

## THE INFLUENCE OF WHOLE SONICATE *BRUCELLA ABORTUS* ANTIGEN ON THE *CANDIDA ALBICANS* INFECTION IN MICE

\*Alwan, M.J, \*\*Al-Oubaidy, S.S. \* Al-Zubaidy, I.A.

\*Zoonosis unit, \*\* Department of pathology and poultry, College Vet, Med. University of Baghdad, Iraq.

**Keywords:** Brucella , post-Vaccine, Candidiasis.

### ABSTRACT

The aim of this study is to evaluate the effect of immunization with whole sonicate *Brucella abortus* antigen on the mice infected with *Candida albicans*, for that, Twenty one white mice ,both sexes, average weight 20-24g, were divided randomly into (3) equal groups.

First group immunized with (0.5)ml of sonicated brucella antigen, two doses, 10 days interval, skin test was done 27 days post-vaccination, 2<sup>nd</sup> group and 3<sup>rd</sup> groups served as positive and negative control group respectively. (30) days post-vaccination, 1<sup>st</sup> and 2<sup>nd</sup> groups were challenged with (0.5)ml of inoculums (1X10<sup>8</sup> yeast/ml), *C. albicans* I/P. 3<sup>rd</sup> group was injected with (0.5)ml of sterile normal saline I/p.

The results showed that the immunized animals revealed cellular immune response, negative fungal isolates and minor pathological changes in compared with positive control. Positive control animals show sever fungal isolates from internal organs with sever pathological changes characterized mainly by polymorphonuclear cells infiltration and multiple foci of granulomatous lesion.

### INTRODUCTION

Candidiasis is the infection caused by species of *Candida*, mostly *C.albicans* which is the most frequently isolated fungal pathogen in humans and animals (1).

*C.albicans* is a normal commensal of the gastrointestinal and genitourinary tracts of human and of various warm-blood animals (2). In immuno-compromised hosts, the transition of *C. albicans* into an opportunistic pathogen is not uncommon, and disseminated candidiasis of endogenous origin may occur (3) .

*C. albicans* pathogenicity depends on a complex array of microorganisms related putative virulence factors, these include the yeast to mycelium transition, antigenic variability, phenotype switching, adheres to host and tissue, cell surface hydrophobicity, molecular mimicry, immunomodulation of host defence mechanisms and production of extracellular enzymes (4). The three most significant hydrolytic enzymes produced by *C.albicans* are secreted aspartic proteinases, phospholipase and lipase. Their hydrolytic activity has a number of possible function in addition to simple role of digesting molecules for nutrition, these enzymes used to host tissue invasion by digesting or destroying cell membrane and by degrading host surface molecules and all these enzyme are able to attack cells and molecules of the host immune system to avoid or resist antimicrobial activity (5)

*Candida* antigen may stimulates specific cell mediated and humoral immune response .Most invasive fungal infection occur in patient with defective cellular immunity but the role of antibodies in resistance to candidiasis is poorly understood (6) The integration of innate and adaptive immune responses is required for efficient control of *C.albicans* (7).

Resistance to *C.albicans* infection is determined by phagocytic effector mechanisms enhanced by Th-1 type CD4 cell cytokines (8). Neutrophils and mononuclear phagocytic cells play importance role in preventing candidal dissemination (9). Th-1 cytokines play essential role in the

activation of mononuclear phagocytic cells, Th-1 differentiation requires the combined effects of different cytokines, including interleukin-12 (10), this cytokine was both required and prognostic for the development of protective Th1 responses to *C. albicans* (11) and acted as an adjuvant in response to a Candida vaccine(12).

*C. albicans* can switch from a unicellular yeast form into various filamentous form.all of which can be found in infected tissues (13), the ability to reversibly switch between these forms is thought to be important for Candida virulence. (14) Filamentous growth form is required to evade the cells of the immune system, whereas the yeast form may be the mode of proliferation in infected tissues (14). Phagocytic cells such as neutrophils, macrophages and dendritic cells rapidly and efficiently phagocytosed both yeast and hyphae of the fungus. In vitro, ingestion of yeasts activated phagocytic cells for IL-12 production and priming of T helper type 1 cells whereas ingestion of hyphae inhibited IL-12 and Th1 priming, and induced IL-10 production (15) IL-10 differentiated nave CD4 T cell to Th2 type cells which produced IL-4 and IL-5 and mostly associated with pathology and disease progression (16).

Safety vaccine against *C. albicans* infection in human and animals was not recognized, the aim of this study is to evaluate the effect of immunization with whole sonicated brucella abortus antigen in mice against *C.albicans* infection.

