Foam cell formation is influenced by oxidized LDL (ox-LDL) levels,

triglycerides, and smaller LDL particle size (sd-LDL). Small, dense LDL is readily recognized by macrophage scavenger receptors, enhancing foam cell development. HDL cholesterol facilitates the removal of LDL, but elevated ox-LDL impairs hepatic LDL receptor activity, thereby contributing to the formation of foam cells.

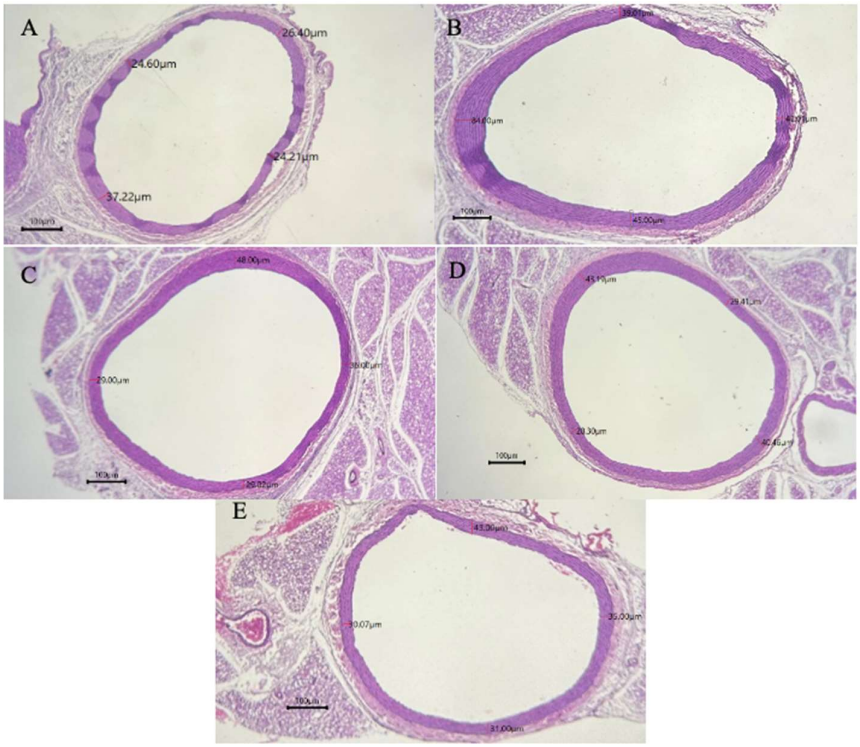

Figure 1. Histopathological features of the thoracic aorta lumen stained with Hematoxylin & Eosin (x40). (A) N; (B) K; (C) P1; (D) P2; (E) P3.

Comparative tests revealed significant differences in total cholesterol ($p = 0.006$) but not in triglycerides ($p = 0.16$) or HDL cholesterol ($p = 0.06$), which may partly explain the lack of differences between the K and treatment groups (Wahyuni, 2015). The LDL cholesterol measurement method used did not specifically assess ox-LDL levels, which are more relevant to foam cell formation. Additionally, the 35-day duration may have been inadequate to observe significant differences, as prolonged exposure is recognized to intensify lipid profile abnormalities, oxidative stress, and foam cell formation (Febrianingsih, 2019; Bujok et al., 2021; Malik & Hu, 2015).

The bioactive components of the flaxseed ethanol extract (phenols, flavonoids, and omega-3 alpha-linolenic acid (ALA)) may have been insufficient to prevent foam cell formation. The lower levels of ALA compared to linoleic acid (LA) and the variability in extract composition due to seed variety, farming practices, and extraction methods (Kauser et al., 2024; Mueed et al., 2022). Groups P2 and P3 did not reveal any foam cells; however, this could be attributed to the rat's overall health rather than the extract itself. Physiological factors, including genetic predisposition, oxidative stress, and chronic inflammation induced by gavage feeding

and fructose water, may also contribute (Chahirou et al., 2018; Juszczyk et al., 2021). Foam cell findings could also be influenced by staining methods, such as Oil Red O and Sudan Black (Gurina & Simms, 2025).

These findings underscore the need for more extended intervention periods and optimized extract formulations to elucidate better the mechanisms by which flaxseed prevents foam cell formation.

