Original Article

An investigation of the inhibitory effects of dendrosomal nanocurcumin on *Candida albicans* and systemic candidiasis in BALB/c mice

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Abstract

Background and Purpose: Use of curcumin, as a promising antifungal agent, is considered an alternative treatment for fungal infections; however, the low solubility of this agent limits its efficacy. Accordingly, in this study, we aimed to evaluate the *in vitro* and *in vivo* antifungal activities of dendrosomal nanocurcumin with improved solubility and bioavailability.

Materials & Methods: The *in vitro* antifungal activities of several *Candida* species, including *C. albicans, C. tropicalis*, *C. krusei, C. parapsilosis*, and *C. dubliniensis,* were evaluated, using the broth microdilution method. In the *in vivo* study, different doses of nanocurcumin (5, 10, 20, and 40 mg/kg) were administered to mice with systemic *C. albicans* infection via intraperitoneal injection. All mice were euthanized at 20 days following the administration of different doses of nanocurcumin. Different organs were extracted for organ culture and histopathological investigation.

Results: Based on the findings, 40 mg/kg of nanocurcumin significantly decreased the fungal load in the evaluated organs; the results were confirmed with histopathological examination. The kidney was found to be the most affected organ with the highest number of severe lesions. Yeasts and pseudohyphae were observed in the blood vessels, kidney, and brain. Also, yeasts were present in the liver, brain, lungs, and heart of the control group.

Conclusion: Although curcumin is generally an excellent antifungal component, its nano-sized form showed more potent properties. Based on the gathered data, dendrosomal nanocurcumin is an effective antifungal agent with good efficacy against disseminated candidiasis. However, further studies are required to evaluate the effects of dendrosomal nanocurcumin on other fungal infections. Also, this agent could be useful for the prevention of fungal infections, such as candidiasis, particularly in high-risk patients.

Keywords: *Candida albicans*, Systemic candidiasis, Antifungal, Nanocurcumin,

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Introduction

The occurrence of fungal infections has significantly increased since 1980's as a consequence of the indiscriminate use of broad-spectrum antibiotics and the growing number of immunocompromised individuals [1, 2]. *Candida* species are major yeasts isolated from clinical infections. The genus *Candida* is composed of several species, which are found in the normal flora of the mucosal oral cavity, gastrointestinal tract, and vagina [2].

Candida species are commensal in humans and animals and remain responsible for several clinical manifestations, such as mucosal and skin overgrowth or bloodstream infection, especially in immunocompromised patients or cases with the elimination of normal flora [3]. Although *C. albicans* is the major cause of invasive infection, other species such as *C. parapsilosis, C. tropicalis,* and *C. glabrata* are also involved [4].

The National Nosocomial Infections Surveillance (NNIS) system report in the United States has introduced *Candida* species as the major cause of infection in immunocompromised patients [5]. The need for the development or improvement of antifungal agents is growing, considering the limited number of these agents, compared to antibacterial drugs.

Azoles are routinely used to combat *Candida*

species. However, owing to their dominant fungistatic activities, fungal resistance may occur, especially due to long-term use. In fact, azole-resistant *Candida* strains still remain a major challenge for clinicians [6]. Furthermore, some antifungal agents exert adverse side-effects such as nephrotoxicity [7].

Recently, several strategies, such as combination therapy and use of herbal medicines, such as allicin, berberine, grapefruit seed extracts, tea tree oil, and curcumin, have been used for the treatment of fungal infections. Among the introduced strategies, curcumin, a phenolic component derived from the rhizomes of *Curcuma longa,* has been widely used, given its anti-cancer, anti-inflammatory, antioxidant, and anti-infection properties.

The *in vitro* antifungal activity of curcumin against several fungal strains, such as *Candida* and *Aspergillus* species, has been confirmed in recent studies [6, 8]. According to the literature, curcumin shows better antifungal activities against *Paracoccidioides brasiliensis* and *Sporothrix schenckii*, compared to fluconazole [9]. Curcumin is believed to exert its antifungal effects through altering the membrane-associated properties of ATPase activity, ergosterol biosynthesis, proteinase secretion, and apoptosis induction [10].

