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Research Paper

Preparation and Evaluation of Antihypercholesterolemic Activity of Atorvastatin Calcium-Maleic Acid Co-Amorphous Solids

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Abstract
Atorvastatin calcium is a statin drug used for antihypercholesterolemic. The oral bioavailability of atorvastatin calcium is relatively low because it is poorly soluble in water. The low oral bioavailability of the drug causes a decrease in its therapeutic effectiveness. This study aimed to increase the solubility of atorvastatin calcium through the formation of co-amorphous solids and evaluate its activity as antihypercholesterolemic. Atorvastatin calcium was prepared into co-amorphous solids with a maleic acid coformer using the spray drying method. Solids characterization was carried out using Powder X-Ray Diffraction (PXRD), Differential Scanning Calorimetry (DSC), Fourier Transforms Infra Red (FTIR), and Scanning Electronic Microscopy (SEM). The solubility test was carried out using the shaking method, while the evaluation of antihypercholesterolemic was carried out in vivo in experimental animals. The results of the analysis of diffractograms, thermograms, FTIR spectra, and micrograph images showed that the atorvastatin calcium-maleic acid solids prepared by spray drying were a co-amorphous solid. The atorvastatin calcium-maleic acid co-amorphous solids had a greater solubility in water ($p<0.05$) when compared to pure atorvastatin calcium. However, the in vivo antihypercholesterolemic activity results in experimental animals showed that the cholesterol-lowering activity of the atorvastatin calcium-maleic acid co-amorphous solids was not significantly different ($p>0.05$) with pure atorvastatin calcium. This phenomenon is thought to be because atorvastatin calcium from co-amorphous solids in solution is more present as a charged fraction, affecting the permeability and absorption process.

Keywords
Atorvastatin Calcium, Antihypercholesterolemic, Co-Amorphous Solid, Maleic Acid

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1. INTRODUCTION

Atorvastatin calcium is a statin drug that is clinically used to lower blood cholesterol levels (Sharma and Mehta, 2019). Atorvastatin calcium inhibits 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, an enzyme that plays an important role in the production of cholesterol in the body (Shaker, 2018; Prihapsara, 2020). Atorvastatin calcium shows very high permeability, but its solubility in water is very low, so its oral bioavailability is only 12-14% (Kwon et al., 2019; Prihapsara, 2020). This can lead to a decrease in its therapeutic effectiveness (Trivedi et al., 2020). Therefore, the solubility of atorvastatin calcium needs to be improved in order to increase its bioavailability and therapeutic effectiveness.

The formation of co-amorphous solids is one method that has been proven to be used to increase the solubility of poorly soluble drugs (Renuka et al., 2017; Wicaksono et al., 2021). Co-amorphous solids are a multicomponent amorphous solid system formed from drug and coformer molecules with small molecular weights (Chavan et al., 2016). Co-amorphous solids can increase the solubility of poorly soluble drugs because their molecular arrangement in solids is in a high internal energy phase so that the dissolution barrier energy is lower (Karagianni et al., 2018). Another advantage of co-amorphous solids is the increase in physical stability due to intermolecular interactions between the drug and coformers molecules that can prevent molecular mobilization so that the recrystallization process does not occur (Chavan et al., 2016). Therefore, co-amorphous solids are very promising as a solid form of poorly soluble drugs.

One method for preparing co-amorphous solids is the spray drying technique. Spray drying is often used for the formation of co-amorphous solids because it is easy to carry out, and the process is fast. Co-amorphous solids resulting from spray drying are spherical particles (Karagianni et al., 2018; Wicaksono

et al., 2021). The spray drying technique is able to regulate the size distribution and morphology of the expected solid particles by controlling the composition of the feed solution and drying speed. An additional advantage of the spray drying technique is that it is continuous manufacturing and easily scaling up (Shi et al., 2019).

