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ANTIFUNGAL ACTIVITY OF CURCUMIN CASPOFUNGIN COMBINATION AGAINST *CANDIDA* SPP. VIA APOPTOTIC INDUCTION

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ABSTRACT

Phytochemicals with drug combinations have led to the discovery of different therapeutics used in fighting off the different types of infections. In this study, I have investigated the synergetic antifungal effect of Curcumin and Caspafungin combination against *C. albicans* and *C. glabrata* by using a checkerboard microdilution assay. Apoptotic effects of curcumin and caspafungin concentrations were evaluated against *C. albicans* and *C. glabrata* by using flow cytometry via annexin V-propidium iodide binding capacity. Synergetic antifungal effect assessment was evaluated via flow cytometry by using checkerboard microdilution assay. Early and late apoptotic effects of curcumin and caspafungin combination (1000 and 1 µg/mL) against *C. albicans* were observed as 8.1%, 77.4% respectively. Early and late apoptotic effects of curcumin and caspafungin combination (1000 and 1 µg/mL) against *C. glabrata* were observed as 6.3%, 77.7% respectively. Apoptotic effects of 1 µg/mL caspafungin and 1000 µg/mL curcumin combinations were 85.5% and 84% against *C. albicans* and *C. glabrata*, respectively. Both *Candida* species showed a synergic effect, according to fractional inhibitory concentration index (FIC ≤0.5). Results revealed that the combination of curcumin and caspafungin exhibited stronger anticandidal activity than monotherapy.

KEYWORDS:
Curcumin, caspafungin, apoptosis, *Candida albicans*, *Candida glabrata*

INTRODUCTION

Candida species have been associated with several opportunistic pathogen of the human gastrointestinal, respiratory and reproductive tracts especially affecting immunocompromised patients [1-4]. There are significant limitations in the existing antifungal drugs, such as antifungal resistance, inadequate activity spectrum, poor bioavailability, low tolerance index, interactions with other drugs, inadequate pharmacokinetic profile and undesirable side effects [5-8]. Especially, *C. albicans* and *C. glabrata* are an emerging pathogen that has become the first and second most frequent cause of candidiasis.

There has been a sharp rise in the incidence of *Candida* infections. Currently available anti-fungal agents have undesirable side effects, toxicity problems and rising drug resistance problems for the treatment of *Candida* infections [7, 9-12]. For this reason, components derived from natural products are an encouraging source of new anti-fungal therapy alternatives.

An optimal anti-fungal agent against *Candida* infections should not have undesirable effects or toxicity [13, 14]. On the other hand, all the antifungals currently in use have some unwanted effects on the gastrointestinal tract, liver, and kidney [7, 15, 16]. For this reason, researchers have concentrated on the potential synergistic activity of phenolic acids with the combination of existing antifungals in order to maximize the antifungal effect. It is a good strategy to evaluate the synergistic effects when MIC values of phenolic acids against *Candida* are highly variable [17, 18]. For example, the synergistic effect of benzoic acid, amphotericin B, itraconazole combination against *C. albicans* has been reported [19]. On the other hand, mechanism of synergistic effect of phenolic acids and conventional antifungal agents is poorly understood. As a result, it is of crucial importance to explore similar synergistic effects revealed by the combined use of other phenolic compounds and antifungal agents.

Polyphenolic product curcumin has been subjected to so many antimicrobial, antioxidant, antitumor investigations all over the world because of extensive traditional usage and low side effects [20, 21]. Reported antimicrobial activities of curcumin against different bacteria, viruses, fungi, and parasites made it a good candidate to enhance the inhibitory effect of existing anticandidal drugs by the way of synergism [22].

In this regard, this manuscript intends to evaluate the potential synergistic antifungal activity of curcumin with the combination of caspafungin as a safer therapeutic strategy against *Candida* species.

MATERIALS AND METHODS

Fungi Strains and Chemicals. *C. albicans* ATCC 90028 and *C. glabrata* ATCC 90030 were taken from American Type Culture Collection (ATCC, Manassas, VA, USA). Caspofungin diacetate (SML0425) and curcumin were acquired from Sigma (St. Louis, MO, USA).

Antimicrobial assay. MIC is described as the lowest concentration of the antimicrobial agent that inhibits the bacterial growth, as detected by the lack of observable turbidity. The microbiological assay was performed according to the Clinical and Laboratory Standards Institute M7-A7 broth microdilution method [23].

Checkerboard microdilution assay for curcumin and caspofungin. Synergistic effect was evaluated by the checkerboard microdilution assay; a two-dimensional range of serial concentrations of test agents, which has been used to evaluate antimicrobial combinations *in vitro*. The tested dilutions were based on the MIC of the two compounds. The checkerboard test was used as the base of the Fractional Inhibitory Concentration (FIC) index calculation [24, 25]. The effects of the combination of caspofungin with curcumin were investigated by the checkerboard broth microdilution method. Drug interaction was classified as synergistic, additive or less-than-additive according to the FIC index, which is the sum of each compound FIC index. The FIC index of each compound was calculated as the MIC of that drug in combined treatment divided by the MIC of the drug used alone. Drug-drug interactions were considered synergistic if the FIC index was ≤0.5, indifferent if the FIC index was >0.5 and ≤4 and antagonistic if the FIC index was > 4.0 [26].

