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INNATE AND CELLULAR IMMUNITY STUDY ON SOME IMMUNOLOGIC PARAMETERS IN MICE VACCINATED WITH COMBINATION OF CANDIDA ALBICANS CELL WALL MANNOPROTEIN AND BRUCELLA REV.-1 VACCINE

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ABSTRACT
A study was carried out to investigate, the immunomodulatory effect of the Mannoproteins of *Candida albicans* cell wall on the immune response of mice vaccinated with *BrucellaRev-1* vaccine and considered as biological immunomodulators. The study included eight groups; the first four subgroups were I: treated with distilled water. II: injected subcutaneously with a dose of (300 µg /ml) cell wall mannoprotein only, III: injected subcutaneously with a dose of (200 µg /ml) cell wall mannoprotein only, IV: mice treated with brucella-*Rev-1* vaccine only. The V, VI were treated with combination of Brucella Rev-1 vaccine and cell wall mannoprotein while VII and VIII groups were injected with the immunosuppressive drug prednisolone prior to the forthcoming treatment 5 days. All these treatments were carried out on day 1, and then the mice were sacrificed on day 8 to estimate serum phagocytic activity by Eliza assay and on day I4 for estimation of lymphocyte transformation by MTT assay. The results demonstrated a clear immunomodulatory effect of the mannoproteins of Candida albicans cell wall (improvement of non-specific, and cellular immune response) of the treated mice vaccinated with *Brucella-Rev-1*. The phagocytic activity (showed a significant increased ($P \leq 0.05$ and $P \leq 0.01$) in serum level in Mannoproteins-treated vaccinated mice in comparison with negative and positive controls groups, and group VI showed a highest increased. In lymphocyte, transformation by MTT assay, an increased index was significantly increased ($P \leq 0.05$ and $P \leq 0.01$) in Mannoproteins-treated vaccinated mice in comparison with negative and positive groups.

KEYWORDS: Mannoproteins, Brucella Rev -1, NTB, MTT assay, Mice.

INTRODUCTION

Brucellosis is one of the most important zoonetic diseases worldwide, resulting in serious economic losses and public health issues. It is caused by intracellular Gram-negative bacteria of the genus *Brucella*, which are responsible for a debilitating disease in humans and a chronic infection in domestic animals (1). In this regard, biological immunomodulator are materials that mediate the effectors mechanisms of the immune system through immune stimulation to a given antigen or potentiate the effectiveness of a vaccine (2). Recent advances in immunology have led to design vaccines to maximize activation of the humoral or cell-mediated branches of the immune system (3). *Candida albicans* has been one of the fungal species that share the interest of investigators in the field of immune modulation. Mannoproteins purified from *C. albicans* and administered to mice before or during immunization with viable *C. albicans* developed a significant increased in both humoral and cellular immune response (4). Furthermore, (5) have demonstrated that immunization with mannan (a mannoproteins fraction) and mannoproteins derived from digested cell walls of *C. albicans* induced resistance to a systemic candidiasis. (6) Were purified cell wall mannoproteins of intact yeast using a simple treatment of yeast with mercaptoethanol and sodium dodecyl sulfate followed by Concanavalin A chromatography. They are found that Cell wall mannoproteins to be the main cause of adherence of *C.albicans*to epithelial cells in the first step of an infection process.(7)was study the immunomodulatory effect of the Mannoproteins of *Candida albicans* cell wall on the immune response of mice vaccinated with *BrucellaRB51* vaccine and considered as biological immunomodulators. The results demonstrated a clear immunomodulatory effect of the mannoproteins of Candida albicans cell wall (improvement of non-specific, and cellular immune response) of the treated mice vaccinated with *Brucella-RB51*.

MATERIAL AND METHODS

There were eight groups in this experiment, which was designed to evaluate the immunomodulator potential of *C. albicans* cell wall mannoproteins in mice vaccinated with *Brucella* Rev-1 vaccine. The total number of animals in these groups was 200 mice (25 mice in each group).