The spray drying method has been successful in preparing atorvastatin calcium co-amorphous solids with maleic acid coformers. The atorvastatin calcium-maleic acid co-amorphous solid results from the spray drying method showed higher solubility than pure atorvastatin calcium (Wicaksono et al., 2021). However, evaluation of the antihypercholesterolemic activity of atorvastatin calcium co-amorphous solid has not been carried out until now. In fact, this information is needed for the formulation of co-amorphous solids into pharmaceutical preparations. Therefore, a study was conducted on the preparation of atorvastatin calcium-maleic acid co-amorphous solids by spray drying technique and followed by evaluation of its activity as an antihypercholesterolemic. The feed solution in the spray drying process was prepared with methanol because it dissolves atorvastatin calcium and maleic acid very well. In addition, methanol is relatively easy to remove from solid products, so it is often used for the preparation of pharmaceutical solids (Ainurofiq et al., 2018; Ashwini et al., 2021; Labib and Nasr, 2021).

2. EXPERIMENTAL SECTION

2.1 Materials

Pharmaceutical grade atorvastatin calcium was obtained from PT Dexa Medica (Palembang, Indonesia), while maleic acid and CMC-Na (synthetic grade) were purchased from Merck KgaA (Darmstadt, Germany). Analytical grade methanol was purchased from PT. Smart Lab (Tangerang, Indonesia). Wistar rats (male, 3-4 months old, bodyweight 100-300 grams) for experimental animals were obtained from the Faculty of Dentistry, University of Jember. The research protocol on experimental animals was approved by the Ethics Committee of the Faculty of Dentistry, University of Jember (Letter number: 1056/UN25.8/KEPK/DL/2020).

2.2 Methods

2.2.1 Preparation of Atorvastatin Calcium–Maleic Acid Co-Amorphous Solids

The co-amorphous solid of atorvastatin calcium was formed by using maleic acid as the coformer with a molar ratio of 1:1. The solids were dissolved in methanol to obtain a solution with a concentration of 5% (w/v). The solution was then dried using a spray dryer (Lab Plant SD-Basic Spray Dryer) with a feed speed of 5 mL/min and air pressure of 6 scales. The inlet and outlet temperatures of the spray dryer were set at 60 and 55°C, respectively. The results of the dry powder were collected for further testing (Wicaksono et al., 2021).

2.2.2 Characterization of Atorvastatin Calcium–Maleic Acid Co-Amorphous Solids

PXRD characterization of the atorvastatin calcium-maleic acid co-amorphous solids was carried out using a Philip Xpert instrument with a CuKα radiation source (λ = 1.5402 A°) whose voltage and current were set at 40 kV and 30 mA. The analysis was performed at an angle of 2θ at 5-50° with a scanning speed of 10°/min. DSC characterization of co-amorphous solids was carried out using a Thermo plus DSC 8230 instrument in a temperature range of 30-200°C. The DSC instrument was run at a heating rate of 10°C/min and a dry airflow of 50 mL/min. FTIR analysis of co-amorphous solids was performed with a Bruker Alpha spectrophotometer. The FTIR scanning was carried out with a resolution of 4 cm^{-1} in the wavenumber range of 4,000-600 cm^{-1}. SEM characterization of co-amorphous solids was carried out using a Hitachi TM3000 instrument equipped with a Hitachi E-1045 ion sputter. The SEM instrument was set at a voltage and current of 15 kV and 12 mA, respectively, while the observations were carried out at the appropriate magnification.

2.2.3 Solubility Test

Solubility test was carried out by shaking method with approximately 25 mg of powder put into a 100 mL erlenmeyer glass, and then 10 mL of distilled water was added. The erlenmeyer glass was shaken with an orbital shaker at 175 rpm for 8 hours at 25°C. The obtained filtrate was filtered using a 0.45 μm cellulose nitrate membrane, and the atorvastatin calcium content was determined by utilizing a UV-Vis spectrophotometer.

2.2.4 Preparation and Treatment of Experimental Animals

The experimental animals were placed in clean cages and acclimatized for a week. The animals were randomly divided into four groups (n=3), namely group I (normal), II (negative control), III (pure atorvastatin calcium), and IV (atorvastatin calcium-maleic acid). The total cholesterol level of the experimental animals was measured (day 0), and then for 14 day, they were given normal feed (group I) and feeding with high-cholesterol feed (groups II, III, and IV). The high-cholesterol feed was made of quail egg yolk at a dose of 10 mL/kg BW and 0.1% propylthiouracil solution, and the administration was done using oral gavage. The total cholesterol level of the experimental animals was measured again (day 15), and then they were given treatment for seven day (group I = normal feed, group II = 0.5% CMC-Na, group II = pure atorvastatin calcium 1.8 mg/kg BW, and group IV = atorvastatin calcium-maleic acid co-amorphous solids 1.8 mg/kg BW). At the end of the treatment (day 22), the total cholesterol level of the experimental animals was measured again (Djamil et al., 2020; Kurniawan and Audita, 2021).