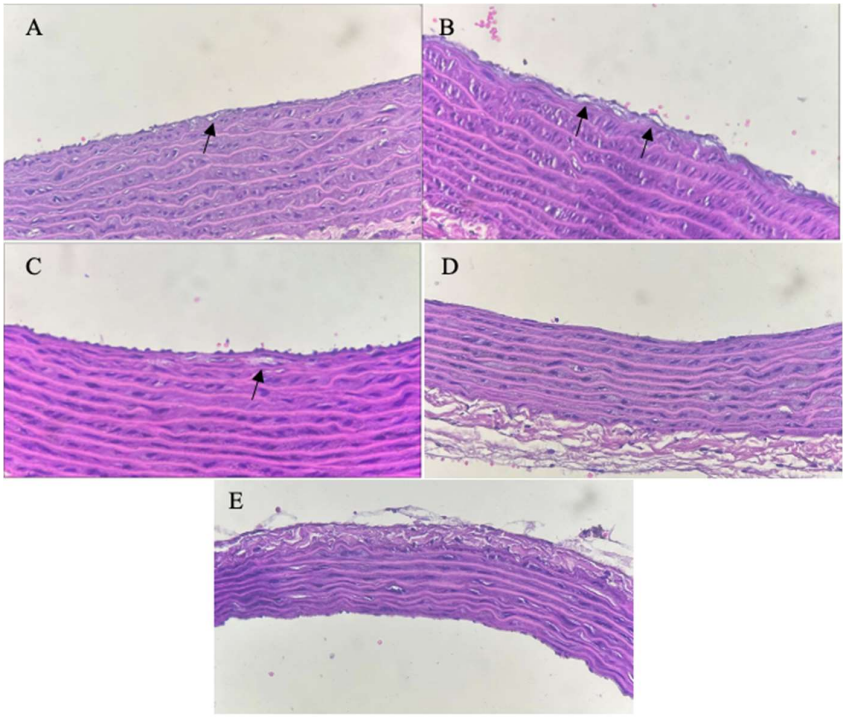

Figure 2. Histopathological features of foam cells in the thoracic aorta tunica intima stained with Hematoxylin & Eosin (x400). (A) N; (B) K; (C) P1; (D) P2; (E) P3. Arrows indicate foam cells (foamy cytoplasm and eccentric nuclei).

This study has several limitations. During the study, six rats (two from each treatment group) dropped out of the study. A statistical analysis remained feasible, as each group retained at least three rats, which is the minimum required for the sample size. The mortalities were likely associated with complications related to the oral gavage procedure, not the extract itself, as there were no signs of toxicity, changes in behavior, or unusual findings. However, other factors, including individual variability or dietary stress, cannot be completely ruled out. Oral gavage can cause aspiration pneumonia, perforation of the esophagus or stomach, and esophageal impaction. According to the Institutional Animal Care and Use Program, gavage was performed twice a day, with at least a four-hour break in between, to maintain stable cortisol levels (Institutional Animal Care and Use Program, 2023). Oral gavage method and fructose water may cause stress, which could lower 5-HT(1A) receptor levels and lead to sudden cardiac death (Chahirou et al., 2018). Flaxseed extract was not toxic, as previous studies had indicated adverse effects solely at significantly elevated doses (>5 g/kg body weight/day)

(Es-said et al., 2022). The lack of quantitative measurement of oxidized LDL (ox-LDL), a critical component in foam cell formation, coupled with the absence of phytochemical analysis of lignans and phytosterols, hinders the understanding of their roles in cholesterol metabolism. Subsequent research should encompass extended study durations, incorporate phytochemical analyses of lignans and phytosterols, and utilize comprehensive lipid profiling, particularly ox-LDL measurements, to enhance the evaluation of flaxseed extract's influence on atherosclerosis progression.

4. CONCLUSION

This study found that flaxseed extract lowered LDL cholesterol levels in Wistar rats on a high-fat, high-fructose (HFHF) diet, particularly at a dose of 400 mg/kg body weight per day. This effect was caused by the bioactive compounds in flaxseed, including phenolics, flavonoids, and omega-6 fatty acids. However, both lower and higher doses failed to offer additional benefits, suggesting a dose-dependent threshold for efficacy. Although LDL cholesterol levels decreased, there were no significant differences in foam cell formation among the treatment groups; this may be attributed to the relatively short study duration and variations in the bioactive components of the flaxseed ethanol extract. In addition, the high-fat, high-fructose (HFHF) diet also significantly elevated LDL cholesterol, confirming its role in promoting dyslipidemia and atherosclerosis.

