NANOSUSPENSION OF *CARICA PAPAYA* L. SEED EXTRACT FOR ANTI-HYPERLIPIDEMIC PROPYL LIPIDS IN HYPERLIPIDEMIC HAMSTERS

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Abstract: *Carica papaya* seeds contain flavonoid compounds, saponins, tannins, and anthocyanins that function as anti-hyperlipidemic. This study aims to determine the effect of giving nanosuspension *C. papaya* seed extract on reducing levels of total cholesterol, LDL-cholesterol, triglycerides, and an increase in HDL-cholesterol in hamster blood induced by high-fat feed. The animals carried out for testing were randomly divided into seven treatment groups, namely the standard group, the opposing group was only given high-fat flour, the positive group was assigned atorvastatin 2.42 mg/kg body weight, and three test groups were given consistent preparations of *C. papaya* seed extract at a dose of 2.4% each; 4,8%; 9.6% and the test group was assigned a trial of *C. papaya* seed extract of 240 mg/kg body weight. Activities the administration of test preparations is carried out for 14 days. Measurement of lipid propyl levels using a clinical spectrophotometer. Test data analysis using one-way ANOVA statistics. All doses of *C. papaya* seed extract nanosuspension preparations were significantly able to lower levels of total cholesterol, LDL-cholesterol, and triglycerides, and increase HDL-cholesterol ($p \leq 0.05$) compared to negative controls in hyperlipidemia hamsters. Dose 3 nanosuspension preparations (9.6%) were significantly able to lower levels of total cholesterol, LDL, and triglycerides and increase HDL-cholesterol levels ($p \leq 0.05$) compared to negative controls and were comparable to positive controls of atorvastatin ($p \geq 0.05$).

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INTRODUCTION

Coronary Heart Disease (CHD) has become the leading cause of death in the world. One of the other risk factors for coronary heart disease is hyperlipidemia (Sulistyowati, 2009). Hyperlipidemia is a condition of excess fats (lipids) in the blood, including cholesterol and triglycerides. Hyperlipidemia, in general, is divided into two subcategories: hypercholesterolemia and hypertriglyceridemia (Harikumar et al., 2013).

Plants that can reduce hypertriglyceridemia include *C. papaya* seeds. *C. papaya* seeds have benefits where the compounds contained in *C. papaya* seeds, namely tannins, saponins, and flavonoids, have the potential as antioxidants (Zhou et al., 2011). The content of compounds found in *C. papaya* seeds includes fatty acids, crude protein, crude fiber, *C. papaya* oil, campaign, benzyl isothiocyanate, benzyl glucosinolate, glucotropacolin, benzylthiourea, caring hentriacontane, β-sitosterol, and the enzyme tyrosine (Yogiraj et al., 2014). Phytochemical analysis of *C. papaya* showed the presence of saponins, alkaloids, tannins, flavonoids, cardiac glycosides, anthraquinones, phlobatinins, anthocyanosides, and phenols (Srivastava & Singh, 2016). Previous studies have shown *C. papaya* seed water extract can lower glucose levels, lipid profiles, or total cholesterol and blood triglycerides in male rats of *Sprague Dawley* (Lusiana et al., 2011; Dawane et al., 2015). The same thing done by Nwangwa & Ekhoye (2013) showed that *C. papaya* seed water extract reduced blood lipid levels in albino rats. According to Inneh et al. (2019), the *C. papaya* seed extract has anti-hyperlipidemic and anti-atherogenic activity

The activity of medicinal plants depends on the release of biologically active compounds. Most of the extracts' active components cannot pass through the lipid membrane of the cells (Ajazuddin & Saraf, 2010). These constraints cause some excerpts not to be used clinically, so optimizing the preparation of natural materials with nanotechnology is necessary. The manufacture of nanostructured systems is expected to strengthen the action of herbal extracts (Bailey & Berkland, 2009). Nanotechnology is an approach to producing particles in nano-size (Chingunpituk, 2007). The manufacture of extract nanoparticles is carried out with a ball mill tool. Milling is carried out inside the chamber with small balls rotating inside. The *C. papaya* seed extract is a viscous extract with Etonol as a solvent; milling is carried out with ethanol carriers. Particle size reduction of *C. papaya* seed extract with Ballmill tool. Particle size reduction results in particles measuring 410.4 nm. This study aims to determine the nanosuspension activity of ethanol extract of 70% *C. papaya* seeds against prolyl lipids of hamster hyperlipidemia.

