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Effect of multicomponent crystal of piperine-nicotinic acid on antihyperlipidemic activity in rats

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Abstract: The alkaloid piperine is the main bioactive compound in black pepper (*Piper nigrum* L) and exhibits antihyperlipidemic activity. Piperine is a Biopharmaceutical Classification System (BCS) Class II compound, which has low water solubility, resulting in low bioavailability. This study aims to examine the effects of multicomponent nicotinic acid-piperine crystals on antihyperlipidemic activity in rats fed a high-fat diet. To increase the effectiveness of piperine, we prepared multicomponent crystals by the solvent-drop grinding method, using nicotinic acid as a co-former. The antihyperlipidemic activity of the preparation was estimated by measuring total cholesterol (TC), total triglycerides (TG), low-density lipoprotein cholesterol (LDLc), and high-density lipoprotein cholesterol (HDLc), using the enzymatic colorimetric method. Rats fed a high-fat diet exhibited an increase in plasma lipid levels. However, rats administered the multicomponent piperine-nicotinic acid crystals at a dose of 40 mg/kg/BW showed significantly (p<0.050) reduced plasma lipid levels. Compared with hyperlipidemic rats, multicomponent crystals of piperine-nicotinic acid decreased TC from 237.8±8.02 mg/dL to 174.53±7.07mg/dL, TG levels from 208.33±5.79 to 85.95±7.41mg/dL, and LDLc levels from 144.225±15.99 mg/dL to 88.55±10.83mg/dL but increased HDLc levels from 51.93±10.92mg/dL to 68.78±2.56 mg/dL. Thus, the results demonstrate that the multicomponent piperine-nicotinic acid crystals lowered TC, TG, and LDLc but increased HDLc.

Keywords: Piperine, nicotinic acid, multicomponent crystals, antihyperlipidemic.

INTRODUCTION

The alkaloid piperine is the main bioactive component in black pepper, giving it a spicy taste (Gorgani *et al*., 2017; Parthasarathy *et al*., 2008). The amount of piperine varies in Piperaceae, ranging from 2% to 7.4% (Zachariah and Parthasarathy, 2008). However, in black pepper, the piperine content reaches 9% (Raman and Gaikar, 2002).

Piperine has various pharmacological activities such as antioxidant (Vijayakumar *et al* ., 2004), hepatoprotective (Sabina *et al* ., 2010), anti-cancer and antitumor (Sunila and Kuttan 2004), anti-inflammatory (Tasleem *et al.*, 2014), bio-enhancing (Patil *et al* ., 2011), and antiulcer effects (Bai and Xu, 2000). Recent research has found that piperine plays an important role in reducing blood cholesterol, triglyceride and glucose levels (Kim *et al.*, 2013).

Hyperlipidemia is a disorder of lipid metabolism, characterized by increased lipid levels in the bloodstream. These lipids include cholesterol, cholesterol esters, phospholipids, and triglycerides or plasma lipoproteins including low-density lipoprotein (LDL) and high-density lipoprotein (HDL) (Eaton, 2005; Karam *et al*., 2017; Shattat, 2014), which are components that regulate the cholesterol balance in the body (Karr, 2017). Increased plasma cholesterol and plasma LDLc cause atherosclerosis and represent a risk for coronary heart disease (Karr, 2017) as well as increased morbidity and mortality in adults (Goodman and Gilman, 2008).

LDL levels are one of the targets of hyperlipidemia treatments (Latimer *et al*., 2016). Statins such as HMG-CoA reductase inhibitors (Sathiyakumar *et al.,* 2018) are often used for this purpose. However, in some patients, the use of statins is considered inadequate (Fox *et al*., 2018; Kuiper *et al*., 2017) because of intolerance and side effects that harm the liver and muscles (Banach *et al.*, 2012).

In this study, piperine was used as an antihyperlipidemic agent. Piperine is a BCS Class II compound (Zaini *et al* ., 2020) and has low solubility in water (Vasavirama and Upender, 2014), thus resulting in low bioavailability (Pachauri *et al*., 2015). To increase the solubility of piperine, here, multicomponent crystals were produced by applying the solvent-drop grinding method, using nicotinic acid as a co-former.