- Group I: mice were injected subcutaneously with a single dose (0.2 ml) of deionized distilled water in day 1.
- Group II: mice were injected subcutaneously with a high dose (300 µg /ml) of mannoproteins in a total volume (0.2 ml) day 1.
- Group III: mice were injected subcutaneously with a moderate dose (200 µg/ml) of mannoproteins in a total volume (0.2 ml) day 1.

- Group IV: mice were injected subcutaneously with a single dose*B*rucella Rev-1 vaccine in day 1.
- Group V and VI: mice were injected subcutaneously with a single dose of *combination of B*rucella Rev-1 vaccinated moderate and high dose respectively in day 1.
- Group VII and VIII: mice were injected subcutaneously with a single dose of prednisone 5 days prior to the combination of the *Brucella* Rev-1 vaccine and moderate and high dose of mannoprotein in day 1.Nitro blue tetrazoliumtestwas carried out for evaluation of phagocytic activity percentage according to a method presented by (8) for all the mice. The method is outlined in a microtiter plate of 96 wells, a peripheral Blood Leucocytes 1^{χ} 10^{6} cell/ well (100 ul Blood, with 175 ul MEM media) was isolated from peripheral blood and add to each well, NBT 1 g / liter was added as indicator for oxygen-dependant phagocytosis by peripheral leucocytes. ELIZA evaluated results at 650-wave length. The phagocytic activity percentage, which was calculated according to the following equation: (9).

$$\text{Phagocytic activity percentage (\%)} = \left(\frac{A - B}{B}\right) \times 100$$

A = Treated groups; B = Negative control group.

The procedure of (8) was followed to assess the lymphocytestransformation activity percentage after an *in vitro* stimulation specific antigen (brucelline) by Lymphocyte Transformation test (MTT). The procedure of MTT assay(3-(4, 5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)measured by Elisa at 490-wave length.

1. The Lymphocyte transformation percentage which was calculated according to the following equation: (9),

$$\text{Phagocytic activity percentage (\%)} = \left(\frac{A - B}{B}\right) \times 100$$

A = Treated groups; B = Negative control group.

RESULTS AND DISCUSSION

The results of NBT index were given in table 1, while the treatment efficiency for each group of treated mice was presented in figure 2. All groups of mice showed different significant increases in the NBT index which represent the phagocytic activity% (195.2%, 103%,149% , 144%, 295% ,183% and 187%, respectively) as compared to group I (0%), which was injected with distilled water (control group). The best treatment efficiency was recorded in-group VI (295%), which included mice that were treated with combination of 300 µg/ kg of Candida albicansmannoproteins and*Brucella* Rev-1 vaccine while lowest treatment efficiency was recorded in-group III (103%) included mice that were treated with 200 µg/ kg of Candida albicansmannoproteins only.

TABLE 1: Nitro blue tetrazolium (NBT) index in mice vaccinated with *Brucella* Rev-1 vaccine and treated with *C. albicans* cell wall mannoproteins

Groups	NBT OD (mean ± S.E. %)*	Phagocytic activity%
I	1.01±0.15 ^{c}	0%
II	2.96±1.03 ^{b}	195.2%
III	2.05±0.98^{b}	103 %
IV	2.52±0.84 ^{b}	149 %
V	2.47±0.81^{b}	144 %
VI	4 .00 ^{a}	295 %
VII	2.88±1.11^{b}	183 %
VIII	2.90±1.09 ^{b}	187 %

*Different letters: Significant difference (P≤0.05) between means of the same column.