RESEARCH METHODS

Seeds Extraction

Fresh *C. papaya* seeds are cleaned of adhering impurities and then washed with water. Soaked seeds are drained, then dried by oven at 60ºC to dry and carry out dry sorting. After drying, the simplicia is mashed using a pollinator until it becomes powder and then sifted with a sieve mesh number 40 weighed. After extraction by maceration, 500 g of *C. papaya* seed powder is put into the macerator, then 70% ethanol solvent is added. Separated macerate using filtration. Do the repetition three times. The Maserati obtained is concentrated using a vacuum rotary evaporator until a viscous extract is received, then evaporated in a water bath with a temperature of 50ºC until a thick section of Ethanol 70% *C. papaya* seeds is obtained.

Examination of Extract Characteristics

The examination of extract characteristics includes an organoleptic test examining the shape, color, smell, and taste of ethanol extract of 70% *C. papaya* seeds. The calculation of amendment is calculated by calculating the amount of section obtained divided by Simplicia powder and then multiplied by 100% (Departemen Kesehatan RI, 2000). Determination of water content using the toluene distillation method (Departemen Kesehatan RI, 2008). The decision of the total level of the calculation against the weight of the test material is expressed in % b / b. This phytochemical screening test was carried out to see the chemical content in the form of alkaloids, flavonoids, tannins, saponins, terpenoids, and steroids contained in the range of *C. papaya* seed extract (Hanani, 2015).

Manufacture of *C. papaya* Seed Extract Nanosuspension

The nanosuspension formula of the *C. papaya* seed extract can be seen in Table 1.

Table 1. Nanosuspension Manufacturing Formula

Ingredient Name	Formula I	Formula II	Formula III	Function
C. papaya seed extract	2,4	4,8	9,6	Active substances
HPMC	1	1	1	Viscosity increase
Tween 80	1	1	1	Surfactant
Nipagin	0,1	0,1	0,1	Preservatives
Na.metabisulfit	0,1	0,1	0,1	Antioksidants
Aquadest ad	100ml	100ml	100ml	Solvent

The manufacture of *C. papaya* seeds nanosuspension extract is carried out by Ready *C. papaya* seed extract, put into ball milling by wet milling method. Then, a nanoparticle of ethanol extract is formed, 70% *C. papaya* seed extract. HPMC is dispersed in hot water at a temperature of 70ºC until it expands. Nipagin is dissolved in hot water first until it becomes a solution. Sodium metabisulfite is dissolved first with water, then HPMC and nipagin solution, stirring until homogeneous. Put tween 80 as a surfactant, enter the *C. papaya* seed extract's nanoparticle results, then go until homogeneous After mixing all, justify the volume with equates until the limit mark (Iskandarsyah & Mutakim, 2010).

Evaluation of the physical properties and physical stability of the nanosuspension *C. papaya* seed extract was carried out for six weeks, including particle size, potential zeta, polydispersity index, particle morphology, viscosity, and acidity of the nanosuspension preparation to determine the character and stability of the nanosuspension particles.

Manufacture of High Cholesterol Feed

High-fat feed comprises 40% quail egg yolk, 10% white butter, and standard meal (hamster pellets) 50%. For the manufacture of high-fat feed, raw quail egg yolks are separated by the whites, then quail egg yolks are added with white butter that has been heated and mixed with hamster standard feed until dough is formed is made into pellets. Feed hamsters 80 g in 1 day about 10 g (Smith et al., 1995).

Test Animal Treatment

The treatment of test animals has received approval from the Ethics Committee for Health Research, Faculty of Medicine, University of Indonesia, No: KET-1257 / UN2. F1/ETIK/PPM.00.02/2019. This study was conducted experimentally with a complete randomized design, using 28 male *Syrian* hamsters divided into seven groups of 4 hamsters per group. The division of the group of hamsters is as follows:

a) Group 1: Normal control, given standard feed.
b) Group 2: Positive control, given high cholesterol feed and Atorvastatin 2,422 mg/kg body weight.
c) Group 3: Negative control, given high cholesterol feed and CMC Na.
d) Group 4: D introduction of *C. papaya* seed extract using a dose of 240mg/kg body weight.
e) Group 5: D introducing high-cholesterol feed and a 2.4% dose of extract nanosuspension preparations.
f) Group 6: Given high cholesterol feed and nanosuspension preparation, extract dose of 4.8%.
g) Group 7: Given high cholesterol feed and nanosuspension preparation, extract dose of 9.6%.