In spite of its promising pharmaceutical characteristics, curcumin exhibits poor aqueous solubility and fast biodegradation [11]. Several approaches, such as treatment with adjuvants, liposomes, and more recently nanoparticles, have been applied to overcome this limitation. According to the literature, dendrosomal nanoparticles markedly enhance the therapeutic efficacy of curcumin by improving its solubility [12]. Therefore, in the present study, we aimed to describe the *in vitro* antifungal activity of dendrosomal nanocurcumin against *Candida* species and systemic candidiasis in mice.

Materials and Methods

Reagents and strains

The reference strains of *C. albicans* (ATCC10231), *C. tropicalis* (ATCC750), *C. krusei* (PTCC 5295), *C. parapsilosis* (ATCC22019), and *C. dubliniensis (CD36)* were used in the present study. To prepare the inoculum, fresh cultures were grown on Sabouraud glucose agar (SGA; Merck Co., Darmstadt, Germany) at 30°C for 48 h. The yeast cells were harvested, washed with normal saline, and adjusted by a hemocytometer (approximately 1×10^8/ml). Moreover, curcumin was purchased from Sigma-Aldrich Co. (USA). The procedures for producing dendrosomal curcumin were performed as described in the literature [12] at the Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran.

In vitro antifungal activity

The strains were cultured on Sabouraud dextrose agar (SDA) at 30°C for 48 h before the experiments. The proper concentration of the prepared *Candida* suspension was adjusted by the heamocytometer. As described by the Clinical & Laboratory Standards Institute (CLSI) document M27-S4 [13], broth microdilution method was used to determine the *in vitro* minimum inhibitory ceontration (MIC) of nanocurcumin against the strains. MIC was determined as the lowest concentration resulting in fungal growth inhibition.

The broth microdilution method was performed in flat-bottomed 96-well microtiter plates, using RPMI-1640 medium, which had been buffered to a pH of 7.0 with 0.165 M of morpholinepropanesulfonic acid (Sigma, USA). Then, 100 µl of nanocurcumin (4%) was added to the first well, containing 100 µl of the medium, followed by twofold serial dilution. An inoculum size of $0.5–2.5\times10^3$ CFU/ml was prepared spectrophotometrically and added to each well. The plates were incubated at 35°C for 48 h. The tests were performed in triplicate on separate plates.

Animals

In total, 25 healthy female BALB/c mice (4-6 weeks old), weighing 18-22 g, were used in this study. The mice were purchased from Pasteur Institute of Iran (Tehran, Iran) and kept in the experimental animal laboratory of Tabriz University. The animals were housed in stainless metal cages and fed standard pellets and water ad libitum (80% humidity, temperature of 37°C) in a 12:12 h light/dark cycle.

Treatment

After one week of acclimatization, the mice were randomly divided into five groups (five mice per group) to receive different treatments. The mice were

infected by intravenous injection of 0.5×10^7 of saline-washed *C. albicans* blastospore reference strain. In group I, the first dose of nanocurcumin (5 mg/kg) was administered via intraperitoneal injection two days before the inoculation of *C. albicans*; the next dose was injected two days after inducing infection in mice.

Treatment groups II, III, and IV received nanocurcumin at doses of 10, 20, and 40 mg/kg, respectively via intraperitoneal injection at 24 h following the injection. Nanocurcumin injection was repeated every 24 h over one week for these treatment groups (II, III, and IV). On the other hand, the control group received no treatments after the intravenous injection of *C. albicans*. All mice were euthanized at 20 days following the administration of different doses of nanocurcumin.

Organ culture

At the end of the exposure, the animals were sacrificed by ether anesthesia. Whole specimens were obtained from the brain, lungs, liver, intestine, kidney, and spleen of mice. A section of aseptically-collected organs was homogenized by a porcelain mortar and pestle, and 0.1 g of the homogenized tissue was placed on SGA in a plate. The plates were incubated at 35°C for 48 h, and the number of colony-forming units (CFUs) per 0.1 g of each tissue was determined. The organ cultures were conducted in duplicate.