Several studies on the formation of multicomponent piperine crystals have been reported (Sari *et al*., 2019;

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Zaini *et al*., 2020a; Zaini *et al*., 2020b). However, studies evaluating the *in vivo* antihyperlipidemic activity of piperine-nicotinic acid multicomponent crystals and their comparative effects with physical mixtures are lacking. The evaluation of drug activity in experimental animals is necessary to confirm drug effectiveness *in vivo*. The antihyperlipidemic activity *in vivo* of multicomponent crystals of piperine-nicotinic acid was determined by observing the plasma lipid profiles (i.e., total cholesterol, total triglycerides, LDL cholesterol, and HDL cholesterol), using the enzymatic colorimetric method.

MATERIALS AND METHODS

Materials

Piperine was isolated from *Piper nigrum L.* at STIFARM Padang, nicotinic acid was purchased from Sigma Aldrich (USA), simvastatin was purchased from PT Kimia Farma, and a total cholesterol kit, total triglyceride kit, LDLc kit, and HDLc kit were obtained from DiaSys, Diagnostic Systems GmbH.

Experimental Animals

Male Wistar rats (*Rattus norvegicus*) aged 2–3 months and weighing 180–250 g were used. A total of 24 rats were obtained from the Animal House, Department of Pharmacology, Faculty of Pharmacy, Andalas University. Before the experiment, the animals were acclimatized for 7 days. The room temperature was set to 22 ± 3°C, with 30–70% relative humidity, and a 12 h light/12 h dark cycle. The room was always kept clean. Animals were given food and water *ad libitum* according to laboratory standards. It should be noted that, during acclimatization, the change in body weight of the animals should not be more than 10% (Badan Pengawas Obat dan Makanan, 2021). This research received ethical approval from the Andalas University Medical Research Ethics Commission, with number 134/UN.16.2/KEP-FK/2023.

Preparation of a High-Fat Diet

The high-fat diet consisted of 10 g of cholesterol, 3 g of propylthiouracil (PTU), and 10 g of a 100 g solution of cholic acid in 100 mL of peanut oil. All ingredients were mixed until a homogeneous emulsion was formed (Vogel, 2002).

Preparation of multicomponent crystals and a physical mixture of piperine-nicotinic acid

Multicomponent crystals of piperine-nicotinic acid at a ratio of 2:1 were prepared using the solvent-drop grinding method. Piperine and nicotinic acid were weighed and crushed together in a mortar, with the addition of ethanol *pa* for ± 15 min until a dry and homogeneous mass was formed. The mixture was then stored in a desiccator (Zaini *et al.*, 2020a).

A physical mixture of piperine-nicotinic acid was prepared with a molar ratio of 2:1. Piperine and nicotinic acid were weighed according to the formula and homogenized in a mortar. The resulting mass was then stored in a desiccator (Yuliandra *et al.*, 2018).

A suspension of the piperine, physical mixture, and multicomponent crystal was prepared with 0.5% sodium carboxymethyl cellulose (Na CMC) in distilled water. The preparations were weighed according to the determined dosage, then dissolved with 0.5% Na CMC, and dispersed until homogeneous.

Experimental design

Experimental animals were randomly divided into six groups: two control groups (normal control (NC) and high-fat diet (HFD)) and four treatment groups (piperine (PIP), a physical mixture of piperine-nicotinic acid (PM), multicomponent crystals of piperine-nicotinic acid (MC), and simvastatin (SIM)). Each group comprised four rats. The NC was given a normal diet with standard food. The other five groups were given a high-fat diet for 7 days (Vogel, 2002; Nawale *et al.*, 2018). Starting on Day 8, the treatment groups were administered the bioactive preparations for 14 days. At the end of the period, all groups were anesthetized and sacrificed to have their blood drawn through the retro-orbital sinus (Badan Pengawas Obat dan Makanan, 2021; Sarnaizul *et al.*, 2013). Blood was collected in a gel activator tube, allowed to stand for 15 min, and centrifuged at 3000 rpm for 15 min to obtain serum. The serum was separated with a pipette to determine the lipid profile.

