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Title of the Abstract: Growth and Pathogenicity Modulation by Cinnamaldehyde and its Derivatives in *Candida albicans*

Abstract

Candida is the most common human fungal pathogen and the cause of invasive candidiasis, the fourth leading cause of nosocomial bloodstream infections. It colonizes mucosal surfaces of the oral and vaginal cavities and the digestive tract and is also able to cause a variety of infections. Although many antifungal agents are avilable they all are inherent toxic and present serious side effects. Research on plant derived molecules has accelerated in recent years due to their low inherent toxicity and side effects. Cinnamaldehyde occurs naturally in the bark of Cinnamon trees and other species of genus *Cinnamomum* like camphor and cassia. It is primarily used in the flavor and fragrance of various types of foods. Cinnamaldehyde is effective in inhibiting growth of yeast and filamentous moulds and is tentatively thought to act by inhibiting ATPases, cell wall biosynthesis, and by changing membrane structure. In the present study we have explored the effect of four natural derivatives of cinnamaldehyde; α-methyl cinnamaldehyde (MECD), *o*-methoxy cinnamaldehyde (MOCD), 4-hydroxy-3-methoxy cinnamaldehyde (HMCD) and 3, 5-dimethoxy- 4-hydroxy cinnamaldehyde (HDMCD), along with cinnamaldehyde against 93 fluconazole-sensitive and 22 fluconazole-resistant *Candida* strains. To better understand the effect of test compounds on growth and pathogenicity of *Candida* we have investigated with the following objectives: a). Determination of MIC and study of growth curve b). Synergistic effect. c). Time kill kinetics. d). (Disc diffusion assay & Spot assay). e). Yeast to hyphal transition. f). Proton extrusion by plasma membrane ATPase g). Measurement of Intracellular pH. h). Microscopic analysis i). Proteinase and Phospholipase secretion. j). Cytotoxic effect. k). Ergosterol biosynthesis. l). Resistance of *Candida* cells to H_2O_2 m). Assay of glutathione, TBARS and oxidative stress related enzyme activities. The MIC_{90} of test compounds against the sensititive and resistant isolates of different *Candida* isolates ranged 50-500 µg/ml. In synergy experiment, it was observed that test compounds showed significant synergism with fluconazole in most of the cases. Disc diffusion assay showed that the yeasts were highly sensitive towards the test compounds. The drug sensitivities for different *Candida* isolates tested by spot assay also revealed that cells show increased sensitivity to test compounds. In growth curve studies it was noticed that increase in

concentration of test compounds leads to significant decrease in growth with suppressed and delayed exponential phases. Time kill assays demonstrated that increase in concentration of test compounds leads to significant killing activity. Inhibition of dimorphism by cinnamaldehyde and its derivatives was observed both in terms of percentage of process induction (% hyphae formed) and hyphal length. In the absence and presence of glucose, the average proton extrusion inhibition caused by test compounds ranged 47%-83% & 25%-54%. Treated cells showed increase in internal acidification. Confocal microscopy results indicated that the mechanism of the drug involves a primary lesion on the cell membrane. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) micrograph of treated cells showed extensive breakage in the cell wall and cell membrane. The proteolytic activity of secreted proteinases and lypolytic activity of secreted phospholipases of tested *Candida albicans* isolates was significantly decreased in presence of sub-MIC concentrations of the test compounds. High cytotoxic effect or growth reducing effect of cinnamaldehyde and its derivatives is seen on candidal cells at MIC_{90} values for short term exposure. The average decrease in ergosterol content for cells grown in MIC of test compounds ranged 40-81%. In resistance to oxidative stress assay significant dose dependent synergistic inhibition was observed with all the five compounds on cells surviving under H_2O_2 induced oxidative stress. The test compound treated cells showed increase in LPO content and increase in the level of primary defence enzymes (SOD & Catalase), while as the level of GSH and other secondary defence enzymes decreased. This work reveals that plant derived molecules used in this study are found to be effective anticandidal agents against various *Candida* isolates. α-methyl cinnamaldehyde is found to be most active and it may be suggested that the substituents on the side chain have greater effects as compared to unsubstituted and ring substituted ones on antifungal activity. Immediate part of this antifungal activity may be originating from inhibition of PM-ATPase and drop of pHi which may inhibit ergosterol biosynthesis. This eventually leads to damage of membrane and cell wall as visible in SEM and TEM results. These essential oil components are also found to substantially inhibit key pathogenicity factors: Hydrolytic enzyme secretion and morphogenesis. Increased ROS and their insufficient detoxification cause the redox imbalance inside the treated cells leading to killing. These results taken together with limited toxicity of cinnamaldehyde make them eligible for further development as antifungals.