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Title of thesis: **"Effect of ferulic acid and its natural derivatives on growth and pathogenicity of Candida spp".**

ABSTRACT:

Infections caused by opportunistic yeasts are increasingly contributing to morbidity and mortality because of AIDS, invasive hospital procedures and increasing use of medical implants. Medical procedures like organ transplantations, prolonged chemotherapy, radiotherapy, metabolic diseases like diabetes mellitus and debilitating diseases like AIDS, tuberculosis further dispose towards opportunistic yeast infection. Candidosis is most common yeast infection and the species associated with it are: *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, *Candida gulliermondii* and *Candida krusei* in order of prevalence. Large numbers of factors are associated with virulence of *Candida albicans* such as functioning of PM-ATPase, secretion of proteinases and phospholipases, morphological transition between yeast and hyphae, and ability to survive under oxidative stress. Both families of antifungal are besetted with resistance and high host toxicity.

AIM OF THE STUDY:

In this study, we investigate and compare the effect of ferulic acid and its two natural derivatives: *para*-coumaric acid and isoeugenol, differing in substitution on benzene ring and polar surface area with the objective of correlating antioxidant power and lipophilicity of these molecules with antifungal activity. All experiments have been performed on several isolates and species, both standard and clinical to ensure that results obtained apply across the board and are not species specific. Potency of molecules has been explored by performing experiments at several concentrations and on both sensitive and resistant isolates/ species. To understand the effect of ferulic acid and its derivatives on growth and pathogenicity of *Candida* we have investigated with the following objectives: a). Determination of minimum inhibitory concentration (MIC) and study of growth at different concentrations. b). Effect of test compounds in solid media (disc diffusion assay & spot assay). c). Cytotoxic effect. d). Microscopic analysis (Confocal imaging, SEM & TEM). e). Ergosterol biosynthesis. f). Proton extrusion by plasma membrane ATPase in presence and absence of glucose. g). Measurement of Intracellular pH. j). Proteinase and Phospholipase secretion. k).Yeast to hyphal transition. l). Resistance of *Candida* cells to H_2O_2 induced oxidative stress by colony forming units count assay. m). Assay of glutathione, TBARS and oxidative stress related enzyme activities.

RESULTS:

Thirty three (33) fluconazole-sensitive and 11 fluconazole-resistant *Candida* strains have

been used in this study by employing standard protocols. Results obtained demonstrated that the ability to kill *Candida* species is concentration dependent. No significant difference was obtained between various isolates and species whether standard, clinical fluconazole sensitive or resistant. Average MIC of three tested molecules against all 44 isolates was 700 µg/ml for ferulic acid, 300 µg/ml for *para*-coumaric acid and 150µg/ml for isoeugenol. Two properties of these molecules: antioxidant activity and lipophilicity could be correlated with their antifungal activity as isoeugenol has both antioxidant groups (hydroxy and methoxy) on benzene ring and it has lowest polar charge area and is thus most active. It appears that by virtue of its increased lipophilicity, it incorporates in membranes in higher amount. Ferulic acid, *para*-coumaric acid and isoeugenol at their highest MIC (against any strain) of 800 µg/ml, 450 µg/ml and 250 µg/ml caused 7.3%, 8.0% and 14.0% hemolysis of fresh human RBC as compared to 20.0% haemolysis caused by fluconazole (32 µg/ml).

CONCLUSION:

Antifungal activity of plant derived phenylpropanoids: Ferulic acid, *para*-coumaric acid and isoeugenol have been investigated against several *Candida* isolates. All three natural antioxidants were found to be effective both in solid and liquid media with MIC_{90} of ferulic acid, *para*-coumaric acid and isoeugenol ranging from 500-800 µg/ml, 200-450 µg/ml and 100-250 µg/ml respectively. Membrane associated properties: ergosterol biosynthesis and PM-ATPase promoted H^+-extrusion and pHi were found to be drastically affected. Yeast to hyphal transition and secretion of proteinases and phospholipases was found to be significantly suppressed by three antioxidants. At tested concentrations, these three antioxidants did not confer any oxidative stress combating advantage on host cells and lead to significant alteration in oxidative stress combating enzymes. Ferulic acid, *para*-coumaric acid and isoeugenol are found to be less toxic against human RBC's as compared to standard antifungal: fluconazole. Finally plant derived antioxidants: Ferulic acid, *para*-coumaric acid and isoeugenol are found to be very active anti-candidal agents. Isoeugenol was most effective followed by *para*-coumaric acid and ferulic acid. This order of effectiveness would be co-related with their polar charged area. These molecules did not confer any oxidative advantage on yeast cells and severely affected growth and tested pathogenicity parameter. Above facts taken together with their low toxicity make ferulic acid, *para*-coumaric acid and isoeugenol potential molecules for development as clinical antifungal.