2.2.5 Measurement of Total Cholesterol Levels of Experimental Animals

The tip of the animal's tail was cut off, and 10 microns of blood were collected in a tube. Blood was placed at 25°C for 10 min

and then centrifuged at 4,000 rpm for 15 min. Blood serum (5 mL) was added with 500 mL of cholesterol reagent (cholesterol oxidase-peroxidase aminoantipyrine phenol/CHOD-PAP) and then incubated at 25°C for 5 minutes. Serum samples and standard solutions were then measured for absorption with Biolyzer 100TM at a wavelength of 546 nm, and total cholesterol levels were calculated. The percentage decrease in total cholesterol levels is calculated by the equation (initial cholesterol levels-end cholesterol levels)/initial cholesterol levels x 100% (Wahjuningsih et al., 2018).

2.2.6 Data Analysis

The data obtained were statistically analyzed by testing for normality using the Shapiro-Wilk test. If the data were normally distributed ($p>0.05$), the analysis was carried out with a significance test using one-way ANOVA and Post-Hoc LSD. If the data were not normally distributed, the analysis was carried out by the Kruskal-Wallis nonparametric test. The data is considered to have a significant difference if the p-value <0.05.

3. RESULTS AND DISCUSSION

3.1 Results of Atorvastatin Calcium-Maleic Acid Solids

The feed solution of atorvastatin calcium-maleic acid for the spray drying process was prepared in a 1:1 molar ratio. The amounts of atorvastatin calcium and maleic acid used were 4.56 and 0.44 grams (total weight 5 grams), respectively, and were dissolved in 100 mL of methanol to obtain a solution with a total concentration of 5% (w/v). The powder yield obtained after the spray drying feed solution was 1.2 grams or about 24% (w/w) of the amount of initial solid material.

3.2 Characteristics of Atorvastatin Calcium-Maleic Acid Solids

The diffractogram of the PXRD characterization is exhibited in Figure 1. Pure atorvastatin calcium has diffraction peaks with relatively low intensity (≤ 1,000 units) at 7.36 and 18.79°, while maleic acid has many diffraction peaks with high intensity (2,000-10,000 units) at 27.9, 32.1, 37.9, and 38.1°. The diffractograms indicated that the starting material of pure atorvastatin calcium is an amorphous solid, while maleic acid is crystalline solid (Lemsi et al., 2017; Lee et al., 2017). The PXRD diffractogram of atorvastatin calcium–maleic acid did not show a diffraction peak, indicating that the solid did not have a certain pattern, so it was an amorphous solid. Single-phase amorphous solid systems consisting of two or more small molecules are referred to as co-amorphous solids (Chavan et al., 2016; Karagianni et al., 2018; Wairkar and Gaud, 2019; Wicaksono et al., 2021).

The DSC thermogram of pure atorvastatin calcium, maleic acid, and atorvastatin calcium-maleic acid is shown in Figure 2. The thermogram curve of pure atorvastatin calcium has shown no sharp endothermic peak but has shown a glass transition temperature (Tg) at 166.1°C. Maleic acid has a DSC curve with a sharp endothermic peak at 142.4°C (ΔH = 269.71 J/g), where the sharp endothermic peak indicates the melting peak of a

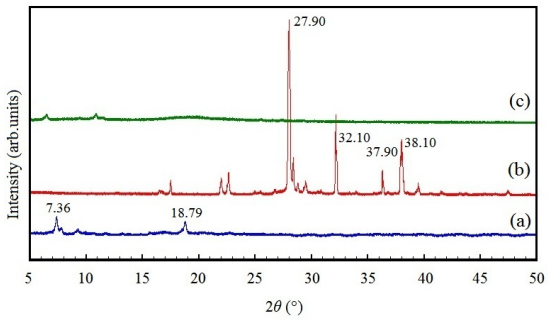

Figure 1. PXRD Difractogram of (a) Pure Atorvastatin Calcium, (b) Maleic Acid, and (c) Atorvastatin Calcium-Maleic Acid Co-Amorphous Solids