Blood Serum Collection

Hamster blood collection is carried out two times, namely on day 29 and day 43, through the orbital sinuses in each group of hamsters. Hamsters are treated using ketamine until unconscious. Previously male hamsters were satisfied first for 12 hours. Blood is put into the microtube and centrifuged for 5 minutes at a speed of 6000 rpm; blood serum will be obtained to check the total cholesterol levels, LDL, HDL, and Triglycerides (Vogel, 2008).

RESULTS AND DISCUSSION

Phytochemical screening is carried out in the thick extract of *C. papaya* seeds, including alkaloids, flavonoids, saponins, tannins, terpenoids, and steroids. The results of phytochemical screening can be seen in Table 2. The compounds in *C. papaya* seeds are tannins, saponins, and flavonoids that function as antioxidants. According to Ulyarti et al., (2017), flavonoids and tannins can be used as biopharmaceutical raw materials.

Table 2. Phytochemical screening results of ethanol extract 70% *C. papaya* seeds

No.	Types of Testing	Results
1.	Alkaloids	+
2.	Saponins	+
3.	Tannins	+
4.	Phenol	+
5.	Flavonoid	+
6.	Triterpenoid	+

+ = exists
- = none

Organoleptic tests and drying shrinkage are carried out to find out the characteristics of powders and extracts. Results can be seen in Table 3.

Table 3. The Characteristic Yield of 70% *C. papaya* Seeds Ethanol Extract

No.	Type	Organoleptic Test			
		Shape	**Odor**	**Taste**	**Color**
1.	Powder	Fine Powder	Distinctive	Bitter	Dark Brown
2.	Extract	Dry	Distinctive	Bitter	Dark Brown

Table 4. Test Results of Water and Ash Content of 70% *C. papaya* Seeds Ethanol Extract

No	Type	Result (%)
1.	Moisture Content	9,84
2.	Ash Content	17,54

***C. papaya* Seed Extract Nanosuspension**

The manufacture of extract nanosuspension is carried out with a ball mill tool. Milling is carried out in the chamber with a small ball rotating inside—particle size reduction results in particles measuring 410.4 nm (Gupta et al., 2012). Meanwhile, the particle test results on the nanosuspension have an average particle diameter size of 300.2 nm - 455.5 nm.

