

Nano-cocrystals of piperine–succinic acid and its antihyperlipidemic effect in male Wistar rats

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Abstract: Background: Piperine, one of the alkaloid compounds from *Piper nigrum* L., has been renowned for its remarkable pharmacological effects, including its antihyperlipidemic activity. However, piperine exhibits poor solubility and impairs pharmacological effectiveness. **Objectives:** The aim of the study was to develop piperine–succinic acid nano-cocrystals and to investigate their efficacy in reducing serum cholesterol and triglyceride levels in male Wistar rats. **Methods:** Nano-cocrystals were prepared by the wet milling method and characterized for solid-state properties. Antihyperlipidemic activity was tested in 24 rats, which were randomly assigned into four groups: negative control group (NaCMC 0.5%), positive control group (simvastatin 0.18 mg/kgBW), intact piperine group and nano-cocrystals piperine–succinic acid group with a dose equivalent to 40 mg/kg of the body weight. Serum total cholesterol and triglyceride levels were measured enzymatically. **Results:** Thermal and X-ray diffraction analyses confirmed the formation of new crystalline structures. The average particle size was 245.5 nm with a polydispersity index of 0.447 and a zeta potential value of -2.51 mV, indicating limited colloidal stability. Despite this, the nano-cocrystals significantly reduced total cholesterol and triglyceride levels compared to intact piperine ($p < 0.05$). The duration of treatment also influenced lipid reduction ($p < 0.05$). **Conclusion:** Although the formulation demonstrated enhanced bioactivity, further optimization of stability parameters is warranted to ensure long-term efficacy.

Keywords: Nano-cocrystals; Piperine; Succinic acid; Total cholesterol; Triglycerides

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INTRODUCTION

Hyperlipidaemia is a metabolic dysfunction marked by abnormal blood lipid levels. It is characterized by elevated levels of total cholesterol, low-density lipoprotein (LDL) cholesterol and/or triglycerides, along with reduced levels of high-density lipoprotein (HDL) cholesterol (Berberich and Hegele, 2022). The prevalence of hyperlipidemia cases is increasing gradually every year due to some influencing factors, including diet and lifestyle. Natural plants have been utilized by ancient civilizations, particularly in culinary practices and traditional medicinal systems such as Ayurveda and traditional Chinese medicine. One of the most prominent spices that is usually used is black pepper (*Piper Nigrum* L.), which has been known for its spicy taste as well as its medicinal properties (Dludla *et al.*, 2023).

Piperine is one of the major compounds found in black pepper and other Piperaceae family. This alkaloid is insoluble or slightly soluble in water molecules (40 mg/L), but readily soluble in alcohol (1g/15 mL) and ether (1g/1.7 mL) (Vasavirama and Upender, 2014). This causes low dissolution of piperine in the gastrointestinal tract which results in oral bioavailability and low *in-vivo* pharmacological effects (Salsabila *et al.*, 2021; Zaini *et al.*, 2020b). Some studies reported that piperine has a variety of pharmacological and therapeutic effects, including analgesic and anti-inflammatory (Quijia and Chorilli,

2020). In addition, piperine has been associated with beneficial effects on the cardiovascular system; it has been reported to increase HDL (Haq *et al.*, 2021), reduce LDL, very low-density lipoprotein (VLDL) and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) levels (Damdindorj *et al.*, 2021).

Several methods have been used to improve the solubility of piperine, including formation of multicomponent crystals of piperine with succinic acid (Zaini *et al.*, 2020b), saccharin (Zaini *et al.*, 2020a), caffeine (Fitriani *et al.*, 2023), combination with 2-hydroxypropyl- β -cyclodextrin (Stasiłowicz *et al.*, 2021), formation of solid dispersion (Fitriani *et al.*, 2024; Thenmozhi and Yoo, 2017; Zaini *et al.*, 2021) and nanoparticles (Bhalekar *et al.*, 2017; Tomar and Kumar, 2023). A previous study showed that the formation of multicomponent crystals of piperine with succinic acid as a cofomer increased up to 3.9 and the dissolution rate to 2 times that of piperine (Zaini *et al.*, 2020a). Nano-cocrystals are a combination of cocrystals with nanotechnology, which was developed based on the Noyes–Whitney equation, decreasing the particle size of the drug leads to an increase in its surface area, thus the dissolution rate is expected to be increased proportionally, which accelerates the completion of insoluble drugs better (Al-Kassas *et al.*, 2017). Therefore, the following research has also explored the formation of nano-cocrystals of piperine-succinic acid using the wet milling method, which could increase the solubility by 12.4-fold and the

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dissolution rate by 7.32-fold compared to intact piperine, respectively (Fitriani *et al.*, 2022).