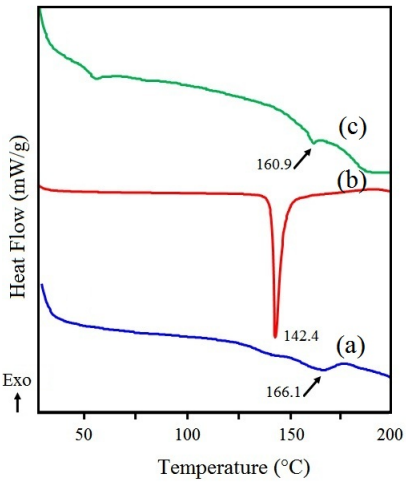

Figure 2. DSC Thermogram of (a) Pure Atorvastatin Calcium, (b) Maleic Acid, and (c) Atorvastatin Calcium-Maleic Acid Co-Amorphous Solids

material. From the results of this DSC test, it can be concluded that pure atorvastatin calcium is an amorphous solid because it has no melting point but has a glass transition temperature, while the maleic acid coformer is a crystalline solid because it has a melting temperature (Lemsi et al., 2017; Lee et al., 2017). The conclusion of the DSC test about the type of the two solids is in agreement with the conclusion of the PXRD test.

On the DSC test, the atorvastatin calcium-maleic acid solids showed a thermogram curve with a Tg at 160.9°C. The results of the DSC test indicated that the atorvastatin calcium and maleic acid molecules in the atorvastatin calcium-maleic acid solid had formed co-amorphous solids, namely a solid that does not have the long-range order of molecular packing (Chavan et al., 2016). Co-amorphous solids have higher internal energy than crystalline solids, so when heated, they do not show a melting point. Co-amorphous solids show the presence of a glass transition temperature (Tg) which is generally located between the melting point or the glass transition temperature

(Tg) of its constituent components (Shi et al., 2019). The DSC test in this study reached the same conclusion as the PXRD test, namely that the atorvastatin calcium-maleic acid solid is a co-amorphous solid.

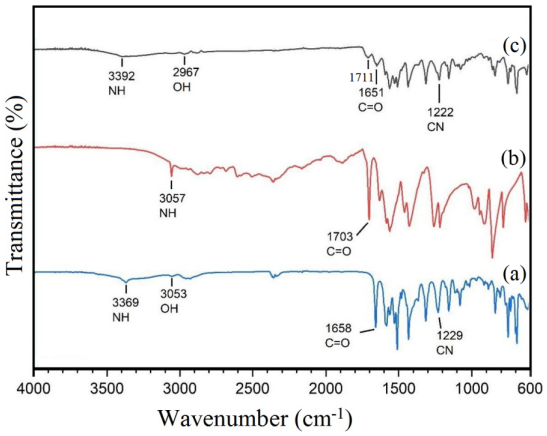

Figure 3. FTIR Spektra of (a) Pure Atorvastatin Calcium, (b) Maleic Acid, and (c) Atorvastatin Calcium-Maleic Acid Co-Amorphous Solids

The FTIR spectra of pure atorvastatin calcium, maleic acid, and atorvastatin calcium-maleic acid are shown in Figure 3. Pure atorvastatin calcium spectra have absorption peaks at 3369 cm^{-1} (N-H strain), 3053 cm^{-1} (-OH stretching), 1658 cm^{-1} (C=O stretching), and 1229 cm^{-1} (C-N stretching). Maleic acid has FTIR spectra showing absorption peaks at 3057 cm^{-1} (-OH stretching) and 1703 cm^{-1} (C=O carboxylic acid stretching) (Samsodien et al., 2017; Wicaksono et al., 2019; Wicaksono et al., 2021). The FTIR spectra of atorvastatin calcium-maleic acid showed absorption peaks as the constituent materials with a shift in wavenumber compared to the starting material. The absorption peak of atorvastatin calcium at 3369 cm^{-1} and 1658 cm^{-1} shift to 3392 cm^{-1} and 1651 cm^{-1}, respectively. Similarly, at the absorption peak of maleic acid, shifts occur at 3057 cm^{-1} and 1703 cm^{-1} to 2967 cm^{-1} and 1711 cm^{-1}. The shifts in the FTIR spectra of atorvastatin calcium-maleic acid indicated intermolecular interactions between the molecules. Therefore, it can be concluded that the atorvastatin calcium and maleic acid molecules in the atorvastatin calcium-maleic acid solid are not a physical mixture but have formed a co-amorphous solid phase (Karagianni et al., 2018).

