INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

COMPARATIVE STUDY BETWEEN IMMUNITY PRODUCED BY HEAT KILLED *CANDIDA ALBICANS* AND *CANDIDA ALBICANS* CELL WALL MANNOPROTEINS ANTIGENS IN MICE

[a]Tareq Jaafar Aljindel, [a]Ihsan Abdullah Kumail & [b]Ishrak Abdull-Amer Alhussieny
[a]University of Al-Muthana/Iraqb
[b]National Center of Drug Control and Research/ Ministry of Health/Iraq

ABSTRACT
A comparative study was conducted to focus on some immunologic parameters in mice immunized with heat killed *Candida albicans* and cell wall mannoproteins. This experiment was carried out in animal Farm of National Centre of Drug Control and Research from March 2011 to June 2012. A total number 120 male and female mice, mice were divided into equal 3 groups, the first group (control) received with distilled water, the second experimental group was vaccinated with cell wall mannoproteins vaccine of *Candida albicans*, and the third group was vaccinated with heat killed *Candida albicans* cells vaccine. Vaccine efficiency was evaluated according to phagocytic activity, delayed type hypersensitivity reaction and anti-Candida antibodies titer in vaccinated mice serum. All treatments were carried out on day 1. Then the mice were scarified and tested at different periods: day 10, oxidative burst reaction by Nitro Blue Tetrazolium test (NBT) was done, at days 14 tests for delayed type reaction of skin, and at day 21 and 28 performed the test for anti-*Candida albicans* level in mice serum by Indirect Immunoflourescent assay. Results revealed that second group recorded significantly ($P \leq 0.01$) higher values in their peripheral blood phagocytic activity by nitro blue tetrazolium test measured by ELIZA and anti-Candida antibodies titer level in mice serum at 21 and 28 days by Indirect Immunoflourescent assay as compared with the control group . Third group results revealed significantly ($P \leq 0.05$) increase in phagocytic activity index of peripheral blood by nitro blue tetrazolium test and the anti-Candida antibodies titer level in mice serum at 21 and 28 days assessed by indirect Immunoflourescent test. In delayed-type hypersensitivity reaction, the index was significantly increased ($P \leq 0.01$) in Mannoproteins- vaccinated mice in comparison with control and second groups, the best results was observed after 24 hours post-Candida protein injection.

KEY WORDS: heat killed *Candida albicans* immunization in rat, Candida cell wall mannoproteins immunization in rat

INTRODUCTION

Candida albicans, an increasingly common opportunistic pathogenic fungus, frequently causes disease in immunocompromised more than immunocompetent human and animals (1). This increase in infections is associated with excessive morbidity and mortality and is directly related to increasing patient populations and animals that are at risk for the development of chronic or serious fungal infections (2). These risk groups include individuals under immunosuppressive therapy such as patients who are undergoing solid-organ or blood and marrow transplantation (BMT); major surgery; those with AIDS, neoplastic disease, advanced age; and those becorne prematurely (3 ,4 and 5) in animals these risk groups include stress factor during transport, heavy used of broad spectrum antibiotics, delivery, radioactive exposure. During the past years an increasing number of mycosis with fatal outcome has been observed (6). The main reasons are (i) an increasing number of intensive patients, (ii) a broad spectrum of prophylactic given antibiotics, (iii) an optimized radio- and chemotherapy resulting in neutropenic phases of cancer patients, (iv) and optimized surgical techniques resulting in a higher number of intensive patients with higher life expectance. Infections with *Candida albicans* show a number of different clinical manifestations (7). The course of infection as well as the prognosis of candidiasis is mainly influenced by the basic illness. In spite of a broad spectrum of different intensive medical treatments, systemic Candida mycosis is often lethal, especially affecting haemato oncological patients. Therefore, a rapid and reliable diagnosis at an early stage of the infection is of great importance. Materials of fungi have been the interest of different investigators around the globe with their aims to establish the immunomodulation potentials of these materials, and some risks associated with attenuated or killed whole-organism vaccines which can be avoided with vaccines that consist of specific purified macromolecules derived from pathogens(8). *Candida albicans* is one of the fungi species that share the interest of investigators in the field of immune modulation. Reported that both *C. albicans*-sensitized and -non-sensitized mice were able to mount immediate-type and delayed-type skin test responses against the cell wall antigens of *C. albicans* but not to the cytoplasmic antigens (7,8,9 and 10). Furthermore (3) 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. Up to now there is no available effective vaccine for protection against Candidiasis in humans and animals,