## MATERIALS AND METHODS

Organisms: organisms used in this study were obtain from the followings:

- 1- The pathogenic *C.albicans* was taken from the stock culture collection of Mycology Division, Collage of Al-nahran Medicine.Yeast cells were grown on Sabouraud glucose agar at 28C, Yeast cells were harvested after 48hrs of culture, centrifuged at 1000 rpm, washed twice in sterile normal saline, counted and diluted to desired concentration. Feature of this strain exhibited according to Lodder (17).The number of viable cells was counted by a haemacytometer.
- 2-*Brucella abortus* 99 was obtained from unit of zoonotic disease unit, College of Vet, Medicine, Baghdad. Sonicated antigen was prepared according to the method which was described by Hiallibartion and Blazkovec (17) and used Sonicater MSE UK. to destruct the bacteria for 15 minutes with ice.

### Experimental design

A total number of 28 white mice, both sexes, were used in this investigation. Their age range between 60-90 days, their weights ranged between 20-34 grams. They were divided into 3 groups:

1- The first group was immunized with 0.5 ml of sonicated antigen (protein concentration 17mg/ml) S/C, two doses with 10 days interval, skin test examination was done at 27 days post-immunization. At 30 days post-immunization, all animals were infected intraperitoneally with a volume of 0.5 ml of inoculums ( $1 \times 10^8$  yeast/ml).

2-The second group was infected with viable *C. albicans* at the same dose and route of first group.

3-The third group was injected S/C with 0.5ml of normal sterile saline

All animals were sacrificed at day 20 post-infection, samples were taken from some internal organs for culture and other samples were fixed in 10% formaldehyed solution for histopathological examination according to Luna (18).

## RESULTS

### Skin test results:

Table:1 showed mean of difference thickness of palm legs of animals at 27 days post-immunization against Brucellin

All the immunized animals remained alive and in good condition. Cultures failed to detect the fungal growth from their internal organs. Two animals died from non-immunized group and all animals gave heavy growth of yeast from kidney, lung, spleen, liver and heart (table: 2).

Pathological examination:

1- Gross examination showed no pathological lesion in the examined organs of immunized animals. Gross lesions of non-immunized animals were mostly confined to the kidney and liver, the lesions characterized by congestion of these organs with white pin-point areas distributed over their surfaces.

2- Microscopic examination:

Non-immunized animals

- Kidney:

Microscopic section revealed congested blood vessels between renal tubules with inflammatory cells mainly neutrophils in their lumens. Bowman's capsules losses its normal histological architecture, atrophy of glomerular tufts and dilated of bowman space, acute cellular degeneration of epithelial lining cells of renal tubules also reported as well as polymorphonuclear cell infiltration in the interstitial tissue of the cortex and around the blood vessels (fig:1).

- Liver:

The lesions in the liver distributed throughout the liver parenchyma, characterized by area of necrosis and disappearance of hepatic cells which replaced by RBCs (fig: 2), in addition to the presence of multiple foci of granulomatous lesions consisted from neutrophils, macrophages scatter in the liver parenchyma (fig: 3). In other section, abscess which consists from dead and live of neutrophils and encapsulated by fibrous connective tissues was seen in the liver capsule and it adhere to the stomach and esophages layer (fig:4,5).

- Lung:

Histopathological findings of the lung consisted from hyperplasia of epithelial lining of mucosa of bronchioles with inflammatory cells infiltration mainly neutrophils and macrophages in the subepithelial layer and in the lung parenchyma (fig: 6)

- Spleen:

polymorphonuclear cells infiltration, hemorrhage and also congestion of blood vessels which contained inflammatory cells in their lumens were seen in the spleen (fig7).

Immunized animals

Histopathological examination of the internal organs showed severe hyperplasia of white pulp of the spleen together with mononuclear cells proliferation around splenic sinuses which appear as a cord-like (fig: 8). No signs of inflammatory lesions observed the lung (fig: 9) lymphocytes aggregate around the central veins (Fig: 10) and blood vessels in the portal area.

## DISCUSSION

The present work aimed to determine the effects of sonicated *Brucella abortus* antigen in immune responses against infection by *C.albicans* in mice.

The results revealed that *Brucella* antigen stimulated cell mediated immunity in immunized animals which showed negative fungal isolates from their internal organs compared with non-immunized animals in which heavy fungal isolates were recovered from their organs

Cell mediated immunity is essential for host protection against virtually all fungal pathogens (19).