The review by Tripathi *et al.* (2022) summarizes that piperine (30–50 mg/kgBW) can improve metabolic disorders by acting as a Melanocortin-4 (MC-4) receptor agonist, enhancing lipid profile, improving insulin signaling and reducing oxidative stress and inflammation. Another study also reported that piperine at a dose of 40 mg/kg in male Sprague Dawley rats with hyperlipidemia has shown a significant decrease in levels of triglyceride, total cholesterol, LDL, VLDL, and an increase in HDL levels in the blood (Shah *et al.*, 2011). Hence, the nano-crystal formulation in this study is intended to enhance the antihyperlipidemic efficacy of piperine. The nano-cocrystal was prepared and characterized as in a previous study, with a difference in the choice of stabilizer, Hydroxypropyl methylcellulose (HPMC) (Fitriani *et al.*, 2022). HPMC acts as an effective steric stabilizer by forming a hydrated polymer layer around nanoparticles, preventing aggregation and recrystallization through surface adsorption and hydrogen bonding between its hydroxyl groups and the drug molecules. This interaction is expected to enhance suspension stability by providing steric hindrance and viscosity control (Touqeer *et al.*, 2022). The antihyperlipidemic effect of piperine–succinic acid nanococrystals was evaluated in male white rats fed a high-fat diet, as this model exhibits lipid metabolism alterations similar to those of human dyslipidemia. The parameters of antihyperlipidemic activity are the percentage reductions in total cholesterol and triglyceride levels, determined by enzymatic reactions.

MATERIALS AND METHODS

Materials

Piperine (BOC Science, USA), succinic acid (Merck, Germany), ethanol pro analysis (Merck, Germany), HPMC (Merck, Germany), ethyl acetate (Merck, Germany), total cholesterol level analysis reagent (Greiner Diagnosti GmbH, Germany), triglyceride analysis reagents (Greiner Diagnosti GmbH, Germany), intact cholesterol (Nippon Fine Chemical, Japan), propylthiouracil tablets 100 mg (PT. Kalbe Farma, Indonesia), simvastatin tablets 10 mg (PT. Kalbe Farma, Indonesia), Na CMC (PT. Alfa Kemika, Indonesia), peanut oil (PT. Alfa Kemika, Indonesia), quail eggs.

Methods

Preparation of nano-cocrystal piperine – succinic acid

Cocrystals of piperine-succinic acid (2:1 molar ratio) were prepared using the slurry method as described in the previous study (Zaini *et al.*, 2020a). Nano-cocrystals of piperine-succinic acid were prepared by wet ball milling (Retsch PM 100; Germany) method as also described in a previous study (Fitriani *et al.*, 2022), using HPMC as a stabilizer. A total of 0.5 g of piperine-succinic acid

cocrystal and 0.05 g of HPMC were dispersed in 10 mL of distilled water. Then put into a chamber containing 30 g of grinding balls. This process formed a nanosuspension at a speed of 400 rpm in 30 minutes. The nanosuspension was then dried in a freeze dryer (Christ Alpha 1-2 Ld Plus; Germany) to obtain nano-cocrystals piperine-succinic acid. The formed nano-cocrystals were then stored in a desiccator at room temperature containing silica gel as the humidity absorbent.

Characterization of nano-cocrystal piperine – succinic acid

Particle size analysis, polydispersity index and zeta potential: Particle size analysis was conducted using a particle size analyzer (Malvern, Zetasizer Nano ZS, UK) with the principle of dynamic light scattering (DLS). The analysis was performed with piperine–succinic acid nano-cocrystals. Nano-cocrystal piperine – succinic acid was dispersed in distilled water and then the particle size was analyzed at a temperature of 25°C.

Thermal analysis with differential scanning calorimetry (DSC): Thermal analysis was conducted using a DSC instrument (Shimadzu DSC-60 Plus, Japan), which was set to a temperature range of 50–250°C and a temperature rate of 10°C/min (Fitriani *et al.*, 2022). Prior to measurement, the sample was placed in an aluminum pan. Analyses were carried out for piperine, piperine-succinic acid cocrystals and nano-cocrystals piperine-succinic acid.

X-ray diffraction pattern analysis: X-ray diffraction (XRD) analysis was performed using a diffractometer (X'Pert PRO, PANalytical, Netherlands) with Cu K α radiation operated at 45 kV and 40 mA. Data were collected over a 2 θ range of 5–40° (Fitriani *et al.*, 2022). The analysis was conducted for piperine, piperine–succinic acid cocrystals and piperine–succinic acid nano-cocrystals.

Drug content analysis

Nano-cocrystals equivalent to 10 mg of piperine were dissolved in 100 mL of ethanol p.a. and measurements were performed in triplicate using a UV–Vis spectrophotometer (Shimadzu UV-1700, Japan) at 342.2 nm (Fitriani *et al.*, 2022). The amount of piperine was then calculated using the linear regression curve and the experiment was performed in triplicate.

Antihyperlipidemic activity study

This study was conducted using an in-vivo experimental approach to assess the antihyperlipidemic effect of nano-cocrystals piperine-succinic acid. The outcome measures included total cholesterol and triglyceride levels, with total cholesterol as the primary outcome. Healthy male Wistar rats aged 2–3 months and weighing 200–250 g were obtained from the Animal House at the Faculty of Pharmacy, Universitas Andalas. Only rats with total cholesterol levels >100 mg/dL after induction were

included in the study. No animals or data were excluded from the analysis. Male rats were selected because estrogen can influence lipid metabolism and potentially interfere with the study outcomes (Xie *et al.*, 2022; Tunsophon and Chootip, 2016).