although there have been many trials to use a combinations of immunomodulators and vaccines to immunpotentiate the immune mechanism in recipient animals (1, 7, 8, 9 ,10 and 11). The immunologists are engaged to design vaccines strategies to maximize the responses of the immune system and to avoid the unfavorable complications resulted from alive and the attenuated vaccines. The present study was designed to compare between some immunologic parameters in mice vaccinated with alive *C. albicans* cells vaccine and cell wall mannoproteins (1, 8, 9, 10 and 11). The parameters of evaluation were Phagocytosis activity test (NBT), delayed-type hypersensitivity reaction test and anti- *Candida albicans* antibodies serum level.

MATERIAL AND METHODS

Present study came out to add some understanding about the role of biological material (*Candida albicans* cell wall mannoproteins) in potentiating the immune response in mice immunized with this biological material. Experimental design is given in table (1).

TABLE 1: Experimental design

Groups	D.W	Heat killed *Candida albicans* vaccine	Cell wall Mannoproteins of *Candida albicans*
I	+	–	–
II	–	+	–
III	–	–	+

All experiments were carried out on 120 male and female albino mice (Blab-c), which were supplied by the National Centre for Drug Control and Research, Baghdad/Iraq. The starting age of mice is rounded (6-8) weeks. They were housed in bio-clean hoods at 20-25ºC with light, dark periods of 14:10 hours. They were fed standard pellets and water, their initial weight was 22 ± 3 grams at the beginning of experiments. Mice were separately caged for a one week preliminary period for acclimatization period. The following culture media were used in carrying out the experiments of the study (agar, Bactodextros, Bacto peptone, Blood agar, Sabourauds dextrose agar, and yeast extract) were the products of Difco Company (U.S.A). A purified ribosomal protein free of lip polysaccharide, which is prepared from *Candida albicans* and composed of 50-70% protein and 15-30% polysaccharide to used for skin test (1) . The dried lyophilized seed of *Candida albicans* strain was supplied by the Zoonotic unit-Veterinary College Baghdad-University/Iraq, the above laboratory receives the strains from the Food and Agriculture Organization (FAO).The Mannoproteins were prepared from the cell wall of a *Candida albicans* as described by(13). Blood phagocytic activity for oxidative burst reaction by Nitro Blue Tetrazolium test (NBT) measured by ELIZA (14) and the phagocytic activity% was calculate as suggest by (15). Delayed type hypersensitivity reaction index in a right foot pad was measured to each mouse in all groups at time zero, each mouse was injected with 50 ul of *Candida albicans* mannoprotein in the right foot pad, then foot Pad swelling was measured at 24 and 48 hours post injection by a digital vernia and given in a unit of millimeter, as suggested by (1). Quantitative Determination of anti-*Candida albicans* antibodies serum Level at 21 and 28 days were carried out by using a mouse anti rabbit antibodies kit (company), which is an indirect Immunoflourescent assay (16). The Statistical Analysis values of the investigated parameters were given in terms of means ± standard errors (S.E.), and differences between means were assessed by analysis of variance (ANOVA), least significant difference (LSD) test, using the computer programmer SPSS version 7.5. The differences were considered significant when the probability value was equal or less than 0.05. Further estimations were also given; they were treated efficiently (17), which were calculated according to the following equation:

$$\text{Treatment efficiency (\%)} = \left(\frac{A - B}{B}\right) \times 100$$

A = Treated groups; B = Negative control group.

RESULTS AND DISCUSSION

The results of Phagocytic activity % by NBT assay of peripheral blood were given in **table (2)**. All groups of treated mice were showed different significant increases ($P \leq 0.01$) in the Phagocytic activity % (166% and 212% respectively) as compared with group I, the highest Phagocytic activity 212 % was reported in group III.

TABLE 2: Phagocytic activity by EIIZA

Groups	Optical density/ wave length 490	Phagocytic activity %
G1	0.80± 0.06 c	0% c
G2	2.13±0.12 b	166% b
G3	2.50±0.11 a	212% a

Different letters point to significant difference($P \leq 0.05$) between means of the same column.