Plasma lipid profile evaluation (TC, TG, LDLc, and HDLc)

Total cholesterol, triglyceride levels, LDL cholesterol, and HDL cholesterol were measured with a clinical spectrophotometer (Microlab 300, the Netherlands) at 546 nm, using a standard enzymatic assay kit. The sample was serum. To measure the cholesterol levels, cholesterol oxidase peroxidase amino phenzon phenol (CHOD PAP) was used as an indicator catalyst for the reaction. Total triglyceride levels were determined by adding glycerol-3-phosphate-oxidase. LDLc levels were measured by precipitating LDL with heparin, and HDLc levels were determined by adding phosphotungstic acid and magnesium, wherein each obtained supernatant was measured enzymatically with CHOD PAP.

STATISTICAL ANALYSIS

The data were statistically analyzed using SPSS v.20. All data are presented as mean ± standard deviation. To determine significant differences, a one-way analysis of variance (ANOVA) followed by Duncan's post-hoc test ($p < 0.05$) was carried out.

RESULTS

Compared to normal controls (NC), 20 male rats fed a high-fat diet (HFD) for a week exhibited significantly

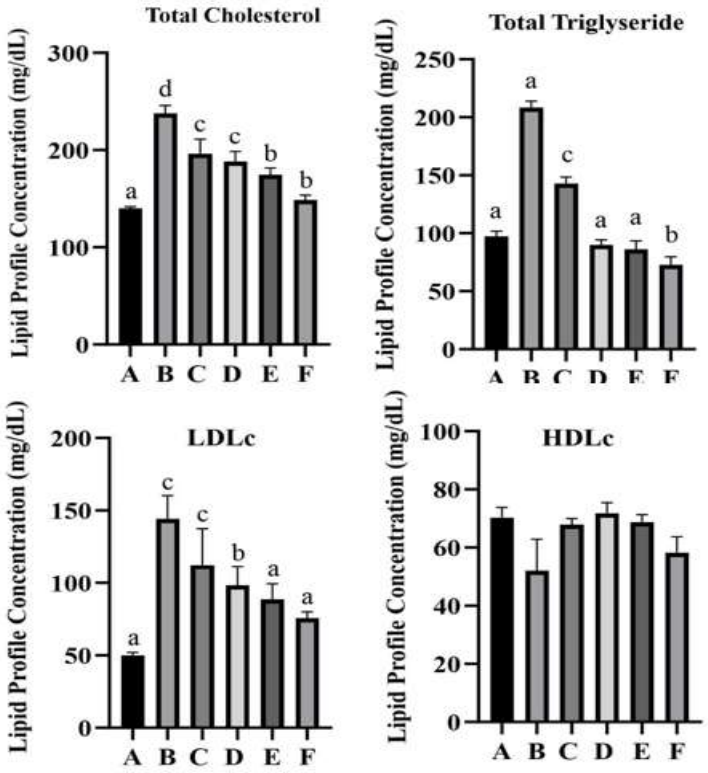

Fig. 1: Plasma lipid concentrations ((a) total cholesterol, (b) total triglycerides, (c) LDL cholesterol, and (d) HDL cholesterol) in rats. (A) Normal control (NC), (B) high-fat diet (HFD), (C) piperine (PIP), (D) physical mixture (PM), (E) multicomponent crystals (MC), and (F) simvastatin (SIM). Different lowercase letters indicate significant differences ($p \leq 0.05$).

increased plasma total cholesterol ($p<0.05$), total triglycerides ($p<0.05$) and LDL cholesterol ($p<0.05$) but lowered HDL cholesterol.

Compared to animals fed an HFD, animals that were administered therapy with piperine (PIP, 40 md/kgBB), a physical mixture of piperine-nicotinic acid (PM, 40 mg/kgBB), multicomponent crystals of piperine-nicotinic acid (MC, 40mg/kgBB), or simvastatin (SIM, 10mg) for 14 days exhibited significantly ($p<0.05$) lowered total cholesterol, total triglycerides, and LDL cholesterol but increased plasma HDL cholesterol. The plasma lipid profiles are summarized in table 1.

DISCUSSION

In this study, multicomponent crystals (MC) of piperine-nicotinic acid were prepared using the solvent-drop grinding method with a ratio of 2:1 mol. The formation of MC is a crystal engineering technique for manufacturing drug preparations. The formation of multicomponent piperine-nicotinic acid crystals is aimed at increasing the solubility and dissolution of piperine compared to pure piperine and its physical mixture. Increasing the solubility and dissolution rate profile results in an increase in the bioavailability of the drug and thus its effectiveness (Desiraju, 2005). Next, we analyzed the effects of the MC of piperine-nicotinic acid on the antihyperlipidemic activity in rats. The results were compared with the administration of pure piperine (PIP), a physical mixture of piperine-nicotinic acid (PM), and simvastatin (SIM).

