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Title of thesis: **"Effect of Curcumin on growth and pathogenicity of candida spp".**

ABSTRACT:

Fungal diseases in humans have increased significantly with increase in population of immunosuppressed and debilitating patients. Among all *Candida* species four most frequently isolated from human infections are *Candida albicans*, *C. tropicalis*, *C. glabrata* and *C. parapsilosis*. *C. albicans* with frequency of about 50% is the most abundant and significant species. Current line of antifungal: Polyenes and Azoles present severe side effects and resistance to them is frequently observed. These limitations emphasize the need to explore and develop new and more effective antifungal with less toxicity. Natural products are attractive prototype for this purpose. Curcumin is the principle curcuminoids of popular Indian spice turmeric and in limited studies it has been shown to have antifungal activity. Curcumin has been shown to enhance antifungal activity of amphotericin B and fluconazole against several *Candida* species and to suppress dimorphism in *Candida albicans.* Mode of antifungal activity of curcumin and its effect on pathogenicity markers of *Candida* has not been reported as yet. In eukaryotic systems curcumin has been shown to inhibit several P-type ATPases: Na^+/K^+-ATPase, H^+/K^+-ATPase and Ca^+-ATPase and has been reported to be a strong antioxidant.

AIM OF THE STUDY:

Using standard protocols and using 41 fluconazole-sensitive and 11 fluconazole-resistant *Candida* strains we have systematically explored effect of curcumin on several growth and pathogenicity parameters. Growth studies in liquid and solid media have been done to check visible effect of curcumin on MIC, and immediate cytotoxcity. Curcumin is reported to affect several P-type-ATPases, accordingly effect of curcumin on PM-ATPase of yeasts in investigated by measuring H^+- extrusion and changes in intracellular pH. Ergosterol measurements are done as it is affected by changes in membrane integrity. Hydrolytic enzyme secretion and yeast to hyphal transition as affected by sub-MIC of curcumin has been

explored as these pathogenicity attributed are influenced by ROS which is known to be affected by curcumin. Direct effect of curcumin on yeast cells has been visualized by confocal and scanning electron microscopy. Curcumin is strong antioxidant; accordingly its effect on oxidative stress combating ability of yeast cells has been examined by externally imposed oxidative stress by employing H_2O_2. Antioxidant combating status of yeast cells in presence of sub –MIC of curcumin has been assessed by measuring lipid peroxidation and GSH content. Activities of enzymes related with oxidative stress superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase and glutathione -S -transferase have been measured to gain better insight. To check the toxicity of curcumin its effect on hemolysis of human RBC has been examined.

RESULTS:

Curcumin is found to be effective both in solid and liquid media with MIC_{90} in the range of 250-500 µg/ml for sensitive and 200-650 µg/ml for fluconazole resistant strains. No significant or systematic difference was seen between various *Candida* species and isolates whether sensitive or resistant to fluconazole. Membrane associated properties: ergosterol biosynthesis, PM-ATPase promoted H^+-extrusion and pHi were found to be drastically affected. Inhibition of PM-ATPase by curcumin corresponded well with DES and DCCD suggesting this pump to be the primary target of curcumin. Yeast to hyphal transition and secretion of proteinases and phospholipases was found to be significantly suppressed by curcumin. At tested concentrations, curcumin did not confer any oxidative stress combating advantage on yeast cells but lead to significant increase in LPO, decrease in GSH and alteration in activities of oxidative stress combating enzymes. Curcumin at 650 µg/ml is found to be less toxic against human RBC as compared to 32 µg/ml fluconazole.

CONCLUSION:

Finally curcumin is found to be very active anti-candidal agent with PM-ATPase as most likely primary target, it did not confer any oxidative stress combating advantage on yeast cells and severely restricted growth both in liquid and solid media. Important pathogenicity attributes of proteinases, phospholipase serection and yeast to hyphal transitions were significantly suppressed by even sub- MIC of curcumin. Above finding taken together with its low toxicity make curcumin a potential antifungal of clinical interest.