Results of Phagocytic activity % by NBT assay had showed a significantly increased ($P \leq 0.01$) percentage in immunized groups, these are in favor of such agreement, and activate murine macrophages which can destroy intracellular bacteria. Nitro Blue Tetrazolium reduction by polymorph nuclear cells may require oxidative metabolism by the hexose monophophate shunt, and is impermeable to cell membrane, but it enters the cell during the process of Phagocytosis, and it is reduced by diaphoreses activity within phagosom (18). Although macrophages and Monocytes possess phagocytic mechanisms in the resting state, these mechanisms can be enhanced, and new

mechanisms can be expressed when they are activated. Activation can occur through exposure to microbial products (*Candida albicans* cell wall mannoprotein) and (heat killed *Candida albicans*), such picture is enhanced by the findings of our study and also confirmed by other investigators (11, 19, 20, 21,22and 23). In contrast to monocytes and Neutrophils, which are important in resistance to early stages of C. albicans infections, more differentiated macrophages activated by cytokines such as gamma interferon participate in the acquired resistance of hosts with *C. albicans*-specific, cell-mediated immunity(24, 25).

The sera of mice in groups I and 11 showed a positive Immunoflourescent reaction at the titer 1:32 and 1:64 respectively comparison with control group, the results were gavine in **table (3)**.

TABLE 3: *Candida albicans* antibodies titer by Indirect Immunoflouresce

Groups	Titer 1/16	Titer 1/32	Titer 1/64	Titer 1/128	Titer 1/256	Titer 1/512
G1	Negative	Negative	negative	Negative	Negative	Negative
G2	Positive	Positive	negative	Negative	Negative	Negative
G3	Positive	Positive	Positive	Negative	Negative	Negative

Such observation suggests that the immunomodulation also involved the humoral immune response, although the pathway may be through the modulation of macrophages and T lymphocytes as both types of cells are required to enhance the B lymphocytes to produce immunoglobulin (8). IFN-γ secretion by TH1 cells also induces antibody-class switching to IgG classes (such as IgG2a in the mouse) that support phagocytosis and fixation of complement. TNF and IFN γ are cytokines that develop inflammation, and it is their secretion that accounts for the association of TH1 cells with inflammatory phenomena such as delayed hypersensitivity .Such findings came to confirm previous results reported by (1, 8, 9, 10, 11 and 10). In table (4) results revealed that DTH index was increased significantly ($P \leq 0.05$) in Mannoproteins-treated vaccinated mice in comparison with control and second groups, a best results was observed after 24 hours post-Candida protein injection.

TABLE 4: Delayed type hypersensitivity reaction.

Groups	At zero time	After 24 hours	BTH index	After 48 hours	BTH index
G1	1.78 ±0.03a B	A 2.25±0.11b	26% b	A 2.11 ± 0.07 b	18% b
G2	1.83±0.03a C	A 2.26 ±0.04b	23% b	B 2.06 ± 0.04 b	12% b
G3	1.79 ± 0.01a C	2.51 ± 0.08a A	34% a	B 2.34 ± 0.04 a	31% a

*Different small letters point to significant difference ($P \leq 0.01$) between means of the same column.
*Different capital letters point to significant difference ($P \leq 0.01$) between means of the same row.

Both *Candida albicans* vaccines were assessed for their effectiveness on immune cell-mediated immunity (CMI), which can be assessed by determining the level of delayed-type hypersensitivity (DTH) response, and the latter one is an important host defense mechanism against candidiasis (26). With this regard, mice treated with A purified ribosomal protein free of lip polysaccharide a significant DTH response in mice immunized with *Candida albicans* antigens comparison with the corresponding control, and the highest thickness was produced after 24 hours of *Candida albicans* proteins injection. These results came to confirm the findings of (2, 9, 11, 27 and 28). In this regard, DTH reactions develop when antigen activates sensitized T_{DTH} cells, and these cells generally appear to be a Th1 subpopulation although T cytotoxic may also be involved (1). A defining cytokine of the TH1 subset, IFN γ activates macrophages, stimulating these cells to increase microbicidal activity, up-regulate the level of class I MHC, and secrete cytokines such as IL-12, which induces T-H cells to differentiate into the TH1 subset. The overall effect of these cytokines is to with draw macrophages to the area of injection and activating them, promoting increased phagocytic activity and increases concentrations of lytic enzymes for more potent killing. As lytic enzymes leak out activated macrophages into the surrounding tissue, the localized tissue destruction can occur. These reactions typically tooks (48-72) hours to develop, which is the time required for initial T_{DTH} cell activation and cytokine secretion to mediate the accumulation of macrophages and the subsequent release of their lytic enzymes(28). From the results reported in the present study, it is possible to point out the effectiveness of mannoproteins of *C. albicans* cell wall used on innate immune response, humoral and cellular immune response in vaccinated mice, and it is possible to conclude that cell wall mannoproteins of *C. albicans* might be a potential vaccine for inducing active immunity against *Candida albicans* infection (26, 28).