**The comparison is between means of the two columns (horizontal comparison).

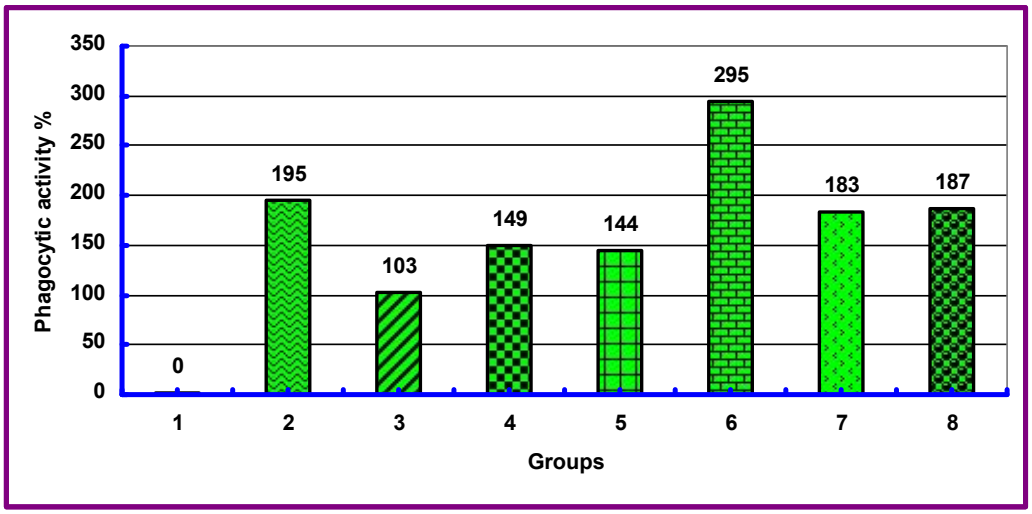

FIGURE 1: Treatment efficiency of NBT index in mice vaccinated with *Brucella* Rev-1 vaccine and treated with *C. albicans* cell wall mannoproteins.

Results of NBT index, which showed a significantly increased percentage in treated mice, are also in favour of such agreement, and IFN-γ can activate murine macrophages to destroy intracellular bacteria. Nitro blue tetrazolium reduction by polymorphonuclear cells may require oxidative metabolism by the hexose monophosphate shunt, and is impermeable to cell membrane, but it enters the cell during the process of phagocytosis, and it is reduced by diphorase activity within phagosome (10,11).

Phagocytic activity by reduction of nitro blue tetrazolium (NBT) to insoluble blue Formozan granules occurs during the stimulus-induced respiratory burst of mature granulocytes, Nitro blue tetrazolium (NBT) test addition of the yellow NBT dye to plasma results in the formation of a NBT–heparin or NBT–fibrinogen complex, which may be phagocytosis by neutrophils (12). Cell wall components from *Candida albicans* were compared to intact cells for their ability to induce natural cytotoxic immunoeffectors in the peritoneal cavity of mice, a soluble mannoprotein extract (MP) stimulated the generation of peritoneal; the NK and macrophage effectors generated by these materials had similar functional and phenotypic properties(13). Intracellular conversion of nitro blue tetrazolium (NBT) toformazan by immunomodulator was used to measure thegeneration of reactive oxygen species and the amount of formazanformed was measured (14).Normal neutrophils show little incorporation of the complex unless they are 'stimulated' to phagocytic activity, e.g. by the addition of endotoxin. This technique was used to measure the degree of 'stimulation' of untreated cells or their capacity for phagocytosis after stimulation.Stimulated neutrophils incorporate the dye complex into phagosome and, after lysosomal fusion, intracellular reduction results in the formation of blue insoluble crystals of formazan.The percentage of phagocytic cells may be determined using a light microscope or, as described below, the total dye reduction may be quantified spectrophotometrically after alkaline DMSO, which reacts with NBT to produce colored diformazan, formed dioxin extraction Super oxide free radical. The methanolic extract of *Portulacaoleracea*scavenges superoxide radical and thus inhibits formazan formation (15). Attachment of the phagocyte to the organism is an important interaction, which may determine whether the uptake is subsequently occurred or not, and whether the killing mechanisms are triggered or not. The attachment can be mediated by two entities; lectins on the organism and lectins on the phagocyte. Of a particular interest in this respect are the complement receptors CR3, P150, and the related molecule LFA-1, which have multiple binding sites specific for different carbohydrate moieties (16). They can bind to B-glucans and to the lipopolysaccharide endotoxin of Gram-negative bacteria and this can lead to complement deposition via the alternative or classical pathways (17). Although macrophages and monocyte possess killing mechanisms in the resting state, these mechanisms can be enhanced, and new mechanisms can be expressed when they were activated. Activation can occur through exposure to microbial products (i.e. *C. albicans* cell wall mannoproteins) and/or materials extracted from plants (i.e. ethanol and aqueous extracts of neem seeds). Such picture is enhanced by the findings of the present study and confirmed by other investigators (18,19). Such immunomodulators can cause a direct activation of phagocytes, or indirect activation through triggering cytokine release from them. Once the organism is internalized, it is exposed to an array of killing mechanisms; oxygen-dependent killing mechanisms (this pathway is also called reactive oxygen intermediates; ROIs) and reactive nitrogen intermediates (RNI) (16). The latter pathway has recently been discovered, and it may be particularly important, because it results in the formation of nitric oxide (NO), which is toxic for bacteria (19). For optimal expression of this mechanism, macrophages need both activation by IFN-γ and triggering by TNF-alpha, and this mechanism enables murine macrophages to kill mycobacterium and probably other intracellular bacteria like *Brucella* (17). Oxygen-independent killing mechanisms are also a further pathway, which may be