*Brucella* antigens stimulated cellular immune response in laboratory animal's model measured by lymphocyte blastogenesis and produce protective cytokines (20, 21)

Phagocytosis of *C. albicans* by macrophages must be accompanied by killing; otherwise, macrophages could promote the dissemination of the pathogen and help the fungus to avoid the immune attack (22). Killing of *C. albicans* can be upregulated in macrophages by cytokines such as interferon gamma (INF- $\gamma$ ) or tumor necrotic factor alpha (TNF-alpha), chemokines, or neuroendocrine products (23). phagocytosis of either form of candida by macrophages and dendritic cells produced sets of cytokines with opposing activities in the developing immune response (24). Macrophage synthesis of IL-12 and TNF-alpha in early infection results in a synergistic stimulation of natural killer cells to synthesize IFN- $\gamma$  (25). This IFN- $\gamma$  activates resident macrophages to become bacteriocidal, particularly through the production of nitric oxide and also induces Th cells to undergo differentiation to CD4+Th1 cells. These Th1 cells synthesize additional IFN- $\gamma$ , thus positively amplifying the host response, the activated macrophages now have an increased ability to present bacterial antigens to these T cells, resulting in their differentiation into armed effectors cells and the sterile eradication of the bacteria (26).

Histopathological results agreed with results of skin test and fungal isolates .No significant lesions in the internal organs of immunized animals compared with no-immunized group.This results suggest that the Brucella antigens may be induced specific or non specific protective immunity against *C.albicans* in mice. Phagocytosis of the brucella antigen activated the macrophages and dendritic cells to secrete proinflammatory cytokines such as IL-1, TNF alpha and IL-12. (27).These cytokines participate both in the control of the fungus and in the induction of protective Th1 immunity (16).TNF alpha is one of the major secretory products of stimulated macrophage with important role in host defense against disseminated candidiasis (28).

IL-12 allows the CD4+ Tcells to differentiate into Th1 cells resulting in phagocytic-dependent immunity clearly represents an important mechanism of anticandidal resistance (29).

The pathological changes in the internal organs of non-immunized animals indicate that a virulence strains of *C.albicans* were used in the present study.phagocytic cells engulfed Candida by a zipper-type phagocytosis (30).Once internalized, yeast were detected in phagolysosomes. where different stages of progressive degradation were seen and may result in an efficient release of fungal peptide for class II-restricted antigen presentation whereas hyphae surviving free in the cytosol may eventually intersect the class I-restricted antigen presentation pathway (31). The activation of CD8+ T cells occurred in response to the filamentous forms of the fungus and activated CD8+ T cells secreted cytokines which induced granulomatous reaction (32), this evidence was in agreement with the present study.

Neutrophils infiltration and necrosis which are seen in some organs of non-immunized animals may be due to virulence factor of *Candida albicans* This fungus secretes hydrolytic enzymes such as phospholipase C that increases penetration into host cell tissues (33).It also play role in perforation of macrophage phagosomes , destruction of the infected cells and spread the Candida to adjacent cells.

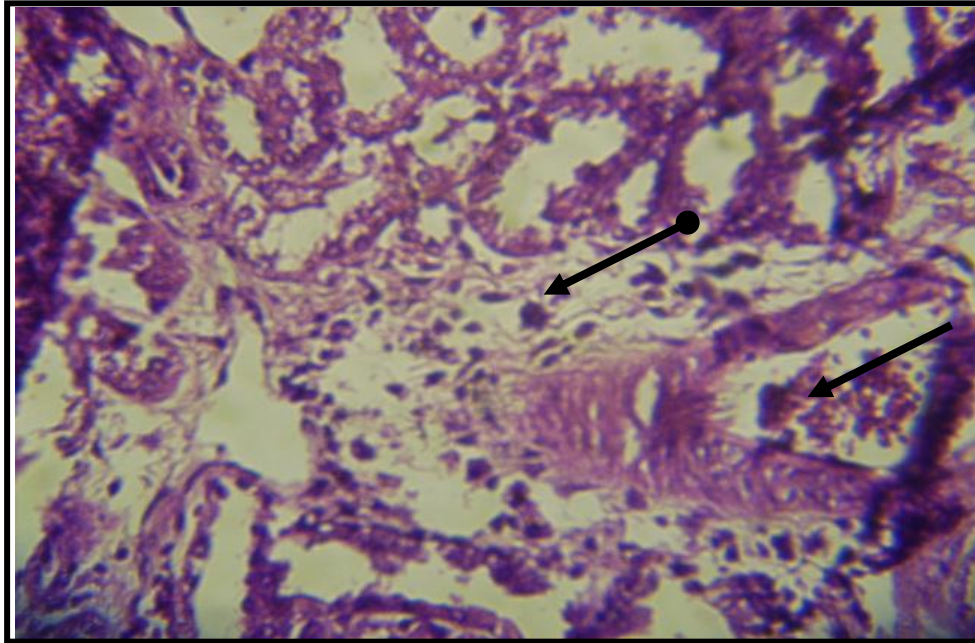


Fig 1: histological section of kidney in animal infected with *C. albicans* showed congestion of blood vessels ← with polymorphnuclear cells infiltration in the interstitial tissue ← (H&EX40).