SEM micrographs of pure atorvastatin calcium, maleic acid, and atorvastatin calcium-maleic acid co-amorphous solids are shown in Figure 4. Particles of pure atorvastatin calcium have needle shapes of varying sizes, whereas particles of maleic acid have an irregular shape. The atorvastatin calcium-maleic acid co-amorphous solids have spherical particles with hollow-looking surfaces. SEM observations have shown that the atorvastatin calcium-maleic acid co-amorphous solids have a different shape and surface topography with its individual constituent

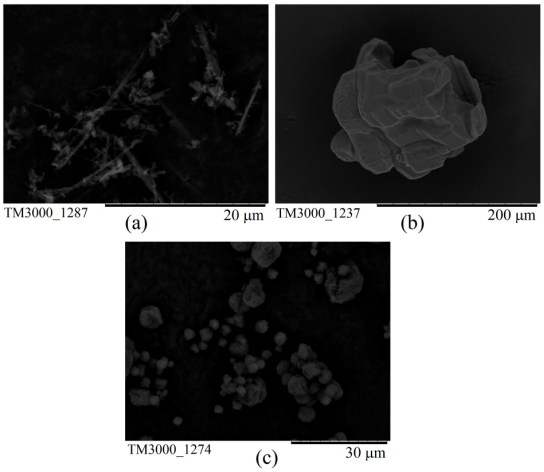

Figure 4. SEM Images of (a) Pure Atorvastatin Calcium (15 kV, X5000), (b) Maleic Acid (15 kV, X500), and (c) Atorvastatin Calcium-Maleic Acid Co-Amorphous Solids (15 kV, X2000)

components, indicating the formation of a new solid phase (Karagianni et al., 2018; Wairkar and Gaud, 2019). Based on the results of characterization using PXRD, DSC, FTIR, and SEM, it can be concluded that the atorvastatin calcium-maleic acid solids prepared by spray drying are a co-amorphous solid.

3.3 Solubility

Pure atorvastatin calcium and atorvastatin calcium-maleic acid co-amorphous solids in the solubility test showed the solubility in distilled water was 236.96±15.64 and 293.26 ± 17.11 mg/L, respectively. The formation of co-amorphous solids of atorvastatin calcium and maleic acid increased the solubility of atorvastatin calcium significantly ($p<0.05$) compared to the solubility of pure atorvastatin calcium. Co-amorphous solids have high solubility because thermodynamically, the solid is in a high energy state, causing the dissolution barrier energy to be lower. Co-amorphous solids do not require energy for crystal lattice rearrangement during the dissolution process (Chavan et al., 2016; Karagianni et al., 2018; Wicaksono et al., 2021).

3.4 Antihypercholesterolemic Activity

Antihypercholesterolemic activity test of atorvastatin calcium-maleic acid co-amorphous solid was carried out in vivo in experimental animals. Assessment of antihypercholesterolemic activity was carried out based on a decrease in total cholesterol levels in the blood of experimental animals before and after sample treatment (Kurniawan and Audita, 2021). Total cholesterol levels on day 15 (after induction) in groups I, II, III, and IV were 91.54 ± 12.38, 68.46 ± 15.61, 107.44 ± 2.61, and 92.57 ± 15.56 mg/dL (Table 1). These results indicate that the induction treatment in experimental animals for 15 day showed a significant increase in total cholesterol levels ($p<0.05$) in the pure calcium atorvastatin group, while in the other group, it did not show a significant increase ($p>0.05$).