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CONFLICT OF INTEREST

All authors declared that this study was carried out without any commercial or financial associations that could be perceived as a potential conflict of interest.

DECLARATION OF GENERATIVE AI IN SCIENTIFIC WRITING

All authors declared that generative AI and AI-assisted technologies were used in preparing this manuscript only to improve language, grammar, and readability. The authors have reviewed and approved the final version of the manuscript and take full responsibility for its content.

REFERENCES

Bruen, R., Fitzsimons, S., & Belton, O. (2017). Atheroprotective effects of conjugated linoleic acid. *British Journal of Clinical Pharmacology*, *83*(1), 46–53. https://doi.org/10.1111/bcp.12948

Bujok, J., Mista, D., Wincewicz, E., Kroliczewska, B., Dzimira, S., & Zuk, M. (2021). Atherosclerosis Development and Aortic Contractility in Hypercholesterolemic Rabbits

Supplemented with Two Different Flaxseed Varieties. *Bioactive/Nutraceutical Compounds in Plant Foods*, *10*(3), Article 534. https://doi.org/10.3390/foods10030534

Chahirou, Y., Lamtai, M., Mesfioui, A., Ouichou, A., Coulibaly, M., Boussekkour, R., & El Hessni, A. (2018). Impact of the Association of a High Fructose Diet and Chronic Mild Stress on Metabolic and Affective Disorders in Male Rat. *Journal of Behavioral and Brain Science*, *08*(04), 157–170. https://doi.org/10.4236/jbbs.2018.84010

Chan, J., Karere, G. M., Cox, L. A., & VandeBerg, J. L. (2015). Animal Models of Diet-induced Hypercholesterolemia. InTech. https://doi.org/10.5772/59610

Djuricic, I., & Calder, P. C. (2021). Beneficial Outcomes of Omega-6 and Omega-3 Polyunsaturated Fatty Acids on Human Health: An Update for 2021. *Nutrients*, *13*(7), Article 2421. https://doi.org/10.3390/nu13072421

Es-said, S., Lafhal, K., Elkhiat, A., Hammoud, M., Regbaoui, N., Ezoubeiri, A., Makbal, R., Sbyea, S., Elhiba, O., Sellami, S., Rais, H., Karim, A., Gamrani, H., Rada, N., Bouskraoui, M., & Fdil, N. (2022). Flaxseed extract reduces tissue accumulation and enhances urinary excretion of chondroitin sulphate in the rat: a possible new path in substrate reduction therapy for mucopolysaccharidosis. *Pharmaceutical Biology*, *60*(1), 879–888. https://doi.org/10.1080/13880209.2022.2068618

Fadli, J. C., Guntarti, A., Salamah, N. (2024). Autentikasi Minyak Biji Jagung *Zea mays* L.: Pengaruh Metode Ekstraksi dan Analisis Komposisi Menggunakan Metode Gas Cromatography-Mass Spectrometry (GC-MS). *Journal of Pharmaceutical Science and Clinical Research*, *9*(2), 256-267. https://doi.org/10.20961/jpscr.v9i2.85367

Farida, Y., Putri, C.I.P. (2016). Efek Penggunaan Simvastatin terhadap Kenaikan Gula Darah Puasa Pasien Diabetes Melitus Tipe 2. *Journal of Pharmaceutical Science and Clinical Research*, *1*(1), 58-65.

Febrianingsih, E. (2019). Pengaruh Pemberian Diet High Fat High Fructose terhadap Jumlah Sel Busa dan Ketebalan Dinding pada Aorta Tikus Sprague Dawley Jantan Model Obesitas Pengaruh Pemberian Diet High Fat High Fructose terhadap Jumlah Sel Busa dan Ketebalan Dinding pada Aorta Tikus Sprague Dawley Jantan Model Obesitas (Thesis). Universitas Brawijaya, Malang.