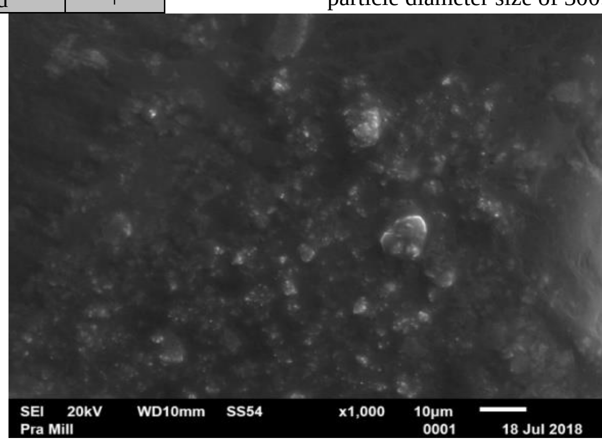

Figure 1. *C. papaya* Seed Extract Before Milling

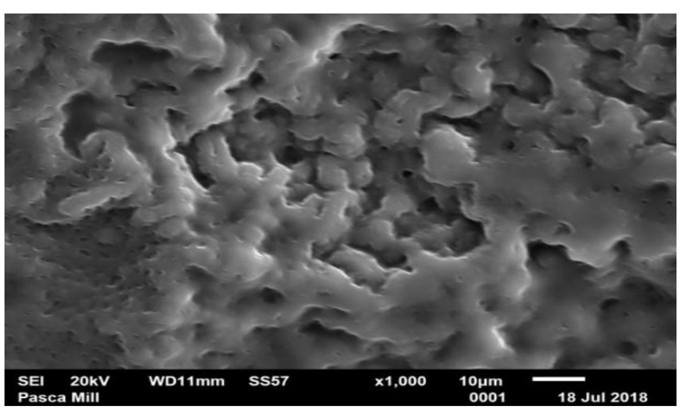

Figure 2. *C. papaya* Seed Extract After Milling

C. papaya Seed Extract Nanosuspension Preparations

Nanosuspension is colloidal dispersions of drug particles in nano-size (Patravale et al., 2004). It can also be defined as a biphasic system consisting of pure drug particles dispersed in an aqueous polymer where the diameter of the suspended particles is less than 1μm in size. Consistency is prepared by mixing *C. papaya* seed extract nanoparticles with HPMC and Tween 80 as a stabilizer that reduces the system's free energy by lowering the interfacial voltage in the design. Besides that, this stabilizer can also prevent nanoparticle aggregation by electrostatic or steric stabilization. The large surface area of *C. papaya* seed extract due to the small particle size produces a high interfacial voltage that can increase the free energy in the system; this shows that the nanosuspension of *C. papaya* seed extract is unstable thermodynamically unstable.

Nanoparticles with a zeta potential of ± 30 mV can prevent aggregation between particles due to charges on the surface of the nanoparticles (Patel et al., 2016). In the formula, there is no potential value of zeta above the ± 30 mV; this means that the procedure can produce a weak repulsive force, as a result of which particles can merge to form aggregates that can precipitate.

The polydispersity index is a parameter that expresses the particle size distribution with values of 0.01 - 0.7 nanoparticles with a narrow distribution and more than 0.7 extensive size distribution (Nidhin et al., 2009). The smaller the value of its polydispersity index, the more homogeneous the particle size spread (Yuan et al., 2008). All formulas obtained a polydispersity index of 0.571, still in the 0.01 - 0.7. This indicates a relatively narrow or homogeneous particle size distribution. No change occurs during six weeks of storage at room temperature.

Viscosity also affects the stability of a suspension system. If the particle charge is negligible, then according to Stoke's law, the sedimentation rate is affected by the diameter and suspending agent. The larger the particle size, the higher the speed of sedimentation rate; on the contrary, the higher the viscosity, the smaller the sedimentation rate. However, if the viscosity is too high, it can cause caking, making the suspension difficult to redispersion (Priyambodo, 2007). The result of the viscosity inspection using the viscometer type first RM spindle no one formula viscosity is 250 rpm. Viscosity is the resistance of a liquid to flow; the higher the viscosity, the more excellent the opposition (Martin et al., 1993). The results of pH measurements in the three formulas carried out; there was no significant difference where the pH of the preparation ranged from 6.5 - 6.7, whereas the standard pH of the suspension, according to Kulshreshta et al. (2009), was between 5-7.

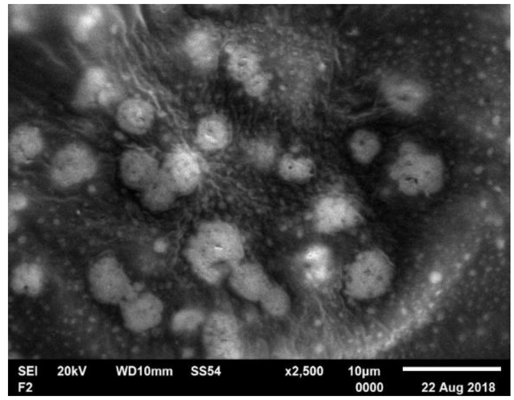

Figure 3. Morphology of Nanosuspension Particles of *C. papaya* Seed Catalysis

Particle surface can be observed using SEM (Scanning Electron Microscopy) with a magnification of 2,500 times; nanosuspension particles look spherical. The particle size in nanosuspension is the most critical parameter because it is responsible for physical stability. Nanosuspension may change the crystal structure, which may become amorphous or polymorphic due to high-pressure homogenization.