Before treatment, rats were acclimatized for 7 days at the Animal House under standard laboratory conditions with free access to food and water to allow adaptation to the environment. Experimental procedures were conducted at the Laboratory of Anatomy and Pharmacology and the Animal house of the Faculty of Pharmacy, Universitas Andalas. All procedures were carried out in accordance with the European Council Directive on the Laboratory Animal Care and Use of Animals (86/609/EEC). Animals were handled with care throughout the study to reduce stress and discomfort and were monitored daily for their general condition. No adverse events or mortality occurred during the experiment. Although no specific humane endpoints were established, the animals were regularly observed for signs of distress or illness.

Sample size was determined using Federer's formula; with four group treatments, six animals per group were required, resulting in a total of 24 rats. The rats were randomly assigned using a computer based random order generator into four groups: A negative control group (Na-CMC suspension), a positive control group (simvastatin, 0.18 mg/200 g BW) (Harini and Astirin, 2009), test group 1 (intact piperine, 40 mg/kg BW) and test group 2 (piperine-succinic acid nano-cocrystals equivalent to 40 mg/kg BW of intact piperine). Potential confounders were minimized by standardizing the timing of treatment administration and measurements, maintaining identical housing and environmental conditions, and applying consistent handling procedures throughout the study. The selection of the piperine dose was based on a previous study (Shah *et al.*, 2011), which demonstrated that piperine effectively reduced total cholesterol, triglycerides and LDL levels while increasing HDL levels in high-fat diet-induced dyslipidemic rats. According to the PubChem Compound Summary (CID 638024), the oral LD₅₀ of piperine in rats is approximately 514 mg/kg body weight, indicating a wide safety margin at the dose used in this study. To induce hyperlipidemia, all animals were administered 1 mL/100 g BW of a high-fat diet for seven consecutive days. The high-fat diet consisted of 10 g pure cholesterol, 3 g propylthiouracil, 10 quail egg yolk and enough peanut oil to make up 100 mL (Vogel, 2008). Following induction, the treatment was administered orally for 15 days. On the 6th, 11th, and 16th days after the treatment began, blood samples were drawn from the tail vein to check total cholesterol and triglyceride levels. Serum samples were analysed using the Cholesterol Oxidase – Peroxidase Aminoantipyrin (CHOD-PAP) method and the Glycerol 3 Phosphate Oxidase-Phenol-Aminophenazone (GPO-PAP) method using photometer 5010 V5+ (Reile, Germany).

Blinding was not applied during group allocation or treatment administration; however, biochemical outcome measurements and data analysis were performed by an investigator blinded to the group allocation to minimize bias.

Statistical analysis

The total cholesterol and triglyceride data were statistically analyzed using a two-way ANOVA (SPSS version 30) followed by Duncan's multiple range test to evaluate the effects of treatment group and administration duration, as well as their interaction, on lipid levels. Normality and homogeneity of variance were assessed using the Shapiro-Wilk and Levene's tests, respectively.

RESULTS

The average particle size of nano-cocrystal piperine-succinic acid is 245.5 nm as seen in Fig. 1, which meets the nanoparticle size requirements of 1-1000 nm.

Thermal analysis revealed a reduction in the melting point of the nano-cocrystals (106.79°C) compared to intact piperine (131.63°C) and the cocrystals (109.43°C), as presented in Fig. 2. A reduction in the fusion enthalpy of piperine-succinic acid nano-cocrystals was observed, as shown in Table 1.

Specific interference peaks for piperine at a diffraction angle of 2θ are at 14.912°, 19.683°, and 22.504°, while for succinic acid, it has specific interference at 16.095°, 20.086°, and 26.170°. HPMC did not show a distinctive and sharp peak due to the amorphous nature of HPMC, as shown in Fig. 3. There were new peaks depicted on piperine-succinic acid cocrystals at 13.989°, 20.086° and 24.532° as seen in Table 2.

The average total cholesterol and triglyceride levels before and after induction are seen in table 3. An increase in total cholesterol and triglyceride levels was observed in rats fed a high-fat diet. Total cholesterol increased from 48.21 ± 2.96 mg/dL to 159.79 ± 7.12 mg/dL, while triglyceride levels increased from 69.25 ± 3.93 mg/dL to 106.25 ± 6.04 mg/dL. These findings confirm that the high-fat diet successfully induced dyslipidemia in the Wistar rats.

The effect of nano-cocrystal on total cholesterol levels was presented in fig. 4 and table 4. The group that administered nano-cocrystals showed the greatest decrease in total cholesterol level. The administration of nano-cocrystals significantly reduced cholesterol levels compared to the intact piperine and simvastatin groups (p<0.05). The relatively similar effect between simvastatin and intact piperine may be attributed to the simvastatin dose used in this study. The dose lies within the lower range typically used in HFD-induced hyperlipidemia models.