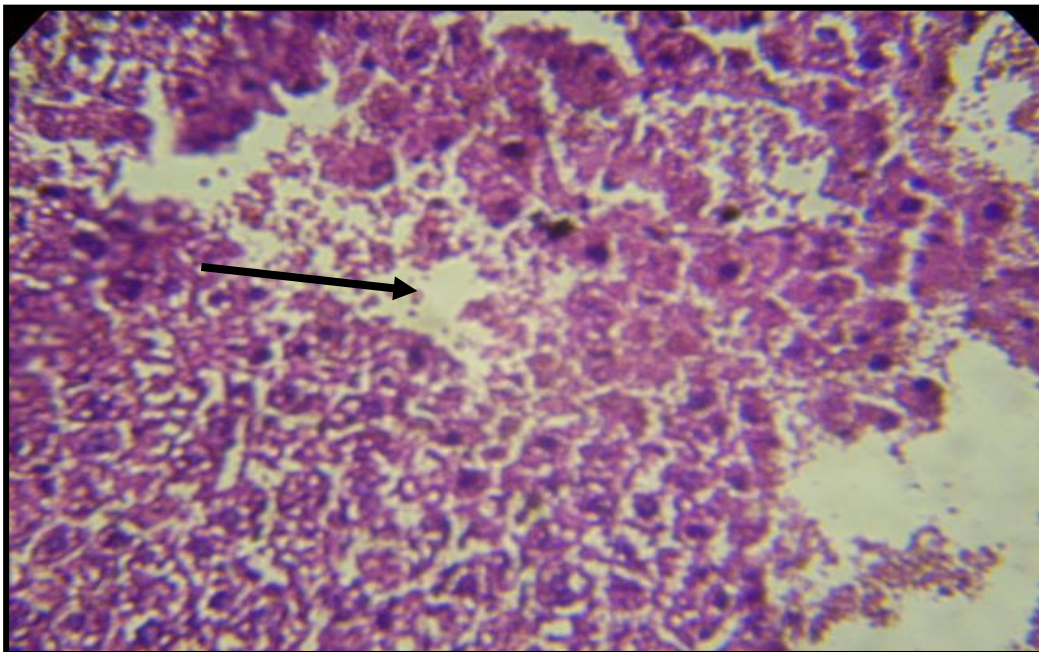


Fig 2: histological section in the liver of animals infected with *C. albicans* reveled necrosis and disappearance of hepatic cells which replaced by RBCs → . (H&EX40).

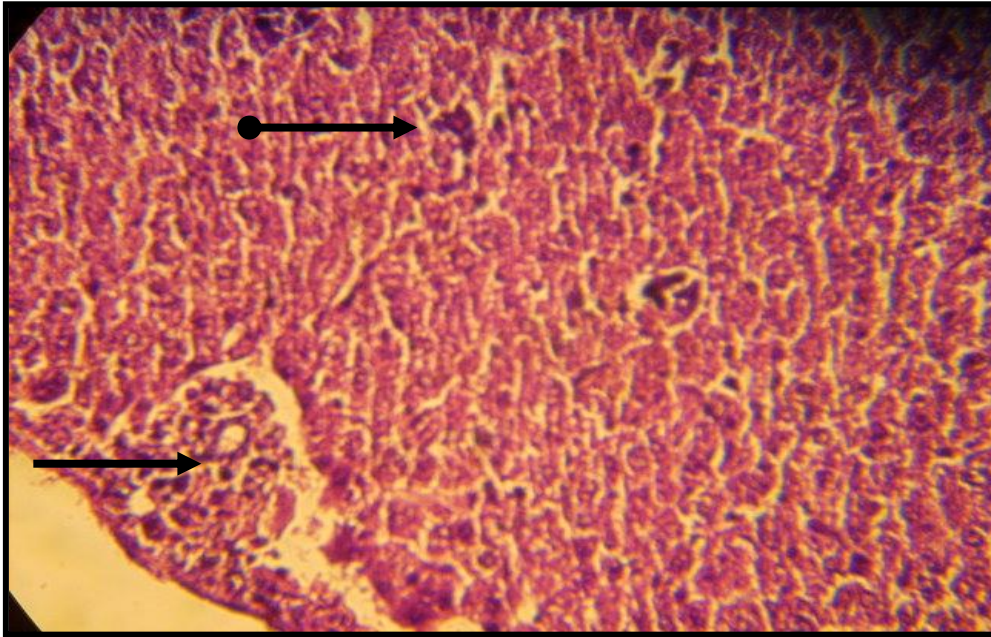


Fig 3: histological section in the liver of animals infected with *C. albicans* explain multiple granulomatous lesion in liver parenchyma → & blood vessels ●→ (H&EX40).