Gileva, O. G., Butolin, E. G., Tereshchenko, M. V., & Ivanov, V. G. (2022). Evaluation of indicators of carbohydrate and lipid metabolism in rats depending on the type of high-calorie diet. *Obesity and Metabolism*, *19*(1), 47–52. https://doi.org/10.14341/omet12712

Gurina, T. S., & Simms, L. (2025). *Histology, Staining*. Florida: StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK557663/

Han, H., Yan, P., Chen, L., Luo, C., Gao, H., Deng, Q., Zheng, M., Shi, Y., & Liu, L. (2015). Flaxseed Oil Containing α-Linolenic Acid Ester of Plant Sterol Improved Atherosclerosis in ApoE Deficient Mice. *Oxidative Medicine and Cellular Longevity*, *2015*, 1–17. https://doi.org/10.1155/2015/958217

Handayani, D., Febrianingsih, E., Desi Kurniawati, A., Kusumastuty, I., Nurmalitasari, S., Widyanto, R. M., Oktaviani, D. N., Maghfirotun Innayah, A., & Sulistyowati, E. (2021). High-Fructose Diet Initially Promotes Increased Aortic Wall Thickness, Liver Steatosis, and Cardiac Histopathology Deterioration, but Does Not Increase Body Fat Index. *Journal of Public Health Research*, *10*(2), Article 2181. https://doi.org/10.4081/jphr.2021.2181

Hardimarta, F. P., Ikawati, K., & Yuniarti, C. A. (2020). Perbaikan Gambaran Lesi Aterosklerotik Dengan Pemberian Ekstrak Beta Vulgaris Pada Tikus Model Diet Atherogenik. *Media Farmasi Indonesia*, *15*(1), 1571-1577. https://doi.org/10.53359/mfi.v15i1.140

Harsa, I. M. S. (2014). Efek Pemberian Diet Tinggi Lemak terhadap Profil Lemak Darah Tikus Putih (Rattus novergicus). *Jurnal Ilmiah Kedokteran*, *3*(1), 21-28.

Indonesian Endocrinology Association. (2021). *Pedoman Pengelolaan Dislipidemia di Indonesia*. Available from: https://pbperkeni.or.id/wp-content/uploads/2022/02/23-11-21-Website-Panduan-Dislipidemia-2021-Ebook.pdf. [Accessed April 5, 2024]

Indonesian Heart Association. (2022). *Panduan Tata Laksana Dislipidemia*. Available from: https://www.inaheart.org/storage/guideline/7f75cffa237e402536f3d425012d985d.pdf. [Accessed April 5, 2024]

Institutional Animal Care and Use Program. (2023). Oral Gavage in Mice and Rats IACUC Standard Procedure. Available from: https://iacuc.ucsf.edu/sites/g/files/tkssra16261/files/wysiwyg/STD%20PROCEDURE%20-%20Misc%20Rodent%20Procedures%20-%20Oral%20Gavage%20In%20Mice%20and%20Rats.pdf. [Accessed 10th January 2025]

Juslim, R. R., & Herawati, F. (2018). *Penyakit Kardiovaskular*. Yogyakarta: Graha Ilmu.

Juszczyk, G., Mikulska, J., Kasperek, K., Pietrzak, D., Mrozek, W., & Herbet, M. (2021). Chronic Stress and Oxidative Stress as Common Factors of the Pathogenesis of Depression and Alzheimer's Disease: The Role of Antioxidants in Prevention and Treatment. *Antioxidants*, *10*(9), Article 1439. https://doi.org/10.3390/antiox10091439

Kanikowska, D., Korybalska, K., Mickiewicz, A., Rutkowski, R., Kuchta, A., Sato, M., Kreft, E., Fijałkowski, M., Gruchała, M., Jankowski, M., Breborowicz, A., & Witowski, J. (2020). Flaxseed (Linum Usitatissimum L.) Supplementation in Patients Undergoing Lipoprotein Apheresis for Severe Hyperlipidemia—A Pilot Study. *Nutrients*, *12*(4), Article 1137. https://doi.org/10.3390/nu12041137

Kauser, S., Hussain, A., Ashraf, S., Fatima, G., Ambreen, Javaria, S., Abideen, Z. U., Kabir, K., Yaqub, S., Akram, S., Shehzad, A., & Korma, S. A. (2024). Flaxseed (Linum usitatissimum); phytochemistry, pharmacological characteristics and functional food applications. *Food Chemistry Advances*, Article 100573. https://doi.org/10.1016/j.focha.2023.100573