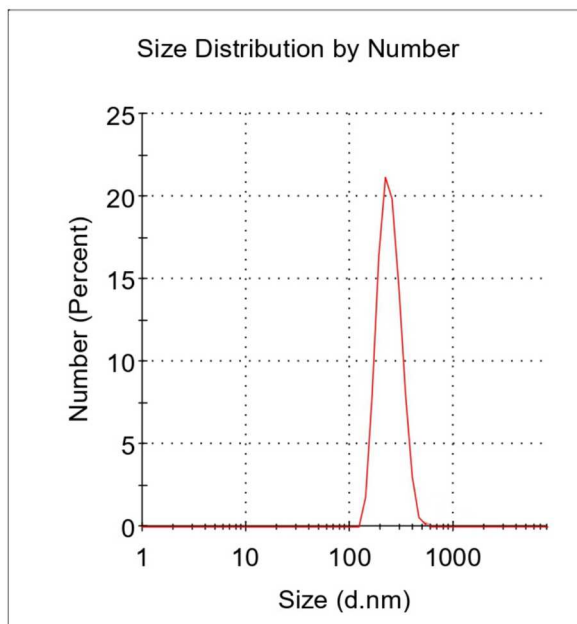


Fig. 1: Particle size analysis of nano-cocrystals

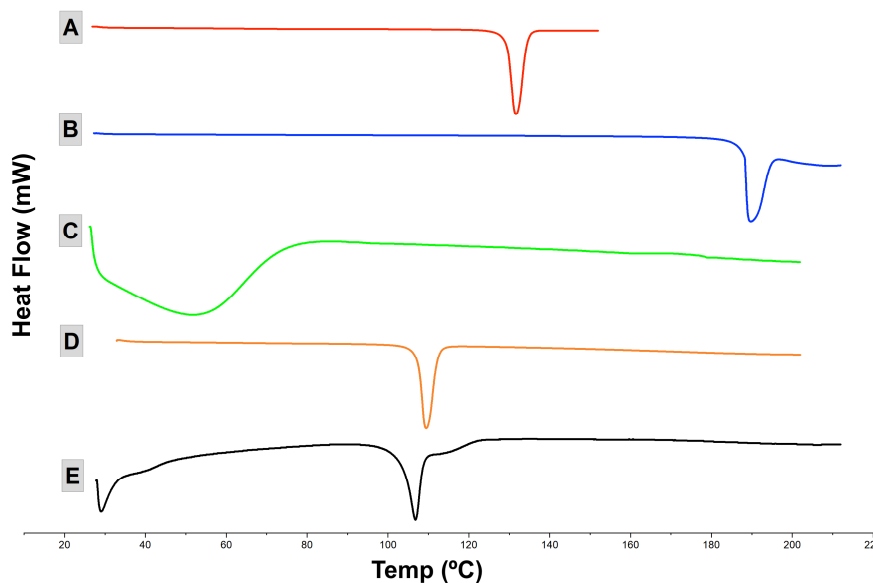


Fig. 2: Thermogram of; (A) Piperine; (B) Succinic acid; (C) HPMC; (D) Co-crystals; (E) Nano-cocrystals

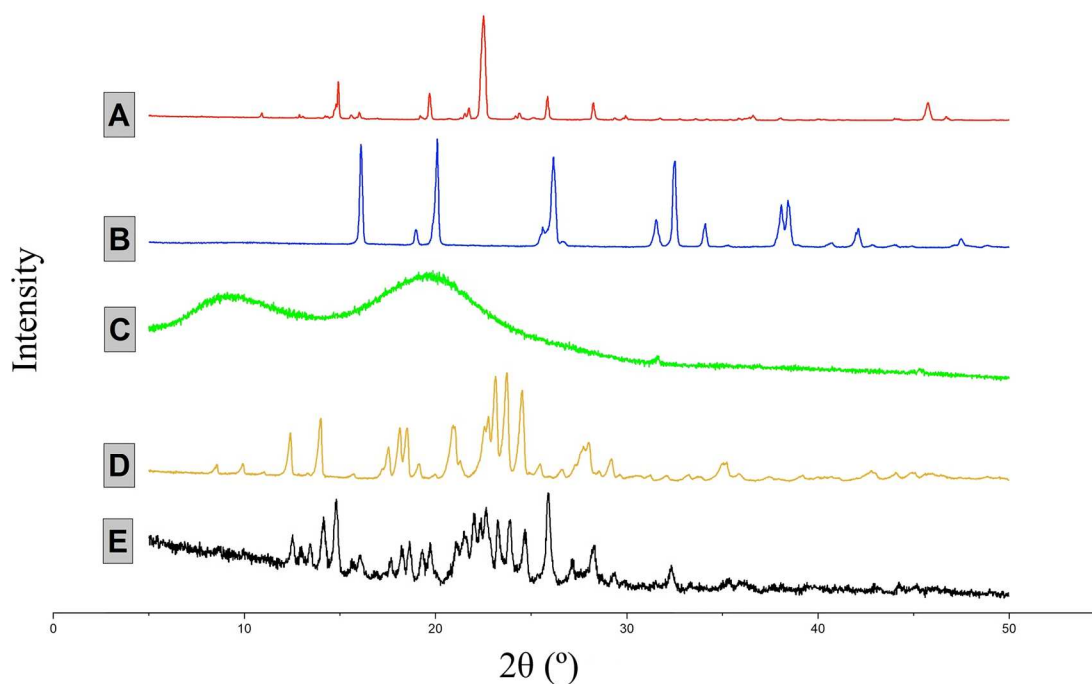
This dosage was selected based on previous studies that reported its efficacy under similar conditions (Harini and Astirin, 2009). Nevertheless, future studies may consider using a higher simvastatin dose to further distinguish its lipid-lowering effect from other treatments. In line with this, prolonged duration of treatment was also associated with a progressive reduction in total cholesterol levels ($p < 0.05$), with the greatest reduction observed on day 16 across all treatment groups.