Hyperlipidemia Hamsters

The study used experimental animals of male Syrian hamsters of the same age and environmental conditions to avoid differences in biological activity. The hamster used is a healthy hamster with signs of clear eyes, clean fur, and regular and active behavior. The selection of hamster model animals is because hamsters have a lipoprotein profile that is more like humans than rats. Hamsters also have an atherogenic lipoprotein profile that can circulate into a non-HDL form and a cholesterol ester transport protein (CETP). This receptor mediates the uptake of LDL lipoproteins through LDL receptors that play a role in producing B-100 apolipoproteins (apo) in the liver and the production of Apo B in the intestine (Smith et al., 1995). Thus, hamsters develop hypercholesterolemia and hypertriglycerides faster when fed cholesterol-rich foods.

The standard feed used contains a moisture content of 13%, protein 21%, ash 7%, crude fat 7%, crude fiber 5%, calcium 0.90%, phosphorus 0.60%. The butter used is Anchor Pure New Zealand Unsalted Butter 10%, each serving (15 g) containing 7 g of saturated fat, 2.5 g of monounsaturated fat, 0.5 g of trans fat, and 30 mg of cholesterol. Butter is one of the saturated fatty acids derived from animal products that contain high fats and is a source of trans fats in the body that are harmful if consumed excessively. Trans fatty acids inhibit the activity of the enzyme Lecithin Cholesterol Acyl Transferase (LCAT), causing a decrease in cholesterol esterification accompanied by an increase in the transfer of cholesterol esters from HDL to LDL. This increases LDL cholesterol levels and decreases HDL cholesterol levels (Mozaffarian et al., 2006).

Total Cholesterol Levels

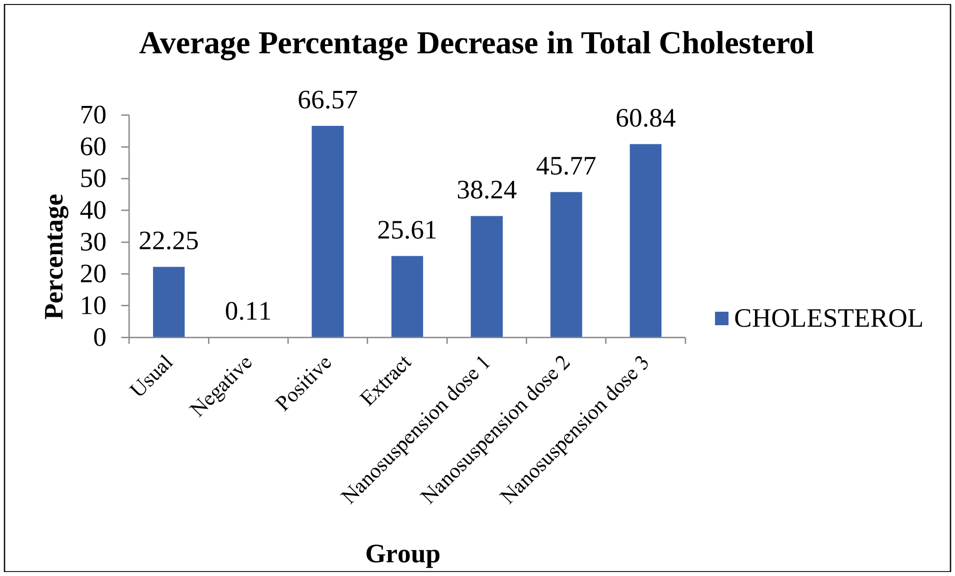

Figure 4. Average percentage decrease in total cholesterol levels

The data results on the percentage of hamsters' total cholesterol levels are entered into the statistics and tested for the normality of the data and their homogeneity. The statistical results show that the data on total cholesterol levels are distributed normally ($p = 0.200$) and homogeneously ($p = 0.274$). Then proceed with the analysis using a one-way ANOVA. From the results of the ANOVA table, the percentage of total cholesterol levels is processed p-value = $0.000 < 0.05$. These results show that the provision of nanosubscribed *C. papaya* seed extract has a meaningful effect on hamster cholesterol levels. The data is then continued with the Tukey test. The Tukey test showed that the percentage decrease in total cholesterol levels was obtained by the nanosuspension group dose III (60.84%), which was comparable to the positive control (66.57%), and differed significantly from the nanosuspension group dose I (38.24%) and dose II (45.77%).

