The Antifungal Activity of Artesunate toward Candida albicans: Two Opposite Activities

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The artemisinin and its derivatives antifungal activity continue to be an interesting research object, with the potential shown to be developed as an antifungal compound. Artesunate, one of the artemisinin derivatives known to have antifungal activity against various pathogenic fungi, including Candida albicans. This study aims to determine the effect of artesunate on antifungal activity toward C. albicans in vitro at concentrations below 1 mg mL−1. The method used is yeast-plate count, with a parameter of observation were the number of C. albicans colonies viable after exposure with artesunate for five days. The concentration of artesunate used was divided into six groups, which were 10; 1; 10 ; 10 ; 10 ; and 10 mg mL−1. Compared to control, a significant decrease in colony counts was only shown at the highest concentration of 10 mg mL−1. Interestingly, at the lowest concentration of 10 mgmL−1, it showed an increase in a number of colonies almost twice of the blank. These results suggest that while at higher concentration of artesunate may inhibit the growth of C. albicans, a lower concentration of artesunate may stimulate their growth.

Key words: antifungal, artesunate, Candida albicans

In addition to its antimalarial activity, artemisinin and its derivatives are known to have various other activities (Pan et al. 2018). Artemisinin is known to have activity against various types of parasitic infections such as Leishmania, Schistosoma, and Toxoplasma (Li and Zhou 2010). Artemisinin and its derivatives are also known to have several other activities such as antiviral (Efferth et al. 2008; Pratama and Gusdinar 2017), antimicrobial (Appalasamy et al. 2014), and even anticancer properties (Willoughby et al. 2009; Li et al. 2008). Artesunate, one of artemisinin derivative with potent antimalarial activity, is currently being developed for the treatment of another disease, including those caused by infection (Loo et al. 2017; Zuo et al. 2016). One of the artesunate properties currently being further investigated is as antifungal, where artesunate is known to have antifungal activity against various types of opportunistic pathogenic fungi such as Candida albicans and Cryptococcus neoformans (De Cremer et al. 2015; Galal et al. 2005). The exact mode of action of artesunate as antifungal itself remains elusive, but several studies have shown that artesunate and other artemisinin derivatives show antiproliferative activity on eukaryotic cells including fungi (Wang et al. 2017; O'Neill et al. 2010). One of the causes is that artemisinin derivatives including artesunate are known to inhibit various cyclin-dependent kinases (CDKs) enzymes induce growth arrest of cell cycle division that are the key factors in

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the proliferation of fungi cells (Zhang et al. 2018; Tran et al. 2014; Ho et al. 2014; Kundu et al. 2015). However, studies conducted generally carried out in doses above 1 mg mL⁻¹, while testing under these doses has never been done before. Thus, the antifungal activity of artesunate in this dose range is still unknown.

This study aims to determine the effect of different concentration of artesunate on the antifungal activity toward C. albicans as well as proving the potency of artesunate as the antifungal compound. Testing was performed with antifungal assay by counting the number of C. albicans viable colonies after administration of exposure to artesunate with multiple levels of dosage compared to controls. A decrease in the number of viable colonies showed that C. albicans cells divide at a lower rate and produce fewer colonies (Kwolek-Mirek and Zadrag-Tecza 2014). Before the test, Growth Promotion Test (GPT), and Sterility Test (ST) are conducted to determine the ability of the medium used to grow C. albicans and its asepticity, respectively (Sutton 2011). The results of this study will reveal how exactly the effect of artesunate exposure on the antifungal activity toward C. albicans.

**MATERIALS AND METHODS**

**Drug and Test Solution.** Artesunate was provided by Guilin Pharmaceutical Co. Ltd, China in the form of sodium artesunate reconstitution and dissolved in sterilized sodium bicarbonate. The test compound is further diluted with physiological saline (sodium chloride 0.9%) sterile solution to obtain 6 concentrations of 10 mg mL⁻¹; 1 mg mL⁻¹; 10⁻¹ mg mL⁻¹; 10⁻² mg mL⁻¹; 10⁻³ mg mL⁻¹; 10⁻⁴ mg mL⁻¹; and 10⁻⁵ mg mL⁻¹, respectively. Each test solution was homogenized with vortex mixer then immediately used within half an hour.

**Fungal Strains.** The C. albicans strains ATCC 10231 used were obtained from Laboratory Microbiology, School of Pharmacy, Bandung Institute of Technology. The strain was grown in Sabouraud Dextrose Broth (SDB) with a pH range of 5.6 ± 0.2 then diluted with SDB until a number of colonies less than 100 CFU mL⁻¹ are obtained. The inoculum suspension of C. albicans then stored in the cold room with temperature -20°C and can be stored up to 1 week.

**Growth Promotion Test and Sterility Test.** The GPT was conducted to determine the ability of the medium used in the observation process to grow the test colony. The test medium used was Sabouraud Dextrose Agar (SDA), which has been known to grow colonies of C. albicans. As much as 1 mL of C. albicans inoculum suspension was added into SDA until a mixture of 15 mL was obtained. The mixture was then homogenized with a vortex mixer for a minute, then poured into a sterile petri dish and made in triplicate. The dishes then incubated at 25°C for five days. Observations were made on the fifth day by counting the number of colonies growing on each dish. All dishes should be able to grow between 30 and 300 colonies.

