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Original Article

INHIBITORY EFFECT OF CURCUMIN INCORPORATED IN CHITOSAN NANOPARTICLE ON α-AMYLASE AND α-GLUCOSIDASE ACTIVITIES

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ABSTRACT

Objective: This research was conducted to determine inhibition activity of curcumin nanoparticles against α-amylase and α-glucosidase enzymes.

Methods: Curcumin nanoparticle was made by ionic gelation method using chitosan as cation, sodium tripolyphosphate as polyanion, and tween 80 as surfactant. Curcumin nanoparticles were tested for inhibitory activity of α-amylase and α-glucosidase enzymes using UV-Vis spectrophotometry at λ= 595 nm and 305 nm, respectively.

Results: Curcumin nanoparticles produced have 198.1 nm of particle size with PdI value of 0.349 and zeta potential value of-8,33 mV. The IC_{50} value of curcumin nanoparticles against α-amylase was 56.140 ppm, while acarbose was 63.32 ppm. While the IC_{50} value against α-glucosidase was 3.95 ppm and 4.11 ppm for curcumin nanoparticles and acarbose, respectively.

Conclusion: It can be concluded that curcumin nanoparticles have great potential as antihyperglycemic by inhibiting α-amylase and α-glucosidase enzymes.

Keywords: Curcumin, Nanoparticle, α-amylase, α-glucosidase

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INTRODUCTION

Diabetes mellitus is a chronic disease that is still a major health problem in Indonesia, it is characterized by hyperglycemia and abnormalities in carbohydrate, fat and protein metabolism [1]. According, to the International Diabetes Federation (IDF), there were 463 million people suffering from diabetes in 2019 and this number is estimated to reach 578 million in 2030 and 700 million in 2045. Indonesia is the sixth country with the most diabetes mellitus sufferers in the world [2].

Synthetic antidiabetic drugs have been used clinically, one of which is acarbose. However, these synthetic drugs have limitations, including side effects such as hypoglycemia, anemia and the long-term use of these drugs can have a disruptive effect on the digestive tract system, such as nausea, vomiting, abdominal pain and bloating [3, 4]. So, alternative treatment is needed to reduce these side effects.

Curcumin is the main yellow polyphenolic compound derived from the rhizome of turmeric (*Curcuma longa*). According to several research, curcumin has pharmacological activity such as anticancer, anti-inflammatory, antioxidant and anti-hyperglycemic by inhibiting α-amylase and α-glucosidase enzymes [5]. However, curcumin is classified into class IV of Biopharmaceutical Classification System (BCS) based on its poor water solubility (4.375 g/ml) and low bioavailability (1,498±0.402 µg h/ml) [6]. Therefore, a technique is needed to improve the properties of curcumin, one of which is nanoparticle.

The technique for forming nanoparticle that is often used is ionic gelation. This technique is based on ionic interaction using chitosan as polycation and tripolyphosphate as polyanion [7]. Polymer-based nanocarriers, such as chitosan nanoparticles, have advantages such as more controlled drug release, increasing drug solubility, being more stable, biodegradable, biocompatible and reducing toxicity [8–11]. This is in line with Ban, *et al.* research in 2020, which showed an increase in the bioavailability of curcumin after being encapsulated in nanoparticles [12]. Research that studies the activity of nano curcumin on α-amylase and α-glucosidase enzymes has not been studied. So, this study aims to incorporate curcumin in chitosan nanoparticles and study its inhibitory activity against α-amylase and α-glucosidase enzymes.

MATERIALS AND METHODS

Materials

Materials used are acarbose (from *Dexa Medica*), chitosan (from Shrimp shell with 97.5% of deacetylation degree), Sodium Tripolyphosphate (Merck), tween 80 (Merck), α-amylase enzyme (CAS No. 9000-90-2 Merck), α-glucosidase enzyme (CAS No. 9001-42-7 Merck), starch (Merck), p-nitrophenyl-α-D-glucopyranoside (Merck).

Curcumin nanoparticle preparation

0.1% curcumin solution was added dropwise to 0.1% chitosan solution, then 2% tween 80 and 0.07% sodium tripolyphosphate were added. Stirring was carried out at a constant speed of 500 rpm for 3 h, and a suspension of chitosan-curcumin-Na TPP was obtained. After that, freeze-drying was carried out using Freeze Dryer-Biobase for 24 h. Then, curcumin nanoparticles produced were characterized consisting of particle size, polydispersity index and zeta potential using *ZetasizerNano ZS* (*Malvern Instrument Ltd., Grovewood, Worcestershire, UK*). Its inhibitory activity was also determined against α-amylase and α-glucosidase enzymes.

