6. SUMMARY

The purpose of the present investigation was aimed to produce chicken egg yolk antibodies against the antigen of *Candida albicans*, *C.tropicalis*, *C.krusei* and to test their potential both *in vitro* and *in vivo* to control the virulence properties of *Candida* sp with respect to oral candidiasis.

The antigens required for the generation of antibodies in the chicken was prepared from the standard strains from MTCC. The 21 weeks old white leghorn chickens were immunized individually for each antigen, their eggs collected and the antibodies were purified. An increase in antibodies against *Candida* sp., was detected in the serum of immunized chicken 7 days after the initial immunization by ELISA. This humoral immunity reached a peak at about 45 days. However, in the egg yolk, the antibody level increased 2 weeks after the initial immunization and persistent increase was observed till 56th day after which the antibody level remained stable till 175th day of immunization. Egg laying capacity of the chickens after immunization was monitored. Protein concentration and total IgY concentration were estimated. The average concentrations of protein were found to be 40.49±0.17mg/ml for *C.albicans*, 41.16±0.67mg/ml and 41.16±0.71mg/ml for *C.tropicalis* and *C.krusei* respectively. A high molecular weight protein band (180 KDa) was visualized in SDS-PAGE which showed the purity of IgY. Specific reactivity of anti-*C.albicans* IgY, anti-*C.tropicalis* IgY and anti-*C.krusei* IgY with antigen of *C.albicans*, *C.tropicalis*, *C.krusei* was determined by the formation of agglutination in the rapid slide agglutination test.

The level of specific IgY titre in egg yolk was estimated by Indirect ELISA and it was found to be 1:50000 on 56th Day and the titer were maintained with booster doses. IgY purified from both immunized and unimmunized eggs were tested for their specific reaction against antigen of *Candida* sp., by ELISA. The specific antibody titre in the immunized egg yolk was significantly higher than the unimmunized egg yolk.

The stability of IgY, when incubated at different conditions, was assessed where the IgY retained its stability at 4°C, 25°C, 37°C and 60°C, at pH ranges between 4 and 9 when compared to the untreated control. IgY was able to retain 80% of its activity in trypsin treatment in contrast to pepsin treatment where it retained 30% of activity in its native form.

IgY antibodies were also evaluated for its cytotoxic effect on KB cell lines and results revealed that they were nontoxic to human cells even at increasing concentrations. The anti-*C.albicans*, anti-*C.tropicalis*, anti-*C.krusei* IgY was tested for its efficacy to inhibit the cell growth of *Candida* sp., by growth inhibition assay. The growth of *Candida* with specific IgY
showed significant reduction when compared with control IgY. The ability of anti-\textit{Candida} IgY to inhibit the adhesion of \textit{C.albicans}, \textit{C.tropicalis}, \textit{C.krusei} to KB cell lines was evaluated by adhesion inhibition assay and they significantly ($P < 0.05$) reduced the adherence of \textit{Candida} sp., to the cell lines in a dose-dependent manner, when compared with the control IgY treated group. The adhesion inhibition ability was increased in the condition where the candidal cells were pre-exposed to the IgY.

Further, the efficacy of anti-\textit{C.albicans} IgY, anti-\textit{C.tropicalis} IgY, anti-\textit{C.krusei} IgY and consortium were evaluated for their neutralization potential in immunosuppressed mice models. Consortium group were able to show significant impact on relative body weight of the infected mice, reduced the tongue lesions and showed lesion score of $0.3 \pm 0.01$ at day 7 after infection which were significantly less when compared with the control group which showed lesion score of $1.3 \pm 0.04$. They showed an effective reduction of the \textit{Candida} load in the mice challenged with the inoculums from the oral swab and given the viable count of $0.7 \times 10^5$ at day 7 which were significantly lower than the initial load at day 1. Moreover, disseminated infection was also significantly reduced. The microscopic examination of progression of infection was studied histopathologically using PAS staining and results revealed that the fungal load was significantly reduced in consortium treated group when compared with control IgY group.

The commercially available mouth rinses were evaluated for their anti-candidal activity and all of them were shown activity with significant differences. These egg yolk antibodies were used to formulate an oral mouth rinse and its activity was assessed. The mouth rinse containing 1\% of anti-\textit{C.albicans} IgY, \textit{C.tropicalis} IgY, \textit{C.krusei} IgY showed a reduction of \textit{Candida} sp., growth by 91\%, 89\%, 90\% respectively; and the mouth rinse containing 0.5\% anti- \textit{Candida} consortium IgY was able to bring a 95\% reduction of \textit{Candida} growth. So, by interfering with the colonization and inhibiting the development, it is possible to prevent its initiation of infection with respect to oral candidiasis.

These egg yolk antibodies were used for formulation as an oral mouth rinse composition and their activity was assessed. They showed a significant reduction in \textit{Candida} growth. Hence, a novel oral formulation incorporating them into consortium could form a platform for the development of a novel drug which will be a reliable, safe, and economic with less or no side effects against Oral Candidiasis as a prophylactic and therapeutic agent. It can be concluded that further human trials on the use of these antibodies can confirm its efficacy and allow its commercialization for human use.