Analysis of apoptosis caused by *Candida* species using flow cytometry. Curcumin in combination with caspafungin concentrations which were analysed against *C. albicans* ATCC 90028 and *C. glabrata* ATCC 90030 based on Annexin V-PI binding capacities in flow cytometry. *C. albicans* ATCC 90028 and *C. glabrata* ATCC 90030 cells ($2x10^6$/mL) were incubated in Sabouraud Dextrose Broth with 1 µg/mL caspofungin and 1000 µg/mL curcumin for 24 hours at 30 °C *C. albicans* ATCC 90028 and *C. glabrata* ATCC 90030 cells were harvested by centrifugation and washed in 0.1 molar potassium phosphate buffer. Annexin V/Propidium Iodide (PI) assays were performed according to the staining kit protocol, using 5 µg annexin V and 5 µg PI at 37 °C for 20 minutes. The cells were analyzed using a BD Accuri C6 flow cytometer (Becton–Dickinson, Mansfield, MA, USA) [27].

RESULTS

The first step of this study was to determine the MIC and FIC index of caspofungin and curcumin, which induced both apoptosis and necrosis, via flow cytometry. The checkerboard microdilution method revealed that curcumin and caspofungin exhibited synergistic antifungal activity against *C. albicans* with an MIC value of 20 µg/ mL and 1 µg/mL, respectively, and against *C. glabrata* with an MIC value of 10 µg/ mL and 2 µg/ mL, respectively. The FIC index of curcumin and caspofungin were shown in Table 1.

The apoptotic effects of caspofungin, curcumin, caspafungin-curcumin combination concentrations after the 24-hour incubation period, were evaluated against *C. albicans* and *C. glabrata* based on Annexin V–PI binding capacities by using flow cytometry (Figure 1). Flow cytometric analyses revealed early and late apoptotic effects of 1µg/mL caspofungin against *C. albicans* as 0.3% and 0.6%, respectively. However early and late apoptotic effects of curcumin against *C. albicans* were found to be increased as 19.7% and 58.2%, respectively (Figure 1). According to results; early and late apoptotic effects of curcumin against *C. glabrata* revealed 0.3% and 1.5%, respectively (Figure 1). The apoptotic effects (early and late) of the curcumin in combination with caspofungin against *C. albicans* and *C. glabrata* were found 88.5% and 84%, respectively (Table 2 and Figure 1). Early and late apoptotic effects of caspafungin and curcumin combination against *C. albicans* were calculated as 8.1% and 77.4% respectively. Early and late apoptotic effects of caspafungin and curcumin combination against *C. glabrata* were computed as 6.3% and 77.7% respectively (Table 2). Results indicate that combination of caspafungin and curcumin triggered the apoptosis of *C. albicans* and C. *glabrata* depending on synergism. The synergistic activity of caspofungin and curcumin caused a drastic reduction in the MIC of *C. albicans* and *C. glabrata.*

Drugs in combination with phytochemical product combinations have been shown to reduce the required therapeutic dose of anticandidal agents and consequently their toxicity if a synergism is present. Caspafungin and curcumin combination demonstrated a significant synergistic antifungal effect against *C. albicans* and *C. glabrata* which may become a promising natural anti-candidal therapy strategy. In order to develop new therapeutic strategies, further research is needed for the suitability and medical credibility of the natural antifungal agent.

In this study, we investigated the synergy of curcumin in combination with caspofungin against *C. albicans* and *C. glabrata* by using a quantitative checkerboard microdilution assay. Synergism was evaluated via a fractional inhibitory concentration index (FIC) of <0.5. To my knowledge, the synergistic anticandidal effect of curcumin and caspafungin