In addition, there was also a reduction in triglyceride levels, which is seen in Fig. 5 and Table 5. The nano-

cocrystal form of piperine also demonstrated a significant reduction in triglyceride levels compared to the groups receiving the intact piperine. Triglyceride levels declined significantly with longer treatment duration, with the largest reductions observed on day 11 and 16 after the treatment began. This indicates that prolonged administration of the nano-cocrystal formulation enhances its lipid-lowering effect, likely due to improved solubility and sustained bioavailability of piperine. Overall, these results support the nano-cocrystal form as a more effective option than conventional formulations.

Table 1: Thermal analysis data of piperine, succinic acid, cocrystals and nano-cocrystals

Samples	Melting point (°C)	ΔH Fusion (J/g)
Piperine	131.63	97.23
Succinic acid	189.74	243.73
HPMC	51.68	118.44
Cocrystals piperine – succinic acid	109.43	74.36
Nano-cocrystals piperine – succinic acid	106.79	8.5

**Fig. 3:** PXR D diffractogram of; (A) Piperine; (B) Succinic acid; (C) HPMC; (D) Multicomponent crystals; (E) Nano-cocrystals**Table 2:** Peak Intensity of piperine, succinic acid, cocrystal and nano-cocrystal in X-ray diffractogram

Position 2θ (°)	Peak intensity			
	Piperine	Succinic acid	Co-crystals	Nano-cocrystals
13.989	-	-	4384.82	361.20
14.912	8162.11	-	-	-
16.095	-	15257.57	-	-
19.683	5760.67	-	-	-
20.086	-	16066.89	-	-
20.918	-	-	3943.85	268.19
22.504	21647.33	-	-	-
24.532	-	-	6159.213	322.26
25.884	-	-	-	669.39
26.170	-	13496.94	-	-

Table 3: Average mean of total cholesterol and triglycerides before and after induction in Wistar rats

Parameter	Mean (mg/dL) ± SE	
	Before induction	After induction
Total cholesterol	48.21 ± 2.96	159.79 ± 7.12
Triglycerides	69.25 ± 3.93	106.25 ± 6.04

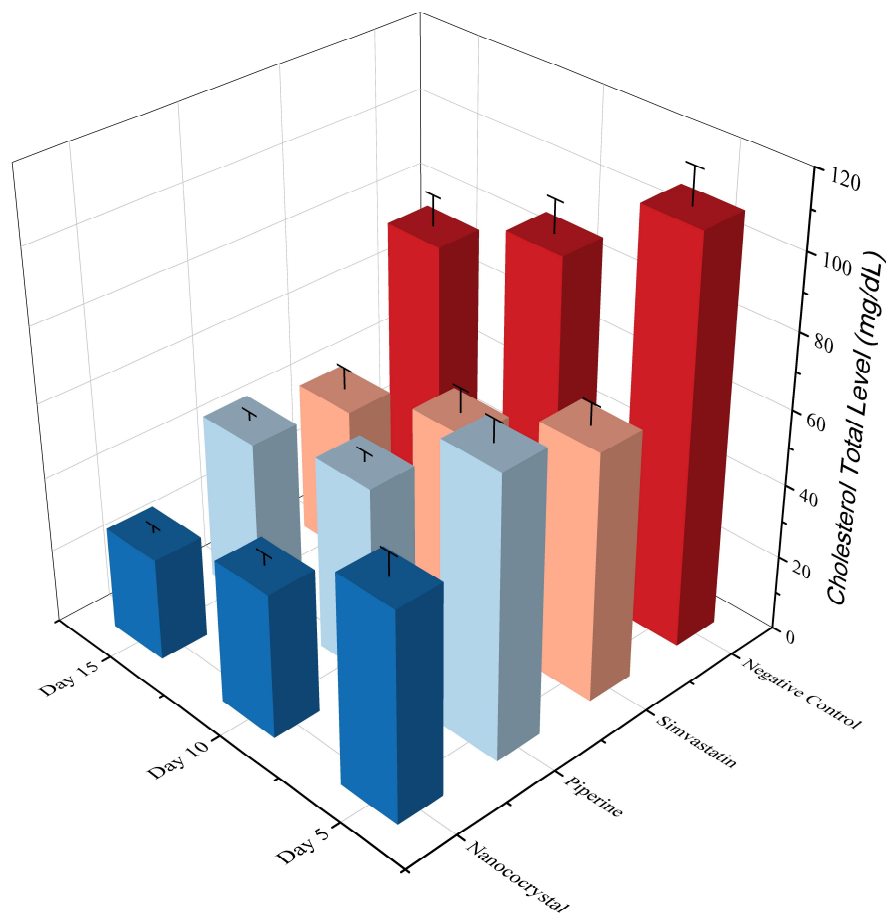


Fig. 4: Total cholesterol levels across experimental groups

Table 4: The result of total cholesterol levels across experimental groups in different days