more important than was previously thought, because many organisms can be killed by cells from patients who cannot produce ROIs (20). It is expected that the isolated mannoproteins are effective immunomodulators. These are in agreement with this conclusion, several researchers suggested the potentianal use of *C. albicans* cell wall mannoproteins in this line of experimental immunology by using different laboratory approaches and animals (10 ,21 , 22,6 ,23 , 24 ,25,7).The results of lymphocyte transformation index were given in table 2, while the treatment efficiency for each group of treated mice were presented in figure 2. mice showed different significant increases in the MTT index which represent the lymphocyte transformation index % (122%, 100%,133% , 144%, 255 % ,122% and 122%, respectively) as compared to group I (0%), which was injected with distilled water (control group). The best treatment efficiency was recorded in-group VI (255%), which included mice that were treated with 300 µg/ kg of Candida albicansmannoproteins prior to the vaccination with*Brucella* Rev-1 vaccine while lowest treatment efficiency was recorded in-group III (100%) included mice that were treated with 200 µg/ kg of Candida albicans mannoproteins only.

TABLE 2: Lymphocyte transformation index in mice vaccinated with *Brucella* Rev-1 vaccine and treated with *C. albicans* cell wall mannoprotein.

Groups	Lymphocyte Transformation Index (mean ± S.E.; %)*	Lymphocyte Transformation activity%
I	0.09±0.02 ^{e}	0%
II	0.20±0.01^{d}	122%
III	0.18±0.02^{d}	100%
IV	0.21±0.007^{c}	133%
V	0.22±0.01^{b}	144%
VI	0.32±0.01 ^{a}	255%
VII	0.20±0.01^{d}	122%
VIII	0.20±0.10^{d}	122%

* Different letters: Significant difference (P≤0.05) between means of the same column.
** The comparison is between means of the two columns (horizontal comparison.

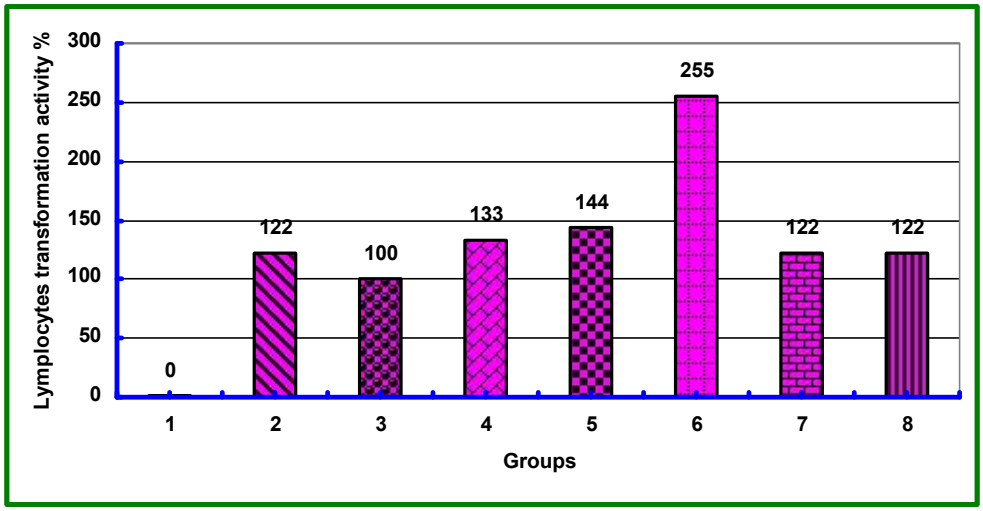

FIGURE 2: Lymphocyte transformation index in mice vaccinated with *Brucella* Rev-1 vaccine and treated with *C. albicans* cell wall mannoprotein.