Table 1. Total Cholesterol Level of Experimental Animals (n = 3)

Groups	Total Cholesterol Level After (mg/dL)			Decrease (%)
	0 day	15 day	22 day	
I	80.51 ± 20.44	91.54 ± 12.38	72.93 ± 7.58	19.75 ± 9.14
II	62.60 ± 13.50	68.46 ± 15.61	57.14 ± 21.27	18.55 ± 16.23
III	81.51 ± 19.05	107.44 ± 2.61	63.33 ± 13.79*	40.83 ± 14.39
IV	83.05 ± 10.85	92.57 ± 15.56	68.46 ± 7.05*	25.36 ± 6.53

Description: I = normal control, II = negative control, III = pure atorvastatin calcium, IV = atorvastatin calcium-maleic acid co-amorphous solids. *: sample treatment significantly reduced total cholesterol levels ($p<0.05$)

The total cholesterol levels of groups I, II, III, and IV after sample treatment (day 22) were 72.93 ± 7.58; 57.14 ± 21.27; 63.33 ± 13.79, and 68.46 ± 7.05 mg/dL, respectively. Total cholesterol levels in groups I and II did not show a significant difference ($p>0.05$), which indicated that the sample treatment procedure had no effect on total cholesterol levels in experimental animals. The treatment of samples in group III and group IV showed a significant reduction in total cholesterol levels ($p<0.05$) in experimental animals. This indicated that the treatment of samples containing atorvastatin calcium has a lowering effect on total cholesterol levels in experimental animals.

Atorvastatin calcium works as an antihypercholesterolemic through the mechanism of inhibition of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase so that the synthesis of cholesterol in liver cell membranes and extrahepatic tissues becomes lower (Shaker, 2018; Sharma and Mehta, 2019). In this study, based on total cholesterol levels before (day 15) and after treatment (day 22), the percentage decrease in total cholesterol levels due to treatment can be determined. The percentage reduction in total cholesterol levels in groups III and IV were 40.83 ± 14.39 and 25.36 ± 6.53%, respectively. Total cholesterol levels in group III showed a greater percentage of total cholesterol reduction than group IV, but statistical analysis using the ANOVA test showed that the percentage reduction in total cholesterol levels was not significantly different ($p>0.05$).

The results of the solubility test showed that the atorvastatin calcium-maleic acid co-amorphous solid had a higher solubility than pure calcium atorvastatin. However, the results of the antihypercholesterolemic activity test showed that there was no significant difference in the percentage reduction in total cholesterol levels in groups III and IV ($p>0.05$). Data on the in vitro solubility of drugs, especially for drugs that are difficult to dissolve in water, cannot fully correlate with activity in vivo (Brake et al., 2017). The in vivo activity of a drug is also influenced by physiological factors such as transit time and stability in the gastrointestinal tract (Dressman and Reppas, 2000). The permeability properties also affect the absorption process and in vivo activity of a drug. Dissolved drug molecules that are charged generally have lower permeability so that their absorption and in vivo activity decrease (Sugita et al., 2021). In this study, the increase in solubility of the atorvastatin calcium-maleic acid co-amorphous solid was not directly proportional to its cholesterol-lowering activity. This phenomenon is thought to be due to the dissolved atorvastatin calcium molecules from the atorvastatin calcium-maleic acid co-amorphous solid forming a charged fraction due to the presence of maleic acid molecules. The formation of the charged fraction causes a decrease in the permeability of atorvastatin calcium molecules so that it affects the absorption process and activity in lowering cholesterol levels (Sugita et al., 2021).

4. CONCLUSIONS

The results showed that atorvastatin calcium with maleic acid coformer using spray drying method produced co-amorphous solids. The solubility of the atorvastatin calcium-maleic acid co-amorphous solid was significantly increased ($p<0.05$) compared to the solubility of pure calcium atorvastatin. However, the antihypercholesterolemic activity in experimental animals was not directly proportional to the results of the solubility test. The total cholesterol level of the experimental animal group treated with atorvastatin calcium-maleic acid co-amorphous solids was not significantly different ($p>0.05$) compared to the group treated with pure calcium atorvastatin. These results are thought to be because atorvastatin calcium from co-amorphous solids in solution forms a charged fraction that affects the permeability properties and absorption process.

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