The ST was performed to ensure that the sterilization process is done successfully so that no contaminants grow in the petri dish due to non-aseptic process. As much as 1 mL of physiological saline sterile solution was added into SDA until a mixture of 15 mL was obtained. The mixture was then homogenized with a vortex mixer for a minute, then poured into a sterile petri dish and made in triplicate. The dishes then incubated at 25 °C for five days. Observations were made on the fifth day by counting the number of colonies growing on each dish. All dishes should not show the growth of the colony.

**Antifungal Assay.** As much as 1 mL of C. albicans inoculum suspension was added to 1 mL of test solution for each concentration. The mixture was then added with SDA until a final mixture of 15 mL was obtained. The mixture was then homogenized with a vortex mixer for a minute, then poured into sterile Petri dishes. Each series of test solution concentration was made in triplicate. The physiological saline sterile solution was used as a blank. The entire process was carried out in the Laminar Air Flow in aseptic conditions.

All Petri dishes were incubated at 25 °C for five days. Observations were made on the fifth day by counting the number of colonies growing on each dish then calculated the mean value of each test solution concentrations.

**RESULTS**

**Growth Promotion and Sterility.** The number of colonies that grow on each petri dish was calculated entirely using a colony counter. All visible colonies were counted regardless of the size of each colony. All dishes on GPT show considerable colony growth with a colony range between 203 to 226 colonies. In contrast to GTP results, all dishes on ST show no colony growth of all Petri dishes (Fig 1, 2). The GPT and ST itself is a mandatory requirement before conducting yeast-plate
count testing, where the test medium must be able to grow the test microbes and on the other hand the working process must be guaranteed aseptic to prevent any contaminants from outside.

Antifungal Assay. All petri dishes except on blanks covered by C. albicans colonies. The calculations of the number of colonies were performed on all parts of the petri dish. The colony growth in the antifungal assay is shown in figure 3 below.

DISCUSSION

Surprisingly, the decrease in the number of C. albicans colonies was significantly demonstrated only by the test solutions with the highest artesunate concentrations of 10 mg mL\(^{-1}\). Besides the number of colonies in the concentration is still not reached half of the number of colonies on the blank, indicating that the IC\(_{50}\) of artesunate against C. albicans is in the range greater than 10 mg mL\(^{-1}\) or equivalent to 26 µM, which is very large value. The results clearly show that the potency of artesunate as an antifungal is relatively weak, especially compared to other derivative compounds of natural products such as those derived from Origanum vulgare, Eucalyptus and Thymus sp like carvacrol, thymol, and α-terpineol (Nazzaro et al. 2017; Gucwa et al. 2018).

More interesting results are at lower concentrations
Fig 3 The result of the antifungal assay. Growth of colonies on one of the petri dish from (A) negative control (blank); (B) artesunate 10 mg mL⁻¹; (C) 10 mg mL⁻¹; (D) Number of C. albicans colonies from all petri dishes from each group of test solutions, with the average number for blank; 10⁻¹ mg mL⁻¹; 10⁻² mg mL⁻¹; 10⁻³ mg mL⁻¹; 10⁻⁴ mg mL⁻¹; 1 mg mL⁻¹; and 10 mg mL⁻¹ were 67.7, 103.7, 80.7, 76.3, 65.7, 64.7, and 38.3 colonies, respectively. The dashed line indicates the average colonies number of the blank.

that are below 10⁻¹ mg mL⁻¹, artesunate actually triggered the growth of the number of C. albicans colonies. At the lowest concentration of 10⁻¹ mg mL⁻¹ even the number of colonies that grow almost 50% more than the blank (103.7 to 67.7 colonies). The cause of the increasing number of C. albicans colonies is still unknown, but it is probably related to transcription regulator Pdr1p and its target genes PDR5 and TPO1 (Alenquer et al. 2006; Fardeau et al. 2007). Another possibility is that artesunate interacts with apoptosis protein regulators in eukaryotic cells as shown in plantaricin E and F (Nurhayati et al. 2015).

Another interesting feature is that the increase in the number of colonies appears to occur linearly to the concentration of artesunate, where smaller artesunate concentrations lead to more C. albicans colonies growth. Unfortunately, the following study is only done at the lowest artesunate concentration of 10⁻¹ mg mL⁻¹. It is interesting to observe how the change in the number of C. albicans colonies at artesunate concentrations smaller than 10⁻¹ mg mL⁻¹, whether the number of colonies is still increasing, as well as the lowest artesunate concentration which still gives an increase in the number of C. albicans. Based on the author’s search to date, no studies have reported this. In summary, we show for the first time an actual antifungal activity of artesunate against colonies of C. albicans, which shows that the antifungal activity of artesunate will only appear at high doses. On the contrary at low doses, artesunate actually increases the number of C. albicans colonies. The results of this study open new possibilities that the use of artesunate at low doses can actually increase the potential for candidiasis due to overgrowth of C. albicans.

ACKNOWLEDGEMENT

This work was supported by the internal grant from Institut Teknologi Bandung. In addition, financial support was also provided by the Institute for Research
and Community Services Universitas Muhammadiyah Palangkaraya. We thank Marlia Singgih Wibowo (Institut Teknologi Bandung) for the help with results interpretation.

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