Groups	Average total cholesterol level (mg/dL) ± SE			
	Day – 6 th	Day – 11 th	Day – 16 th	Average
Negative control	108.67 ± 9.79	87.50 ± 8.79	74.83 ± 8.29	90.33 ± 6.02 ^a
Simvastatin	66.50 ± 5.78	52.17 ± 6.62	41.33 ± 6.02	53.55 ± 4.17 ^b
Intact piperine	75.17 ± 6.04	52.17 ± 2.68	45.33 ± 2.28	57.56 ± 3.79 ^b
Nano-cocrystals	56.17 ± 6.34	38.83 ± 3.18	27.67 ± 1.98	40.89 ± 3.21 ^c
Average	76.17 ± 5.13	57.67 ± 4.66	47.30 ± 4.43	

^{a,b,c}: Within a column, means with different superscript letters are significantly different (p<0.05)

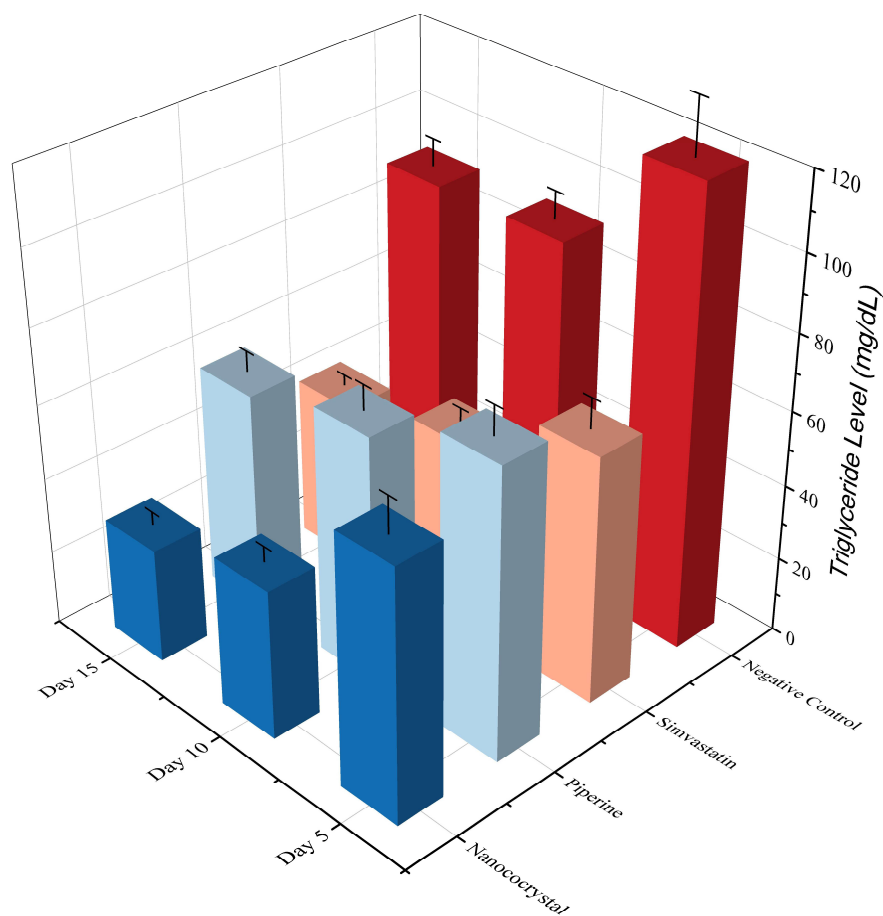


Fig. 5: The profile of the total triglyceride level in different groups

Table 5: The results of the triglyceride in different groups on different days

Groups	Average triglyceride level (mg/dL) \pm SE			
	Day – 6 th	Day – 11 th	Day – 16 th	Average
Negative control	120.5 \pm 14.49	91.33 \pm 7.44	91.17 \pm 7.37	101 \pm 6.52 ^a
Simvastatin	65.83 \pm 8.16	49.83 \pm 3.97	42.83 \pm 2.83	52.83 \pm 3.78 ^b
Intact piperine	77.17 \pm 7.73	65.83 \pm 6.78	53.83 \pm 5.42	65.61 \pm 4.31 ^c
Nano-cocrystals	67.00 \pm 9.47	40.00 \pm 4.20	30.50 \pm 3.43	45.83 \pm 5.07 ^b
Average	82.63 \pm 6.70	61.75 \pm 4.87	54.58 \pm 5.30	

^{a,b,c} Within a column, means with different superscript letters are significantly different ($p < 0.05$).

DISCUSSION

During the milling process in the nano-cocrystal preparation, repeated deformation processes occur, which destroy the cocrystals. The cocrystal particles are trapped between the colliding zirconium balls; as a result, the cocrystals will undergo a process of breaking into nano sizes (Fitriani *et al.*, 2022). The polydispersity index (PI) is

a measure of particle size distribution, ranging from 0 to 1. Values approaching zero indicate a uniform particle size distribution, whereas values above 0.5 reflect high heterogeneity (Fitriani *et al.*, 2022). The polydispersity index of the formed nano-cocrystals is 0.447, which indicates that the nano-cocrystals formed have a particle size distribution that tends to be homogeneous.