The lymphocyte transformation test (LTT) has been proven useful especially in the diagnosis of drug-induced allergic disorders. An in vitro test is because lymphocytes, which have been sensitized by a certain antigen, transform into blasts and proliferate when they are again exposed to this antigen. This proliferation is determined by measurement of the incorporation of [3H]-thymidine or bromodeoxyuridine into replicating DNA. The test has the advantage over skin tests of avoiding re-exposure of individuals, and it was, therefore, hoped that it might also help to diagnose metal allergies and especially sensitization toward beryllium (26). However, the LTT measures only the sensitization of lymphocytes, but not the effector reaction, i.e., there may be positive results in exposed individuals even in the absence of clinical symptoms. Different research groups for the evaluation of various cell-mediated immune reactions have applied the test. The principle of the LTT is because lymphocytes,

which have been sensitized by a certain antigen "memory cells", transform into blasts and proliferate when they are again exposed to this antigen. According to the different biological mechanisms occurring in a transforming cell, there are several chemical and physical methods to measure this transformation into blasts, as, for instance, determination of metabolic processes, biochemical alterations such as protein biosynthesis, or the synthesis of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA). The LTT, which measures the replication of DNA (27). The MTT [3-(4, 5-dimethyl-2-thiazolyl) -2, 5-diphenyl -2H- tetrazolium bromide] test has been used earlier on *Candida albicans* after treatment with heat, formaldehyde, hypochlorous acid or amphotericin B (28) and to determine the sensitivity of *Candida albicans* and *Aspergillus* spp . to therapeutic anti-fungal agents. (29) MTT-reduction was demonstrated to correlate with the viability of the antifungal agent-treated *Candida* cells on culture. (29, 28)also used the MTT-reduction to investigate the effects of anti-*Candida* monoclonal antibodies in vitro; that appears to be the only report based on the use of MTT for this purpose. That specific antibody mediates anti-*Candida* immunity *in vivo* by inhibition of adherence to host cells or surfaces and by the promotion of phagocytosis and intra-phagocytic killing. The MTT (3-[4, 5-dimethyl-2-thiazolyl] -2, 5-diphenyl -2H- tetrazolium bromide)-reduction method as a test of the viability of fungi was used to investigate the effect of complement, normal serum and immune serum on these two species of *Candida* that are of increasing importance as opportunistic pathogens. We report that normal rabbit serum or strain-specific, polyclonal anti- *Candida* rabbit antibody, with or without guinea pig complement, did not cause the reduction of total cell-mass or of the viability of either C. albicans or C. krusei, in vitro as determined by the MTT-reduction test. Complement alone without specific antibody, also, had no such effect on these two Candida species (The investigations described here with *C. albicans* and *C. krusei* and their strain-specific antibodies, were done to investigate the effect of sera containing strain-specific anti- *Candida* antibodies on the viability of *C. albicans* and *C. krusei, in vitro* . The tests were based on the capacity of viable cells to reduce MTT to formazan that was assayed by spectrophotometric quantization of optical density (OD) after its extraction with acid-propane, with the OD taken as a measure of the metabolic status and the total, viable mass of the *Candida* cells. (30).

REFERENCES

[1]. Vidya L. Atluri, Mariana N. Xavier, Maarten F. D. , Andreas B. D., and Renée M. T.(2011) . Interactions of the Human Pathogenic *Brucella* Species with Their Hosts.Annual Review of Microbiology 2011. Vol. 65: 523-541.