Kawakami, Y., Yamanaka-Okumura, H., Naniwa-Kuroki, Y., Sakuma, M., Taketani, Y., & Takeda, E. (2015). Flaxseed oil intake reduces serum small dense low-density lipoprotein concentrations in Japanese men: a randomized, double blind, crossover study. *Nutrition Journal*, *14*(1), Article 39. https://doi.org/10.1186/s12937-015-0023-2

Kinanti, A. P., Lestari, A., Nabilah, Z. M., Maulida, R., Widiastuti, T. C., Kiromah, N. Z. W. (2023). Uji Aktivitas Antidiabetes Ekstrak Etanol Daun Ganitri (Elaeocarpus ganitrus Roxb.) Pada Tikus Wistar Jantan (Rattus norvegicus) Yang Diinduksi Streptozotocin. *Journal of Pharmaceutical Science and Clinical Research*, *8*(1), 139-151. https://doi.org/10.20961/jpscr.v8i1.64771

Kumar, N., Aithal, A. P., & Verma, S. (2021). Morphohistological analysis of the prevalence and distribution of atheroma in the abdominal aorta and its branches: a cadaveric study.

Journal Vascular Brasileiro, *20*, Article e20210014. https://doi.org/10.1590/1677-5449.210014

Kurniawan, D. W., Lestari, N. D., Sulistyo, H., Cacu. (2022). Pengaruh Pemberian Kombinasi Ekstrak Etanol Brotowali, Sambiloto, Meniran dan Kayu Manis Terhadap Histopatologi Glomerulus Tikus Model Hiperglikemia. *Journal of Pharmaceutical Science and Clinical Research*, *7*(3), 294-306. https://doi.org/ 10.20961/jpscr.v7i3.51501

Li, M. Q., Jiao, Q. Z., Xin, W. Q., Niu, S. L., Liu, M. M., Song, Y. X., Wang, Z. G., Yang, X. Y., & Liang, D. G. (2022). The Emerging Role of Rho Guanine Nucleotide Exchange Factors in Cardiovascular Disorders: Insights into Atherosclerosis: A Mini Review. *Frontiers Cardiovascular Medicine*, *8*, Article 782098. https://doi.org/10.3389/fcvm.2021.782098

Maghfiroh, R. M., Hariani, D., & Khaleyla, F. (2022). Efektivitas Ekstrak Daun Pepaya Jepang (Cnidoscolus aconitifolius) terhadap Kadar Kolesterol dan Histologi Aorta Mencit Hiperkolesterolemia. *LenteraBio*, *11*(1), 89–100. https://doi.org/10.26740/lenterabio.v11n1.p89-100

Malik, V. S., & Hu, F. B. (2015). Fructose and Cardiometabolic Health. *Journal of the American College of Cardiology*, *66*(14), 1615–1624. https://doi.org/10.1016/j.jacc.2015.08.025

Ma'rufi, R., & Rosita, L. (2014). Hubungan Dislipidemia dan Kejadian Penyakit Jantung Koroner. *JKKI*, *6*(1), 48–52.

Mercola, J., & D'Adamo, C. R. (2023). Linoleic Acid: A Narrative Review of the Effects of Increased Intake in the Standard American Diet and Associations with Chronic Disease. *Nutrients*, *15*(14), Article 3129. https://doi.org/10.3390/nu15143129

Ministry of Health, Republic of Indonesia. (2018). *Laporan Riset Kesehatan Dasar*. Available from: https://repository.badankebijakan.kemkes.go.id/id/eprint/3514/1/Laporan%20Riskesdas%202018%20Nasional.pdf. [Accessed April 24, 2024].

