Rashmi Ojha

Name of Supervisor: Dr.Luqman Ahmad Khan

Name of CoSupervisor: Dr.Nikhat Manzoor

Department of Biosciences

Faculty of Natural Sciences

Jamia Millia Islamia

Title of Thesis: "Effect of Vitamin C on growth and pathogenicity of *Candida albicans*"

Thesis Abstract

Candida infections, both superficial and systemic have increased dramatically over the last three decades. A number of factors have been implicated to be associated with the virulence properties of *C. albicans.* Vitamin C or Ascorbate is known to carry out a number of biochemical functions most of which are a consequence of its ability to donate one or two electrons. There are a number of situations both natural and interventional where *Candida* is exposed to varying concentrations of vitamin C. Effect of this exposure has been reported variedly from being assisting candidal survival to being antifungal. To better understand the effect of vitamin C on growth and pathogenicity of *Candida*, following objectives have been investigated: a). Determination of minimum inhibitory concentration (MIC) and study of growth curve at different concentrations. b). Cytotoxic effect of Vitamin C (CytoScan WST-1 Cell Cytotoxicity Assay). c). Proteinase and Phospholipase secretion. d). Proton extrusion by plasma membrane ATPase in presence and absence of glucose (autotitrometer), isolated H^+ ATPase activity. e). Ergosterol quatitation. f). Yeast to hyphal transition (inverted microscopy). g). Resistance of *Candida* cells to H_2O_2 induced oxidative stress by cfu count assay. h). Assay of glutathione, TBARS and oxidative stress related enzymatic activities.

No growth promotion was observed at any concentration of vitamin C. No change in growth pattern was seen up to 6mg/ml vitamin C. Growth pattern was affected from 8mg/ml concentration onwards. Tetrazolium assay reinforced the results above. No immediate cytotoxic

effect was noted for concentrations up to 6mg/ml. Minimum inhibitory concentration (MIC_{80}) of vitamin C was determined to be 30mg/ml against three *C. albicans* strains by broth dilution method. Important virulence attribute of proteinase and phospholipase secretion are found to decrease at high concentrations of vitamin C. glucose stimulated H^+extrusion was significantly inhibited by vitamin C, which indicates that less growth could be due to less nutrient import. Ergosterol content was significantly reduced by vitamin C, 47% reduction was seen at MIC_{80} indicates that ergosterol biosynthesis is not the primary site of action of vitamin C. Both yeast to hyphal transition and hyphal length were strongly inhibited by vitamin C even at low concentrations. Decrease in hyphae could be correlated with anti-oxidant activity of vitamin C. vitamin C treated cells showed strong resistance to H_2O_2 induced killing. This resistance was more at low vitamin C concentration on stochiometric basis, indicating that antioxidant effect is more prominent at low concentrations. It appears that at high concentrations deleterious effects of vitamin C override the benefits of antioxidant effects. Vitamin C decreases lipid peroxidation in lower concentration ranges. Superoxide dismutase activity increases with increase in vitamin C. no corresponding increase in catalase was observed. It appears that deleterious effects of vitamin C at high concentrations are countered by high H_2O_2 through hormosis effects. Low GSH and glutathione reductase levels at high vitamin C could be explained by inhibition of glucose 6P dehydrogenase. Clinical implication of the study for direct application of vitamin C is that it should be employed only at high concentrations. Low concentrations of vitamin C may make *Candida* more resistant to oxidative stress induced killing.