LDL-cholesterol levels

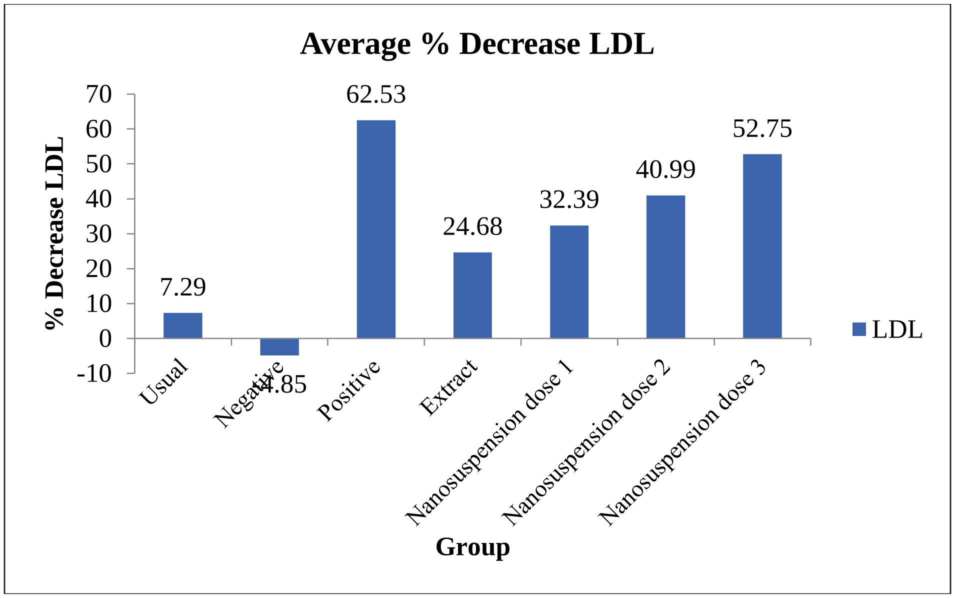

Figure 5. Average percentage decrease in LDL cholesterol levels

The average percentage decrease in LDL levels in figure 2 shows that the positive control group (62.53%) gave the most significant percentage decrease compared to other groups. The negative group showed a percentage decrease of -4.85%, which means that the negative group experienced a continuous increase in LDL levels because the negative group was not given test preparations, only given high fat diet foods. The nanosuspension group at dose 3 (52.75%) gave the largest percentage reduction in LDL levels when compared to the nanosuspension group at doses 1, 2 and the extract group. This also shows that there is an increase in the decrease in LDL levels after being made into nanosuspension preparations when compared to extract preparations.

Data LDL levels were statistically tested, and the normality test results obtained Asymp values. Sig. (2-tailed) = 0.200>α (0.05), the data is normally distributed. The homogeneity test results obtained a Sig value of 0.293 > α (0.05) so that the data varied homogeneously, then continued with analysis using one-way ANOVA. The results of the ANOVA table against the percentage decrease in LDL levels obtained sig values. 0.000 < 0.05. This shows that there are significant differences between treatments. According to Legis (2017), Phenolic compounds in natural ingredients have been shown to have antioxidant effects and can inhibit the oxidation of LDL (Saputri et al., 2017). It can be concluded that the administration of test preparations influences the decrease in hamster LDL levels. Then the analysis continued with the Tukey test. For the Tukey test, the decrease in LDL levels data showed that the negative group differed significantly from the group given the test preparation and the normal group. It can be concluded that the dose three nanosuspension groups have an activity of lowering LDL levels equivalent to atorvastatin as a comparison substance. Phenol extract can also lower glucose, triglyceride, and LDL cholesterol levels and increase energy expenditure and oxidation of weight loss (Terra et al., 2009; Sari et al., 2021). Thus, the nanosuspension of ethanol extract of 70% *C. papaya* seeds dose III lowered LDL levels equivalent to the positive control, with a percentage of reducing LDL cholesterol levels by 52.75%.

Triglyceride Levels

Based on the statistical tests using SPSS, the normality test results obtained Asymp values. Sig. (2-tailed) = 0.054 > 0.05) so the data is distributed normally. The homogeneity test results obtained a Sig value of 0.173 > 0.05) so the data varied homogeneously, then continued with analysis using a one-way ANOVA. The Tukey test results showed a difference in the percentage of the significant decrease in triglyceride levels in the entire group of test preparations. A negative group obtained a Sig value of α < (0.05). This shows that nanosuspension preparations of the *C. papaya* seed extract can significantly reduce triglyceride levels against hamster hyperlipidemia induced by a high-fat diet.