[2]. Farid A. Badria, B. R. Mikhaeil, Gala T. M., and Mohamed M.A. (2003). ImmunomodulatoryTriterpenoids from the oleogum Resin of Boswelliacarterii bird wood. Z. Naturforsch 58:505-516.

[3]. Grimble R.F., Grimble G.K., 1998. Immunonutrition: role of sulfur amino acids, related amino acids and polyamines. *Nutrition,* 14, 605-10.

[4]. Ronald E.G and Judith E.D. (1994) .Lack of effect of Candida albicansMannan on development of protective immune responses in experimental murine Candidiasis. Infection and immunity. :738-741.

[5]. ShigetoshiM.,MasahiroEndo,Toshiaki no-ue,Masahir - o kurasawa,Yokouno,HideharuSaito,Ikunoshin Kato, and KazutochTakesako(2000).Immunization with the Candida albicans membrane fraction and in combination with fluconazole protects against systemic fungal infections. Antimicrob.Agentschemother 44(2):243-247.

[6]. Farahnejad, R. M .; Frozandeh, M. ; Paknejad M.; KashanianS.andRajabi M,H. ,June 2005, Preparation and Characterization of a Monoclonal Antibody Against Mannoprotein of *Candida albicans*. *Issue* 3: 24(3): 146-151.

[7]. Al-jindeel T J (2011). The use of Cell Wall Mannoproteins of Candida albicans as immunomodulators in mice vaccinated with Brucella RB51. Al-Al Unbar University-Medicine Collage.

[8]. Zakaria, Z. A. ; Rofiee M. S. ; Teh L. K. ; Salleh M. Z.; Sulaiman M. R. and M. N. Somchit1(2011). Bauhinia purpurea leaves' extracts exhibited in vitroantiproliferative and antioxidant activities. African Journal of Biotechnology Vol. 10(1), pp. 65-74.

[9]. Perez-Serrano J, Denegri G, Casado N, RodrigueZ,Caaberio F(1997) In vivo effect of oral albendazolesulphoxide on development of secondary echinococcosis in mice . Int J parasitol 27: 1341-1345.

[10]. Mizutani, S. ; Endo, M.; Toshiaki, M. K. ; Yoko, H.S. ; Ikunoshin, K. and Takesako, K. (2000) . Immunization with the *Candida albicans* membrane fraction and in combination with fluconazole protects against systemic fungal infections. Antimicrob. *Agentschemother* 44:243-247.

[11]. Balwinder, D.S., Singh, G.K. and Chauhan, R.S. 2005. Affect of immuplusa herbal immunomodulator on paraspecific immune responses in chicks. Isah2005-Warsaw Poland, 2:60-64.

[12]. Kumar R., Singhal L.K., Singh B.p, Rana N., Singh D.D. and Chauhan R.S. (2003). Immuplus up regulates immune response to FMD vaccine in calves .Livestock international 7(1o):11-15.

[13]. Lucia S, Pierfrancesco M.,Maria B., Luciana T.,Francesco B., Antonio C., (1988). Cell Wall Components of *Candida albicans* as

Immunomodulators: Induction of Natural Killer and Macrophage-mediated Peritoneal Cell Cytotoxicity in Mice by Mannoprotein and Glucan Fractions. *J. Microbiology*: 134:1265-1274.

[14]. Angelica S. V, Craig D. A, Corneliu N. C, Rudolf I. S, Steven H. Z. (2001). Altered mitochondrial function and overgeneration of reactive oxygen species precede the induction of apoptosis by 1-O-octadecyl-2-methyl-*rac*-glycero-3- phosphocholine in p53-defective hepatocytes. Faseb J. 15-1739.

[15]. Sanja SD, Sheth NR, Patel NK, Patel D, Patel B.(2009) . Characterization And Evaluation of Antioxidant Activity of *Portulacaoleracea*. Int J PharmaPharmaceut Sci.; 1:74–84.

[16]. Kuby, J (2003*) Immunology, Text book, 3 $^{rd}_{Ed.}$, W.H. freeman and company U.S.A., New York.*

[17]. Zahid, I.R., Seng-hua, H. and chen-wenxiao, A. (2007). Adjuvant effects of saponins on animal immune responses (review). Department of Parasitology, Sindh Agriculture University, Tando jam, 70060, Pakistan.