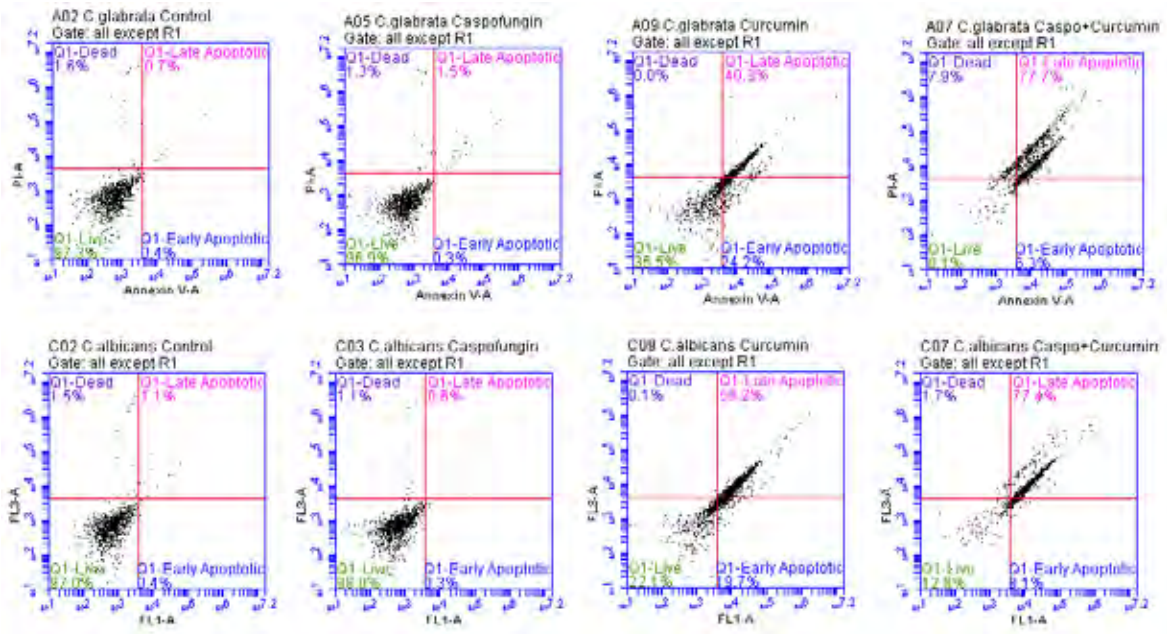

FIGURE 1
***C. albicans* and *C. glabrata* apoptosis assay stained with Annexin V-propidium iodide using flow cytometry.**

TABLE 1
Curcumin and caspofungin application against *C. albicans* and *C. glabrata*.

	Curcumin (µg/mL) (A)	Caspofungin (µg/mL) (B)	Combination (µg/mL) (A+B) (B+A)	Fractional Inhibitory Concentration Index (FIC) > 0.5-< 0.5
C. albicans	20	1	1.25 0.25	0.3125
C. glabrata	10	2 2	2.5 0.25	0.375

TABLE 2
Comparison of percentages of viable, early apoptotic, late apoptotic, and necrotic cells on *C. albicans* and *C. glabrata* stained with annexin V–propidium iodide using flow cytometry.

	***C. albicans* (%)**	***C. glabrata* (%)**
Control		
Alive	97	97.3
Early apoptotic	0.4	0,4
Late apoptotic	1.1	0,7
Necrotic	1.5	1,6
Caspofungin (1µg/mL)		
Alive	98	96,9
Early apoptotic	0.3	0.3
Late apoptotic	0.6	1,5
Necrotic	1.1	1,3
Curcumin (1000 µg/mL)		
Alive	22.1	35,5
Early apoptotic	19.7	24,2
Late apoptotic	58.2	40,3
Necrotic	0.1	0
Caspofungin (1µg/mL)+ Curcumin (1000 µg/mL) combination		
Alive	12.8	8.1
Early apoptotic	8,1	6,3
Late apoptotic	77.4	77.7
Necrotic	1,7	7,9

against *Candida* species has never been investigated via flow cytometry.

DISCUSSION

One of the alternative strategies to fight off candidiasis is the use of natural products such as phenolic acids. Another possibility is to combine existing anticandidal drugs with phytochemicals to enhance the efficacy and lessen the severity of the side effects of drugs. Antifungal activity and synergistic effect of curcumin in combination with caspafungin against *Candida* species have been demonstrated. The present study highlights the possible use of the synergistic drug-herb combinations for the treatment of candidiasis.

In this research, I demonstrated that curcumin could enhance the fungicidal activity of caspafungin against *C. albicans* and *C. glabrata*. The checkerboard microdilution assay was used to examine the relationship between curcumin and caspafungin in terms of their growth inhibitory activities against *C. albicans* and *C. glabrata*.

An *in vivo* study on systemic candidiasis in mice demonstrated that following the treatment with honokiol and fluconazole, the survival rate was 100% while a monotherapy showed only a survival rate of 80% to fluconazole and 20% to honokiol, respectively. Furthermore, the synergism of these two compounds led to a notable reduction in *C. albicans* counts in mouse kidneys compared with the fluconazole treatment alone [7, 28].

Synergism of curcumin with fluconazole, amphotericin B and itraconazole against *C. albicans* has been reported in the literature [21]. Studies have also demonstrated a significant synergism between other known antifungals (fluconazole, amphotericin B and itraconazole) and phenolic compounds (punicalagin, carvacrol, epigallocatechin-gallate, thymol, curcumin) against *C. albicans* [7]. To my knowledge, the synergistic anti-fungal activity of curcumin and caspafungin against *C. albicans* and *C. glabrata* has never been investigated via flow cytometry.

As a result, this manuscript evaluated the synergistic anticandidal effect of curcumin and caspofungin combination against *C. albicans* and *C. glabrata*. Drug-herb combinations synergistic effects may provide a promising therapeutic strategy in controlling opportunistic fungal infections, especially against *Candida* species. The combination of curcumin with caspafungin exhibited a stronger fungicidal activity than monotherapy against *C. albicans* and *C. glabrata*. It can be hypothesized that this anticandidal effect is probably not produced by a single compound. Consequently considering synergistic antifungal activities and the lack of studies related to natural products, curcumin is a promising source of molecules with antifungal properties. Further investigations are needed to clarify the synergistic antifungal mechanism of curcumin and caspafungin.

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