The zeta potential value obtained from the nano-cocrystal piperine-succinic acid particles was -2.51 mV. This value is outside the range of zeta potential requirements, which is about ± 30 mV. The zeta potential value of nanoparticles can be influenced by several factors, including surface chemistry, particle size, particle concentration, pH of the medium, temperature, solvent and ionic strength. Values below ± 30 mV tend to be unstable and form aggregates due to interactions among particles, including van der Waals, hydrogen-bonding, and hydrophobic interactions (Kumar and Dixit, 2017).

The melting point of the nano-cocrystals was lower than that of intact piperine, succinic acid and the cocrystals. Thermodynamically, melting point characteristics of a crystalline phase reflect intermolecular bonding and lattice energy, which can affect aqueous solubility and dissolution rate (Zaini *et al.*, 2020a). The fusion enthalpy value of nano-cocrystals was 8.5 J/g, while the fusion enthalpy values of piperine, succinic acid and cocrystal were 97.24 J/g, 243.73 J/g and 74.36 J/g, respectively. The reduction in enthalpy suggests that the nano-cocrystals require less energy, likely due to decreased crystallinity associated with particle size reduction.

X-ray diffraction analysis

XRD analysis is a prominent technique to characterize the crystallinity of solid phase, which can differentiate the crystalline, semi-crystalline, and amorphous phases (Thenmozhi and Yoo, 2017). As seen in Table 2, new peaks were observed, consistent with previous study (Fitriani *et al.*, 2022). The difference in the cocrystal diffraction pattern with the diffraction pattern of piperine and succinic acid indicates the formation of new crystalline phase (Zaini *et al.*, 2020a). The results of the nano-cocrystal piperine-succinic acid diffractogram showed a decrease in peak intensity. Broad XRD peaks mean low crystallinity and lower XRD peaks indicate lower crystallinity degree.

The amount of piperine levels in nano-cocrystal was 100.76% \pm 0.139. The results obtained are in accordance with the determination of acceptable levels, which also met the specification range (98–102%) (Quijia and Chorilli, 2020). This result is important in calculating the dose given to the white rats.

The hyperlipidemia condition in rats was induced by administering a high-fat diet that consisted of pure cholesterol, propylthiouracil (PTU), quail egg yolk and peanut oil (Vogel, 2008). This method was used for its proven ability to elevate blood lipid levels, including total cholesterol and triglycerides, rapidly and consistently (Hassan *et al.*, 2023). In this research, propylthiouracil (PTU) is used as an inducer of hypercholesterolemia due to its ability to block iodine oxidation in the thyroid gland, which reduces thyroid hormone production and leads to a hypothyroid state. When thyroid hormone levels fall,

cholesterol secretion declines, causing blood cholesterol levels to rise (Duntas and Brenta, 2016). Pure cholesterol provides an external source of cholesterol, while quail egg yolk and peanut oil also contain fats, cholesterol and cholesterol-promoting compounds that contribute to the development of hyperlipidaemia (Alam *et al.*, 2023). This method is effective, repeatable and closely resembles important aspects of hyperlipidaemia in humans. It produces notable increases in cholesterol levels in a short amount of time, which makes it ideal for evaluating lipid-lowering treatments (Hassan *et al.*, 2023).

Total cholesterol and triglyceride levels were chosen as the primary parameters in this study as these two parameters are recognized as the key predictors of cardiovascular risk. Total cholesterol reflects the total load of circulating cholesterol, including LDL, HDL and VLDL fractions, while triglycerides are the main form of stored fat in plasma (Reyes-Soffer, 2021; Visseren *et al.*, 2021). Elevated levels of both parameters can cause major heart problems; thus, by monitoring these markers, it can assess how effectively a treatment works in lowering lipid levels and, in turn, reducing the risk of cardiovascular complications (Visseren *et al.*, 2021). Simvastatin is used as a comparison, as it is a well-known HMG-CoA reductase inhibitor, the key enzyme involved in cholesterol biosynthesis. This drug acts by inhibiting the enzyme HMG-CoA reductase in cholesterol biosynthesis in the liver, which can significantly reduce plasma total cholesterol, LDL and ApoB levels and also cause a small decrease in plasma triglycerides and a small increase in plasma HDL levels (Kim *et al.*, 2023; Shattat, 2015).

In this study, the nano-cocrystal formulation produced the greatest reduction in total cholesterol and triglyceride levels when compared to intact piperine and low-dose simvastatin, with the most notable effects observed on days 11 and 16. These results align with previous studies showing that improving piperine's solubility and absorption enhances its bioavailability, thereby increasing its therapeutic effect (Tripathi, Ray and Mishra, 2022). Another study also demonstrated that a multicomponent piperine-nicotinic acid crystal effectively reduced total cholesterol, triglycerides and LDL-C in high-fat diet-induced rats over 15 days (Octavia *et al.*, 2023). Similarly, Hussain *et al.* (2023) reported that nano-piperine exhibited superior cardioprotective effects compared to intact piperine in cyclophosphamide-induced toxicity in rats.