Moss, J. W. E., Davies, T. S., Garaiova, I., Plummer, S. F., Michael, D. R., & Ramji, D. P. (2016). A Unique Combination of Nutritionally Active Ingredients Can Prevent Several Key Processes Associated with Atherosclerosis In Vitro. *PLoS ONE*, *11*, Article e0151057. https://doi.org/10.1371/journal.pone.0151057

Mueed, A., Shibli, S., Korma, S. A., Madjirebaye, P., Esatbeyoglu, T., & Deng, Z. (2022). Flaxseed Bioactive Compounds: Chemical Composition, Functional Properties, Food Applications and Health Benefits-Related Gut Microbes. *Foods*, *11*(20), Article 3307. https://doi.org/10.3390/foods11203307

Pizzini, A., Lunger, L., Demetz, E., Hilbe, R., Weiss, G., Ebenbichler, C., & Tancevski, I. (2017). The Role of Omega-3 Fatty Acids in Reverse Cholesterol Transport: A Review. *Nutrients*, *9*(10), Article 1099. https://doi.org/10.3390/nu9101099

Polat, E. S., Citil, O. B., & Garip, M. (2013). Fatty acid composition of yolk of nine poultry species kept in their natural environment. *Animal Science Papers and Reports*, *31*(4), 363–368.

Poznyak, A. V., Nikiforov, N. G., Starodubova, A. V., Popkova, T. V., & Orekhov, A. N. (2021). Macrophages and Foam Cells: Brief Overview of Their Role, Linkage, and Targeting Potential in Atherosclerosis Macrophages and Foam Cells: Brief Overview of

Their Role, Linkage, and Targeting Potential in Atherosclerosis. *Biomedicines*, *9*(9), Article 1221. https://doi.org/10.3390/biomedicines9091221

Putri, D. A. (2018). Pengaruh Pemberian Ekstrak Etanol Biji Rami (Linum usitatissimum L.) dengan Fibroblast Growth Factor (FGF) Dari Telur Ayam Terfertilisasi Terhadap Kadar Glukosa Darah Mencit Putih Jantan Hiperglikemia (Thesis). STFI Yayasan Perintis, Padang.

Rosmala, A. Z., Asmara, I. G. Y., & Widiastuti, I. A. E. (2018). Perbedaan Hasil Pengukuran Kadar Kolesterol LDL antara Metode Direk dan Indirek dengan Menggunakan Rumus Friedewald pada Tikus Putih (Rattus norvegicus). *Unram Medical Journal*, *7*(3), 1-4. https://doi.org/10.29303/jku.v7i3.178

Sanches, S. C. L., Ramalho, L. N. Z., Augusto, M. J., da Silva, D. M., & Ramalho, F. S. (2015). Nonalcoholic Steatohepatitis: A Search for Factual Animal Models. *BioMed Research International*, *2015*, 1–13. https://doi.org/10.1155/2015/574832

Shahidi, S., Mahmoodi, M. S., Komaki, A., & Sadeghian, R. (2022). The comparison of omega-3 and flaxseed oil on serum lipids and lipoproteins in hyperlipidemic male rats. *Heliyon*, *8*(6), Article e09662. https://doi.org/10.1016/j.heliyon.2022.e09662

Susanti, N., Rahmawati, E., & Aprinda, R. K. (2019). Efek Diet Tinggi Fruktosa terhadap Profil Lipid Tikus Rattus Rattus norvegicus Strain Wistar. *Journal of Islamic Medicine*, *3*(2), 26–35.

Veonika, D. A., Wiboworini, B., Muthmainah. (2024). Cholecalciferol Effects on Lipid Profile of Experimental Animals: A Scoping Review. *Journal of Pharmaceutical Science and Clinical Research*, *9*(1), 33-44. https://doi.org/10.20961/jpscr.v9i1.73400

Wahyuni, S. (2015). *Dislipidemia* (1st ed., Vol. 1). Denpasar: Udayana University Press.

World Health Organization. (2021). *Cardiovascular diseases (CVDs)*. Available from: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds). [Accessed July 24, 2024].

Xu, L. Z., Gao, F., Yang, Z. L., Han, L. J., & Liu, X. (2016). Discriminant analysis of terrestrial animal fat and oil adulteration in fish oil by infrared spectroscopy. *International Journal of Agricultural and Biological Engineering,* *9*(3), 179–185. https://doi.org/10.3965/j.ijabe.20160903.2239

Yang, C., Xia, H., Wan, M., Lu, Y. F., Xu, D. F., Yang, X., Yang, L. G., & Sun, G. J. (2021). Comparisons of the effects of different flaxseed products consumption on lipid profiles, inflammatory cytokines and anthropometric indices in patients with dyslipidemia related diseases: systematic review and a dose–response meta-analysis of randomized controlled trials. *Nutrition & Metabolism*, *18*(1), Article 91. https://doi.org/10.1186/s12986-021-00619-3