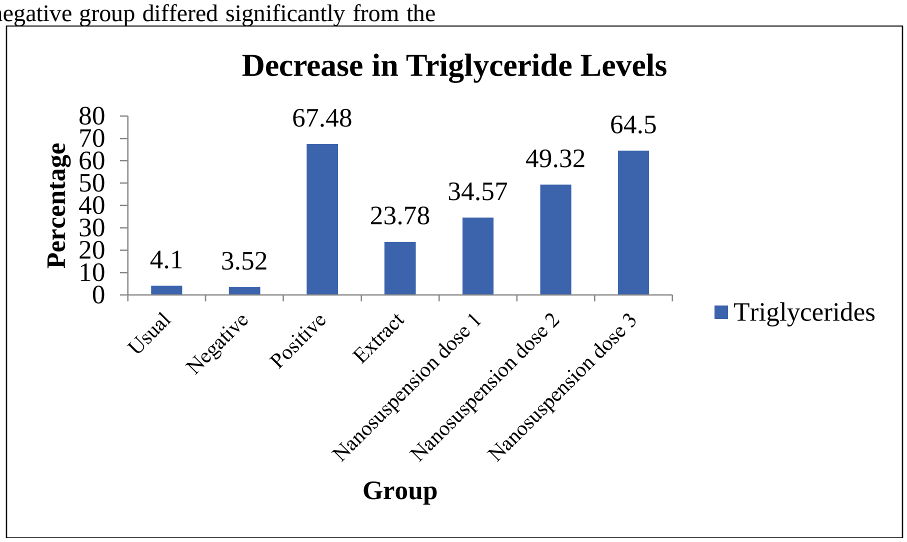

Figure 6. Average percentage decrease in Triglyceride levels

Based on the Tukey test, when compared to the negative group, there was also a significant decrease in the percentage of triglyceride levels in all groups except the normal group. The decrease in triglycerides is not very specific because there is also a decrease in triglyceride levels in the normal group. These results showed that the administration of nanosuspension preparations of *C. papaya* seed extract dose 3 had a meaningful effect on reducing hamster blood triglyceride levels because it was comparable to the positive control of atorvastatin. Based on figure 6, it can be seen that the percentage decrease in triglyceride levels increased in the nanosuspension preparation group of *C. papaya* seed extract of all dose variations (34.57%; 49.32%, and 64.50%) and the positive control group (67.48%) when compared with the negative group (3.52%) and the normal group (4.1%).

HDL-cholesterol levels

The average percentage increase in HDL levels was then carried out normality and homogeneity tests. Based on the data obtained from the results of the standard distribution test for HDL levels (α = 0.071), the homogeneity test (α = 0.233) shows that the distributed data is regular and homogeneous (α >0.05).