Histopathology

At the end of the exposure, the collected organs were examined for gross pathological lesions. The portions of different organs were fixed in 10% neutral buffered formalin. After routine histological procedures, paraffin blocks were divided into 5 µm thick sections, stained with hematoxylin and eosin (H&E) and periodic acid–Schiff (PAS) methods. Afterwards, they were examined under a light microscope for the assessment of *C. albicans* foci, blastoconidia, and pseudohyphae in the organs and evaluation of the intensity of the inflammatory response.

Results

MIC results

The MICs for curcumin and nanocurcumin are presented in Table 1. The antifungal effects of nanocurcumin were at least twice as high as curcumin.

Organ culture

The results of organ culture for the brain, liver, kidney, and spleen are presented in Table 2. All the organs yielded *C. albicans* colonies. The fungal burden in the control group was significantly higher, compared to nanocurcumin recipients in a dose-dependent manner. The highest frequency of *Candida* species was observed in the kidneys, followed by the brain, lungs, and spleen. Based on the findings, only 40 mg/kg of nanocurcumin was able to eliminate fungal infection.

Histopathology

No case of mortality was reported in mice throughout the four-week period of the experiment. Under light microscopy, the negative control group showed no lesions. On the other hand, in other groups, lesions with varying severities were observed. The kidney was found to be the most vulnerable region for *Candida* infection, showing major histopathological changes; other affected organs included the liver, heart, lung, and brain.

In the infected groups, changes in the kidney consisted of hyperemia, hemorrhage, hydronephrosis, tubular degeneration, neutrophil infiltration, tubulointerstitial nephritis with lymphocytes and plasma cells, multifocal lesions of interstitial nephritis, necrosis surrounded by neutrophils and macrophages, and granular and hyaline casts.

Fungal organisms were detected in the pelvis and blood vessels. The lungs showed hemorrhage and emphysema with infiltration of neutrophils and macrophages in the alveolus. The fungi were observed in blood vessels, mostly as blastoconidia. The observed lesions included myocardial necrosis and hemorrhage in the heart; however, no fungal organism was found in the heart.

Table 1. Minimum inhibitory concentrations (MICs) of curcumin and nanocurcumin against *Candida* species

Candida species	MIC (mg/ml)	
	Nanocurcumin	Curcumin
C. albicans	0.5	2
C. tropicalis	0.5	1
C. krusei	1	2
C. parapsilosis	0.5	1
C. dubliniensis	0.5	1

Table 2. Comparison of the efficacy of different concentrations of nanocurcumin on disseminated candidiasis in BALB/c mice

Nanocurcumin dose	Mean CFU/0.1 g				
	Kidney	Liver	Spleen	Lung	Brain
Nanocurcumin (5 mg/kg)	16.8	9.2	2.2	7.2	9.4
Nanocurcumin (10 mg/kg)	17	8.2	1.4	6	7.8
Nanocurcumin (20 mg/kg)	11.6	3.6	0	1.2	2.4
Nanocurcumin (40 mg/kg)	0.6	0	0	0.2	0.2
Untreated (control)	17.4	10.2	2.2	9.2	12.6

The liver showed fatty changes and necrotic areas surrounded with lymphocytes and macrophages; also, blastoconidia of *C. albicans* were present in the samples. In the corpus callosum,axonalswelling,leukoencephalopathy, and gliosis were observed. Furthermore, in all treatment groups, histopathological lesions with varying severities were observed, based on the received treatment.

Discussion

Evaluation of antifungal agents used for the treatment of disseminated candidiasis has revealed treatment failure, occurrence of side-effects, and high rate of disease relapse. Consequently, major efforts have been made to identify natural agents in order to overcome these opportunistic infections. Several studies have investigated the antimicrobial activity of curcumin and its analogues against pathogenic fungi, bacteria, viruses, and protozoa.