REFERENCES

[1]. 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.

[2]. Correia, A., Lermann, U., Teixeira, L., Cerca, F., Botelho, S., Gil da Costa, R. M., Sampaio, P., Gartner, F., Morschhauser, J., Vilanova, M., Pais, C. (2010). Limited Role of Secreted Aspartyl Proteinases Sap1 to Sap6 in *Candida albicans* Virulence and Host Immune Response in Murine Hematogenously

Disseminated Candidiasis. Infect. Immun. 78: 4839-4849.

[3]. Rodaki, A., Bohovych, I. M., Enjalbert, B., Young, T., Odds, F. C., Gow, N. A.R., Brown, A. J.P. (2009). Glucose Promotes Stress Resistance in the Fungal Pathogen *Candida albicans*. Mol. Biol. Cell 20: 4845-4855.

[4]. Morato-Marques, M., Campos, M. R., Kane, S., Rangel, A. P., Lewis, C., Ballinger, M. N., Kim, S.-H., Peters-Golden, M., Jancar, S., Serezani, C. H. (2011). Leukotrienes Target F-actin/Cofilin-1 to Enhance Alveolar Macrophage Anti-fungal Activity. J. Biol. Chem. 286: 28902-28913.

[5]. da Silva Dantas, A., Patterson, M. J., Smith, D. A., MacCallum, D. M., Erwig, L. P., Morgan, B. A., Quinn, J. (2010). Thioredoxin Regulates Multiple Hydrogen Peroxide-Induced Signaling Pathways in *Candida albicans*. Mol. Cell. Biol. 30: 4550-4563 .

[6]. Lopes da Rosa, J., Boyartchuk, V. L., Zhu, L. J., Kaufman, P. D. (2010). Histone acetyltransferase Rtt109 is required for *Candida albicans* pathogenesis. Proc. Natl. Acad. Sci. USA 107: 1594-1599.

[7]. Goupil, M., Trudelle, E. B., Dugas, V., Racicot-Bergeron, C., Aumont, F., Senechal, S., Hanna, Z., Jolicoeur, P., de Repentigny, L. (2009). Macrophage-Mediated Responses to *Candida albicans* in Mice Expressing the Human Immunodeficiency Virus Type 1 Transgene. Infect. Immun. 77: 4136-4149.

[8]. Martinez, J. P.; Gil, M .L; Lopez-Ribot, J .L .and Chaffin, W. L. (1998). Serologic Response to Cell Wall Mannoproteins and Proteins of *Candida albicans*. Amer. Soc. for Microbiol. , 11: 121-141.

[9]. 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. Agents chemother 44:243-247.

[10] Viudes, A.;Perea, S. nd Lopez-Ribot, J. L .(2001). Identification of continuous B-cell epitopes on the protein moiety of the 58-kilodalton cell wall mannoproteins of *Candida albicans* belonging to a family of immunodominant fungal antigens. Infect. Immun. , 69:2909-2919.

[11] Al-Lammi, T. J.(2009). Study the effect of Neem and the cell wall of mannoproteins of *Candida albicans* as immunomodulators on vaccination of mice with Brucella vaccine-Rev-1vaccine.Ph.D.thesis, University of Baghdad, Iraq.