Inhibitory activity on α-amylase enzyme

The resulting nano curcumin and acarbose as a comparison were made with concentrations of 100, 80, 60, 40 and 20 ppm, then 250 µl of each was taken and put into a test tube, 250 µl of α-amylase which is containing 0.2 U/ml was added and 500 µl phosphate buffer with 6.9 of pH were put into a test tube and then incubated at 37 °C for 30 min. Then, 250 µl of 1% starch solution as a substrate and 250 µl iodine was added sequentially. Absorbance was measured at 595 nm by using UV-Visible spectrophotometer Thermo Scientific-*Genesys* 10S UV-Vis [5].

Inhibitory activity on α-glucosidase enzyme

A total of 500 µl of phosphate buffer was put into a test tube then 250 µl of nano curcumin (or acarbose) and 250 µl of the α-glucosidase enzyme were added into vial. Then, it was incubated for 15 min at 37 °C. After the incubation period was complete, 250 µl of p-nitrophenyl-α-D-glucopyranoside (PNPG) substrate was put into test tube. After that, it was incubated again for 30 min. Then, 1 ml of $Na_2C_2O_3$ was added to stop the reaction and absorbance was measured by using a UV-Visible spectrophotometer at 305 nm [13].

RESULTS

Curcumin which has been made in the form of suspension of nanoparticle was characterized including Particle Size, Polydispersity Index (PdI) and Zeta Potential. The result of particle size analysis (fig. 1) shows the average particle size of curcumin nanoparticles produced was 198.1 nm with a polydispersity index value of 0.349.

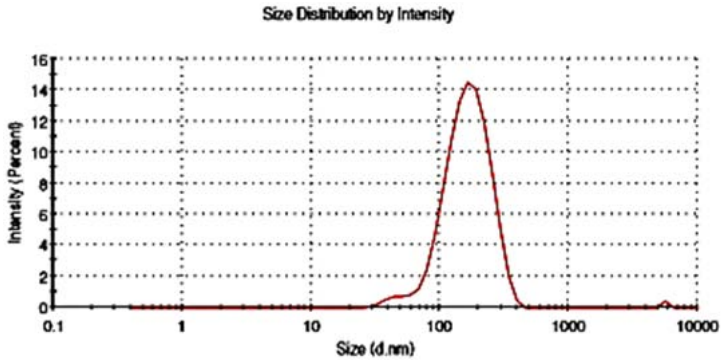

Fig. 1: Particle size distribution of nano curcumin

In addition to particle size, an important characterization of nanoparticles is zeta potential. Zeta potential value of nano curcumin was-8.33 mV, the distribution graph of zeta potential can be seen in fig. 2.

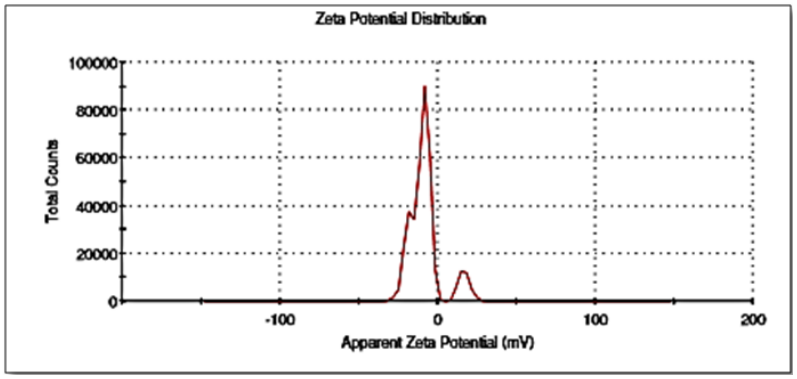

Fig. 2: Zeta potential of nano curcumin

In this study, an *in vitro* test was conducted to determine the inhibitory activity of α-amylase enzyme by observing the decrease in the intensity of blue color in the iodine-starch complex, which indicates the reduction of the starch substrate due to hydrolysis by α-amylase enzyme. The results showed that nano curcumin had smaller IC_{50} value of 56.140 µg/ml, compared to acarbose, which was 63.32 µg/ml. The inhibitory activity of nano curcumin and acarbose can be seen in fig. 3.