In the present study, we characterized the *in vitro* antifungal activity and effects of different doses of dendrosomal nanocurcumin on histopathological results and fungal burden in an experimental model of systemic candidiasis among BALB/c mice. Curcumin, given its useful properties such as adequate safety and extended antimicrobial activity, has attracted researchers' attention. In the present study, dendrosomal nanocurcumin, as a more soluble form of curcumin, was used in order to increase its efficacy.

In this regard, Babaei *et al.* (2012) by using *in vitro* and *in vivo* models reported enhanced bioavailability and anti-cancer efficacy of dendrosomal curcumin, compared with void curcumin due to enhanced solubility and improved curcumin uptake mediated by dendrosomes [14]. Overall, dendrosomes are found to be safe components with no adverse side-effects on natural cells. In this study, we observed that 0.5-1 mg/ml of dendrosomal curcumin could significantly affect the growth of *C. albicans* and non-*C. albicans* species.

Furthermore, Bhawana *et al.* (2011) prepared nano-sized curcumin, using the wet-milling technique. The results showed improved water solubility (without any surfactants), attributed to the narrow size and larger surface area of particles, resulting in enhanced dissolution and bioavailability [15]. The *in vitro* antimicrobial assay using agar dilution and well-diffusion methods revealed that nanocurcumin has more significant antimicrobial activities, compared to curcumin against the selected bacteria and fungal strains (e.g., *Penicillium notatum* and *Aspergillus niger*). This finding could be attributed to the smaller particle size and higher water solubility of nanocurcumin (without any modifications in its structure) [16].

Based on the present findings, the antifungal activity of dendrosomal nanocurcumin was at least twice as good as curcumin against *C. albicans* and non-*C. albicans* species. In line with the current research, better solubility and bioavailability of nanocurcumin and its marginal advantage over curcumin in combating some bacterial strains have been reported in different studies [16, 17].

According to the literature, curcumin is well tolerated by the body even at high doses [16, 17]. However, this agent has low bioavailability and its uptake is limited to epithelial cells of the intestine [18], resulting in a decline in systemic exposure and pharmacological activity. Although in the present study, pretreatment (5 mg/kg) and some post-treatment (10, 20 mg/ml) doses did not result in a significant decline in fungal burden in systemic candidiasis, 40 mg/kg of dendrosomal curcumin significantly decreased the fungal load, compared to the control group.

Dendrosomal nanocurcumin is probably absorbed more easily into the bloodstream

than curcumin. In line with the present study, Sharma *et al.* (2010) reported that 100 mg/kg of curcumin was unable to decrease the fungal load. However, its combination with piperine (an inhibitor of hepatic and intestinal glucuronidation) significantly eradicated the fungal load in the kidney [10].

In the present study, most colonies were recovered from the kidney, although other organs were affected, as well. In a previous study on systemic candidiasis in a dog model, lungs were found to be the most affected organs with the highest fungal burden. The observed discrepancy might be attributed to the use of different animal models, pharmacodynamic and pharmacokinetic factors, and timing and dosage of antifungal regimens. Histopathological findings also confirmed the accuracy of organ culture results, as no fungal structure was found in the group receiving 40 mg/kg of nanocurcumin.

In conclusion, the gathered data suggested that dendrosomal nanocurcumin is an effective antifungal agent with good efficacy against disseminated candidiasis. However, further studies are required to evaluate the effect of dendrosomal nanocurcumin on other fungal infections. Also, this agent could be helpful for the prevention of fungal infections, such as candidiasis, particularly in high-risk patients.

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Authors' Contributions

F.K. and E.B. designed the research, F.K and J.A. supervised the study, and F.K. and J.A. edited the final manuscript. SJ.E. and G.H. performed the *in vitro* tests, while F.K. and E.B. performed the *in vivo* tests.

Conflicts of Interest

The authors declare no conflicts of interest.

Financial Disclosure

There was no financial interest related to the materials of the manuscript.

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