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

[13] Farahnejad, Z. R. M .; Frozandeh, M. ; Paknejad M.; Kashanian S.and Rajabi M,H., (2005). Preparation and Characterization of a Monoclonal Antibody Against Mannoprotein of *Candida albicans*. Issue 3: 24(3): 146-151.

[14] 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.

[15] Sanja, S.D. ; Sheth,N.R.; Patel N.K.; Dhaval, P. and Biraju, P. (2009).Characterization and evaluation of antioxidant activity of Portulaca oleracea . International Journal of pharmcy and pharmaceutical Science .74-84.

[16] World Health Organization (WHO) (1997). WHO Guidelines for the safe transport of infectious and diagnostic specimens, WHO,Geneva,Switzerland, Who/EMC/79.3 WHO.

[17] Perez–Serrano, J.; Denegri, G.; Casado, N. And Rodrigues-Caabeiro, F. (1997) In vivo effect of oral abendazole and abendazole sulphoxide on development of secondary echinococcosis in mice. Int. J. Parasitol., 27: 1341-1345.

[18] Balestrieri, B.; Maekawa, A.;Xing, W.; Gelb, M. H.; Katz, H. R. and Arm, J. P. (2009). Group V Secretory Phospholipase A2 Modulates Phagosome Maturation and Regulates the Innate Immune Response against *Candida albicans*. J. Immunol. 182: 4891-4898.

[19] Chandra, J.; McCormick, T. S.; Imamura, Y.; Mukherjee, P. K. and Ghannoum, M. A. (2007). Interaction of *Candida albicans* with Adherent Human Peripheral Blood Mononuclear Cells Increases C. albicans Biofilm Formation and Results in Differential Expression of Pro- and Anti-Inflammatory Cytokines. Infect. Immun. 75: 2612-2620.

[20] Wellington, M.; Dolan, K. and Krysan, D. J. (2009). Live *Candida albicans* Suppresses Production of Reactive Oxygen Species in Phagocytes. Infect. Immun. 77: 405-413.

[21] Snelgrove, R. J.; Edwards, L.; Williams, A. E.; Rae, A. J. and Hussell, T. (2006). In the Absence of Reactive Oxygen Species, T Cells Default to a Th1 Phenotype and Mediate Protection against Pulmonary Cryptococcus neoformans Infection. J. Immunol. 177: 5509-5516.

[22] Balish, E.; Warner, T. F.; Nicholas, P. J.; Paulling, E. E.; Westwater, C. and Schofield, D. A. (2005). Susceptibility of Germfree Phagocyte Oxidase- and Nitric Oxide Synthase 2-Deficient Mice, Defective in the Production of Reactive Metabolites of Both Oxygen and Nitrogen, to Mucosal and Systemic

Candidiasis of Endogenous Origin. Infect. Immun. 73: 1313-1320.

[23] Newman, S. L.; Bhugra, B.; Holly, A. and Morris, R. E. (2005). Enhanced Killing of *Candida albicans* by Human Macrophages Adherent to Type 1 Collagen Matrices via Induction of Phagolysosomal Fusion. Infect. Immun. 73: 770-777.

[24] Coste, A.; Lagane, C.; Filipe, C.; Authier, H.; Gales, A.; Bernad, J.; Douin-Echinard, V.; Lepert, J.-C.; Balard, P.;Linas, M.-D.; Arnal, J.-F.; Auwerx, J. and Pipy, B. (2008). IL-13 Attenuates Gastrointestinal Candidiasis in Normal and Immunodeficient RAG-2-/- Mice via Peroxisome Proliferator-Activated Receptor-{gamma} Activation. J. Immunol. 180: 4939-4947.

[25] Vonke, A. G.; Netea, M. G.; van Krieken, J. H.; Iwakura, Y.; van der Meer, J. W. M. and Kullberg, B. J. (2006). Endogenous Interleukin (IL)-1{alpha} and IL-1{beta} Are Crucial for Host Defense against Disseminated Candidiasis. The Journal of Infectious Disease 193: 1419-1426.

[26] Kuby, J(2009) . Immunology, Text book,3rd Ed W H freeman and Company USA New York .

[27] 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.

[28] Donatella, P.; Patrizia, L.; Anna, R. ; Silvia, S.; Alessandra, C.; Stefano ,P.; Francesco, B. and Anna V. (2008). A *Candida albicans* mannoprotein 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.