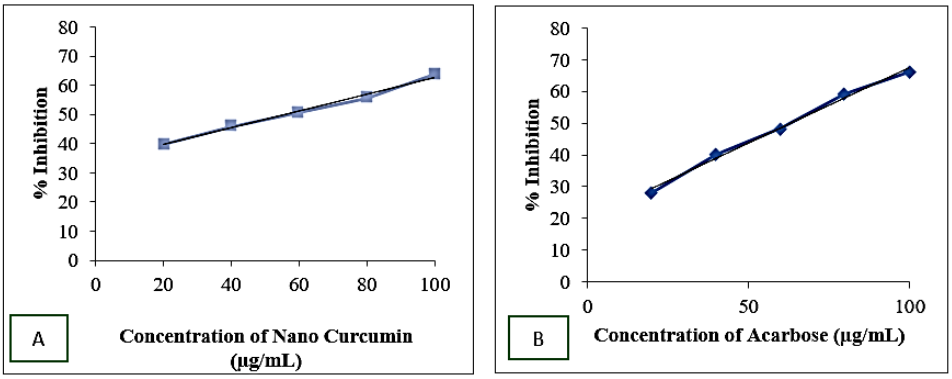

Fig. 3: Inhibitory activity of α-amylase for (a) nano curcumin (b) acarbose; n=1

Inhibitory activity of α-glucosidase can be observed from the decrease in absorption at 305 nm, which indicates the absorption of p-nitrophenol formed. The substrate used was PNPG as a model that represents carbohydrates in the body, where the enzyme will break down the substrate into glucose and p-nitrophenol [5]. The result of this study showed that nano curcumin has a smaller IC_{50} value of 3.95 µg/ml compared to acarbose of 4.11 µg/ml, it is shown in fig. 4.

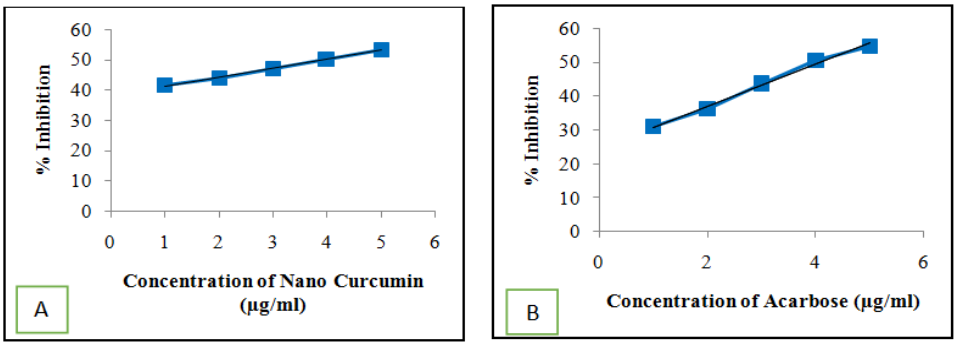

Fig. 4: Inhibitory activity of α-glucosidase for (a) nano curcumin (b) acarbose; n=1

DISCUSSION

Particle size and distribution are important characteristics of nanoparticles [14]. The nanoparticles have a particle size of 10-1000 and the particle size for pharmaceutical raw materials is between 200-400 nm [15]. The curcumin nanoparticles obtained are 198.1 nm so that they meet the nanoparticle range and can be used as pharmaceutical raw materials.

The polydispersity index of nano curcumin has met the requirements of 0.01 to 0.7. This explains that curcumin nanoparticles are monodispersed. This polydispersity value also explains that curcumin nanoparticles have good homogeneity because they have a polydispersity value<0.7, while polydispersity values >0.7 have poor homogeneity. Particle stability is indicated by the homogeneity of the particle size; the more homogeneous the nanoparticle the more stable it is [16].

Nanoparticles have charge characteristics that need to be known to show the stability of a colloidal system; this can be indicated by the zeta potential value. Nanoparticles with zeta potential values less than-30 mV and greater than+30 mV have higher stability [17]. The results of this study indicate a potential zeta value of-8.33 mV. Dispersion systems with low zeta potential values are easier to form aggregates [14].

The results showed that the greater concentration used, the more compounds that played a role so that the percentage of inhibition was higher because of the inhibition of the α-amylase enzyme by curcumin nanoparticles so that the glucose product produced was little so it could reduce blood sugar levels in diabetics. Parameters of inhibitory activity on a compound expressed in IC_{50} which is the concentration of the compound or sample that has an inhibitory activity against enzyme by 50%. The smaller the IC_{50} value, the better the inhibitory activity of a sample [18].

The results showed that curcumin nanoparticles had smaller IC_{50} value than acarbose, it is meaning that the percent (%) inhibition of α-amylase and α-glucosidase enzymes activity by curcumin nanoparticles was higher than acarbose. This shows that when curcumin nanoparticles are added to α-amylase and α-glucosidase enzymes, these curcumin nanoparticles will stick to the active site of the enzyme because they have similarities in the substrate structure [19]. The other research of Agada., *et al.* shows that inhibition of α-amylase and α-glucosidase activity by *Carica papaya* seed extract was 76.96 and 75.78 ppm of IC_{50} value, respectively [20]. It shows that curcumin nanoparticles had better inhibitory activities for both enzymes.

CONCLUSION

It can be concluded that the activity of nano curcumin against alpha-amylase and alpha-glucosidase enzymes is very strong with IC_{50} values of 56.140 and 3.95 µg/ml, respectively. So that nano curcumin has great potential as an antihyperglycemic agent.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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