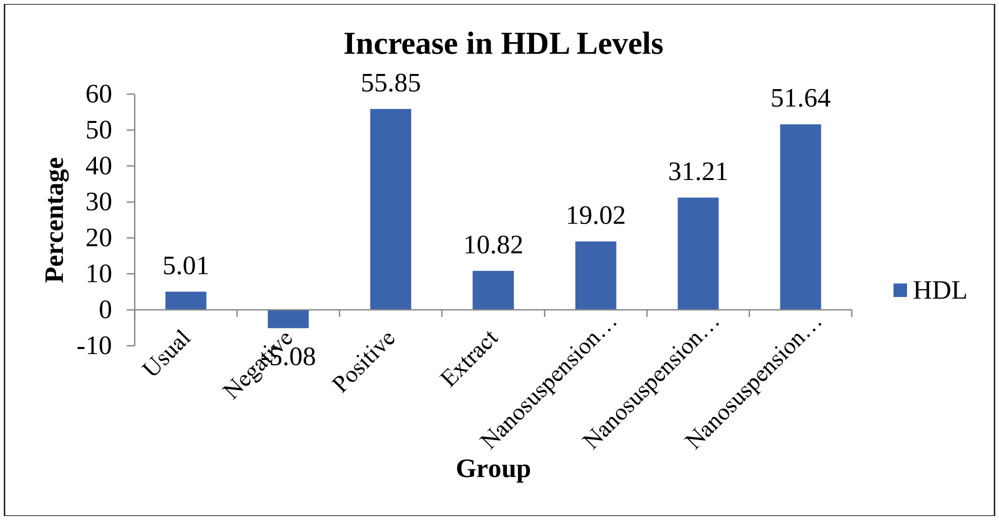

Figure 7. Average percentage increase in HDL cholesterol levels

Data on the percentage increase in HDL levels continued with a one-way ANOVA test with a significance level of 95% ($\alpha = 0.05$) and obtained a Sig value. 0.000<0.05 indicates the effect of treatment or meaningful differences on the increase in HDL levels of each group. Based on the results of the Tukey test, there was a significant difference (<0.05) between the negative control group and the positive control; all extract preparations and nanosubscribed nanosuspension of doses 1, 2, and 3. There was a significant difference between nanosuspension doses 1, 2, and 3 indicating a difference in the activity of the three doses. In this case, the nanosubstitution group of dose three *C. papaya* seed extract had a movement of increasing HDL levels comparable to the positive control group. Figure 7 shows that the negative control group showed the smallest percent increase in HDL levels compared to the other group of -5.08%. The small percentage is due to the induction of high-fat feed but not given test preparations. N-standard control occurs due to physiological factors in hamsters who are not given test preparations or a low-fat diet.

All groups of test preparations showed different results with hostile control groups on reducing total cholesterol levels, LDL cholesterol, triglycerides, and increasing HDL cholesterol levels. *C. papaya* seeds have benefits where the compounds contained in *C. papaya* seeds, namely tannins, saponins, and flavonoids, have the potential as antioxidants (Zhou et al., 2011). Flavonoids are phenol compounds among the secondary metabolites in plants that function as antioxidants (Ningrum et al., 2021; Zuraida et al., 2017). Flavonoids are antioxidants that capture radicals and maintain a balance between oxidants and antioxidants in the body. Flavonoids can improve the endothelial function of blood vessels and are hypolipidemic, anti-inflammatory, and antioxidants. According to Sari et al. (2021), flavonoids can decrease the accumulation of lipids in the heart, reduce glucose absorption, and inhibit the breakdown of polysaccharides into monosaccharides. The high flavonoid content in *C. papaya* seeds can lower total liver cholesterol, blood triglycerides, and LDL cholesterol in hamster hypercholesterolemia. Antioxidant compounds in *C. papaya* seeds are thought to increase the secretion of bile acids to reduce levels of total cholesterol, triglycerides, and LDL cholesterol.

CONCLUSION

The nanosuspension of the *C. papaya* seed extract can be made through a suspension with the combined stabilizer formula of HPMC and Tween 80, which has the highest viscosity with the smallest particle size. All doses of nanosubstantial preparations of *C. papaya* seed extract were significantly able to lower levels of total cholesterol, triglycerides, LDL cholesterol, and increase HDL cholesterol compared to negative controls ($p \leq 0.05$) in hyperlipidemia hamsters. Nanosuspension preparation dose 3 (9.6 %) was significantly able to lower levels of total cholesterol, LDL cholesterol, and triglycerides and increase HDL cholesterol levels compared to the positive control of atorvastatin ($p \geq 0.05$).

ETHICAL APPROVAL

All experimental protocols were approved by the Ethics Committee for Health Research, Faculty of Medicine, University of Indonesia, No: KET-1257/UN2. F1/ETIK/PPM.00.02/2019 and were performed in accordance with ethical standards for the care and use of laboratory animals.

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Conflict of interest: We declare this study no potential conflict of interest.

Informed consent: Informed consent was obtained from all participants included in the study.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to the conception and design, analysis, and interpretation of data, and critical review of the manuscript. HD conception and interpretation of the results, and wrote the manuscript. EW carried out the design of the study, performed the statistical analyses, and the revision of the manuscript. PML performed statistical analyses and helped rewrite the manuscript substantially during the revision process. HH was involved in drafting the manuscript. SS helped revise the manuscript. All authors have been involved in revising the manuscript critically for important intellectual content. All authors have read and agreed to the published version of the manuscript.

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