Providing a high-fat diet for one week significantly increased TC, TG, and LDL in serum and reduced HDL compared to the lipid profile of the NC group. Hence, the experiment proved successful in establishing a hyperlipidemia model (table 1).

The model was used to evaluate the pathophysiology of hyperlipidemia disorders and the action of drugs to treat them. Consistent with previously reported literature (Nawale *et al*., 2018), here, rats fed a high-fat diet for 7 days (Sarnaizul *et al.,* 2013; Newsletter *et al*., 2018) and 14 days (Bao *et al.,* 2012) showed significantly increased serum levels of TC, TG, and LDLc but decreased serum levels of HDLc. These are the four critical biomarkers of hyperlipidemia. Manesai *et al.* (2012) reported that the administration of a high-fat diet increased TC levels by up to 68%.

Table 1: Total cholesterol (TC), total triglycerides (TG), LDL cholesterol, and HDL cholesterol in serum.

GROUP	Total cholesterol (mg/dL)	Total triglycerides (mg/dL)	LDL cholesterol (mg/dL)	HDL cholesterol (mg/dL)
Normal control (NC)	139.75 ± 2.23^{a}	97.2 ± 4.48^{b}	50.03 ± 2.05^{a}	70.28 ± 3.49
High-fat diet (HFD)	237.8 ± 8.02^{d}	208.33 ± 5.79^{d}	144.23 ± 15.99^{c}	51.93 ± 10.92
Piperine (PIP)	192.15 ± 15.01^{c}	142.63 ± 5.79^{b}	112.28 ± 25.09^{c}	55.35 ± 12.32
Physical mixture (PM)	188.05 ± 10.59^{c}	89.68 ± 4.55^{b}	98.33 ± 1285^{b}	71.75 ± 3.69
Multicomponent crystal (MC)	174.53 ± 7.07^{b}	85.95 ± 7.41^{a}	88.55 ± 10.83^{a}	68.78 ± 2.56
Simvastatin (SIM)	148.4 ± 5.26^{b}	72.8 ± 6.81^{a}	75.6 ± 4.54^{a}	58.25 ± 5.45

Different letters on the same column indicate significant differences ($p \leq 0.05$).

The administration of PIP, PM, and MC lowered cholesterol levels significantly ($p < 0.05$) compared to the HFD group (fig. 1a). Based on the statistical analysis, MC showed better effects for reducing total cholesterol levels than PIP and PM, whereas MC had a comparable effect to that of SIM.

Furthermore, PIP, PM and MC significantly ($p<0.05$) reduced TG levels compared to the HFD group (fig. 2b). Duncan's tests showed that there was a significant difference between the HFD group and the groups treated with the bioactive preparations, whereas the MC and PM groups exhibited the same effect as the SIM group.

The treatment groups had lower LDL cholesterol levels compared to the HFD group (fig. 1c). Nevertheless, the MC group showed less LDL cholesterol reduction compared to the PIP and PM groups. One-way ANOVA revealed that the treatment groups showed a significant ($p < 0.05$) decrease in plasma LDL cholesterol levels. Duncan's test revealed that there were significant differences between the MC and HFD groups and between the PM and HFD groups, whereas MC exhibited the same effect as the SIM and NC groups.

Moreover, the treatment groups also showed increased HDL cholesterol levels compared to the HFD group (fig. 1d). The MC group showed higher HDL levels than the PIP group but the same activity as the NC group.

In summary, the multicomponent crystals of piperine-nicotinic acid (MC) decreased total cholesterol, total triglycerides, and LDL cholesterol but increased HDL cholesterol more effectively than pure piperine (PIP) and the physical mixture (PM). This finding indicates that a multicomponent crystal formation of piperine-nicotinic acid can be a potential antihyperlipidemic drug candidate.

CONCLUSION

The multicomponent crystals of piperine-nicotinic acid produced by the solvent-drop grinding method showed antihyperlipidemic activity by reducing total cholesterol, total triglycerides, and LDL cholesterol but increasing HDL cholesterol.

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