The cholesterol- and triglyceride-lowering effects of piperine can be explained mainly by three mechanisms: enhancing the activity of lipoprotein lipase (LPL), suppressing HMG-CoA reductase and increasing bile acid excretion. LPL plays a key role in breaking down triglycerides in lipoproteins into fatty acids and glycerol, which can then be stored in adipose tissue or used as an energy source. When LPL activity increases, triglyceride-rich lipoproteins are cleared more efficiently, leading to

reduced triglyceride levels. On the other hand, HMG-CoA reductase is the rate-limiting enzyme in cholesterol biosynthesis, converting HMG-CoA into mevalonate, a precursor of cholesterol. Inhibiting this enzyme slows down cholesterol production, which ultimately lowers total cholesterol levels (Cui *et al.*, 2021).

Based on this study, in the negative control group, a decrease in total cholesterol and triglyceride levels was also observed on days 11 and 16 after treatment. This phenomenon is expected, as the body possesses intrinsic regulatory mechanisms to reduce circulating lipids over time through metabolic clearance and homeostatic feedback. Cholesterol is cleared from the body through two primary pathways. The first involves direct transport via the liver's ABCG5/G8 receptors, which move cholesterol into the gallbladder. The second pathway converts cholesterol into bile acids that are later eliminated through feces. Nearly 98% of bile acids undergo conjugation with glycine or taurine, a process that enhances their solubility and facilitates efficient reabsorption. This regulation ensures optimal fat digestion while maintaining bile acid homeostasis within the enterohepatic circulation. (Du *et al.*, 2020).

Triglycerides stored in fat tissue are catabolized by hormone-sensitive lipase found in fat tissue and catalyze triglyceride reserves into fatty acids and glycerol. These fatty acids and glycerol are then transported to active tissues, where they are oxidized to generate energy (Richard *et al.*, 2020). When entering the tissue, glycerol is converted into glycerol-3-phosphate and will enter the glycolytic pathway to break down glucose to produce energy. Fatty acids that have gone through the beta oxidation process produce acetyl-CoA, which will enter the Krebs cycle and produce energy (Wang *et al.*, 2021).

The enhanced efficacy of piperine observed in this study is directly linked to the improved solubility obtained through nano-cocrystallization. By forming piperine-succinic acid nano-cocrystals, the surface area is increased and particle size reduced, leading to faster dissolution and higher apparent solubility compared with intact piperine (Fitriani *et al.*, 2022; Fitriani *et al.*, 2024). This effect is further supported by recent work on piperine-nicotinic acid multicomponent crystals showing improved dissolution rates (Octavia *et al.*, 2023) and piperine-caffeine crystals (Fitriani, Simbolon and Zaini, 2023). These physicochemical improvements create a larger concentration gradient across the intestinal membrane, thereby enhancing drug absorption and systemic bioavailability. As a result, the higher plasma levels of piperine achieved with the nano-cocrystal formulation correspond closely with its improved pharmacological performance. This study suggests that the co-crystallization process may enhance the antihyperlipidemic activity of piperine. Nano-cocrystal piperine can increase solubility and bioavailability, thereby likely increasing

systemic exposure and pharmacological effect. The increase in the potency of piperine in the form of nano-cocrystals is as expected, therefore nano-cocrystals of piperine-succinic acid have potential as a drug to lower total cholesterol and triglycerides.

Although these results highlight the strong potential of the nano-cocrystal system, some limitations should be considered. This study only assessed total cholesterol and triglycerides, without evaluating HDL, LDL, or VLDL, which could provide a more comprehensive picture of lipid modulation. Future studies should therefore explore a broader lipid profile assessment. Comparative studies using higher simvastatin doses or other standard lipid-lowering drugs are also recommended to further validate efficacy.

CONCLUSION

Nano-cocrystal of piperine-succinic acid were successfully prepared by wet milling, which was supported by solid-state characterization. The evaluation of its activity against antihyperlipidemic presented a significant effect in lowering both total cholesterol and triglyceride levels. Prolonged duration of treatment was also correlated with a steady decline in total cholesterol and triglyceride levels. However, this study did not evaluate HDL and LDL, which may provide a more comprehensive understanding of lipid modulation. Future studies are recommended to include these lipid fractions.

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Authors' contributions

Lili Fitriani, Nurul Hidayah, Rini Agustin, Elsa Badriyya and Erizal Zaini: Conceptualized and designed the study, as well as drafted the manuscript; Lili Fitriani, Nurul Hidayah and Elsa Badriyya: Contributed to data generation and data analysis; Lili Fitriani, Elsa Badriyya and Erizal Zaini: Revised the manuscript. All authors reviewed and approved the final version of the manuscript and take full responsibility for the integrity and accuracy of the work.

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Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical approval

The animal testing procedure was approved by the ethics commission of the Faculty of Medicine, Universitas

Andalas (Approval number: 659/UN.16.2/KEP-FK/2022). This study was performed in adherence with the ARRIVE guidelines. See supplementary file for the ARRIVE checklist.

Conflict of interest

The authors declare no conflict of interest.

Supplementary data

<https://www.pjps.pk/uploads/2026/06/SUP1780487237.pdf>

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