[18]. Thakurataap, B.P., Mukherjees, H.T.K., Patra, A. and Bag, P.K. 2007. Antibacterial, ansecretory and antihemorrhagic activity of *Azadirachtaindica*used to treat cholera and diarrhea in india. *Journal of Ethanopharmacology,* 21:37.

[19]. Khan, S.A. and Aslam, J. (2008) . Study on the effect of neem (*Azadirachtaindica*) leaves smoke in controlling airborne bacteria in residential premises. *Current Research in Bacteriology,* 1:64- 66.

[20]. Takahashi, H. 2003. Antigen presentation in vaccine development. C*omparative Immunology,Microbiology and infectious Diseases,* 5:309-328.

[21]. Tansho, S. A.; Mizutani, S.; Yasuo, S.; Kazutoh, O. T. and Hideyo, Y. (2002). Protection of mice from lethal endogenous *Candida albicans* infection by immunization with Candida membrane antigen. *Microbiol. mmunol* . , 46:307-311.

[22]. Trinel, P. A. ;Jouault T. , Cutler, J. E. , and Poulain ,D. (2002).β-1,2-Mannosylation of Candida albicans Mannoproteins and Glycolipids Differs with Growth Temperature and Serotype. *Infect. Immun*., 70(9): 5274–5278.

[23]. Savolainen, J. and Johannes, A.R .(2006) . Antigen Binding Molecules in Patients with Invasive Candidiasis Increased Levels of *Candida albicans*Mannan-Specific T-Cell-Derived. *Nature* 13:47-474.

[24]. Sandini, S. ;Lavalle, R.; Debernardis, F.; Macri, C. and Cassone, A. (2007). The 65 KDamannoproteinsgene of *Candida albicans* encodes a putative B-glucanaseadhesine required for hyphal morphogenesis and experimental pathogenicity*J. Immunol*. , 178:2171-2181.

[25]. Donatella, P.; Patrizia, L.; Anna, R. ; Silvia, S.; Alessandra, C.; Stefano ,P.; Francesco, B. and Anna V. (2008). A Candida albicansmannoprotein deprived of its mannan moiety is efficiently taken up and processed by human dendritic cells and induces T-cell activation without stimulating proinflammatory cytokine production.*Amer. Soc. for* ,76: 4359-4367.

[26]. Khosravi, A.R.; Franco, M.; Shokri, H.; Yahyaraeyat, R. (2007). Evaluation of the effects of*Zatariamultiflora*, *Geranium pelargonium*, *Myrth*and *Lemon* essences on immune system function in experimental animals. *J.Vet.Res*. 62 (4), 119-123.

[27]. Levitz SM, Diamond RD.(1985) . Mechanisms of resistance of *Aspergillusfumigatus* Conidia to killing by neutrophils in vitro.J Infect Dis. 1985 Jul;152(1):33-42.

[28]. JahnB, MartinE ,Stueben A, and Bhakdi S . (1995). Susceptibility testing of Candida albicans and Aspergillus species by a simple microtitermenadione-augmented 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide assay. J ClinMicrobiol. 1995 March; 33(3): 661–667.

[29]. Moragues, M.D, Omaetxebarria, M.J, Elguezabal, N, Sevilla, M.J, Conti, S, Polonelli, L, Pontón, J (2003) A monoclonal abtibody directed against a Candida albicans cell wall mannoprotein exerts three anti-C. albicans activities. Infect. Immunol. 71, 5273–5279.

[30]. Arseculeratne SN, Atapattu DN, Kumarasiri R, Perera D, Ekanayake D, Rajapakse J. (2007). The use of mtt [3-(4, 5-dimethyl-2-thiazolyl) -2, 5-diphenyl -2h-tetrazolium bromide]-reduction as an indicator of the effects of strain-specific, polyclonal rabbit antisera on *Candida albicans* and *C. krusei*. *Indian Journal of Medical Microbiology, Vol. 25, No. 3, July-September, 2007, pp. 267*