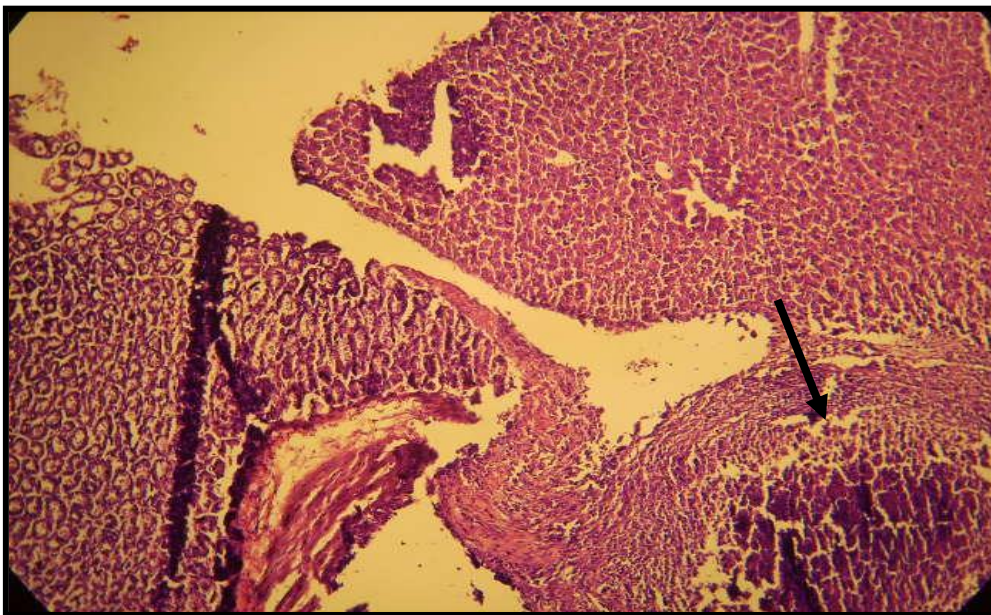
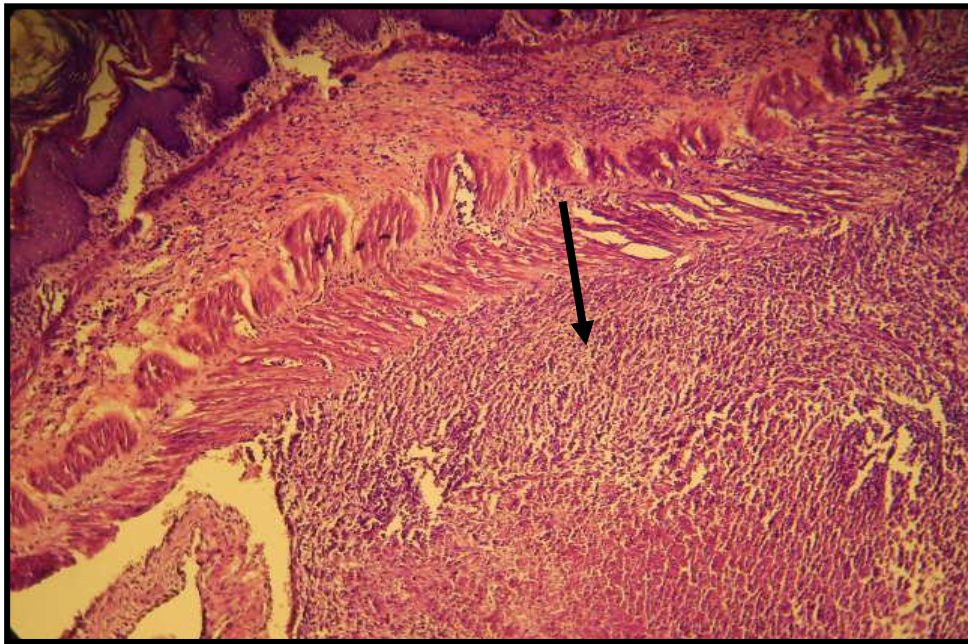
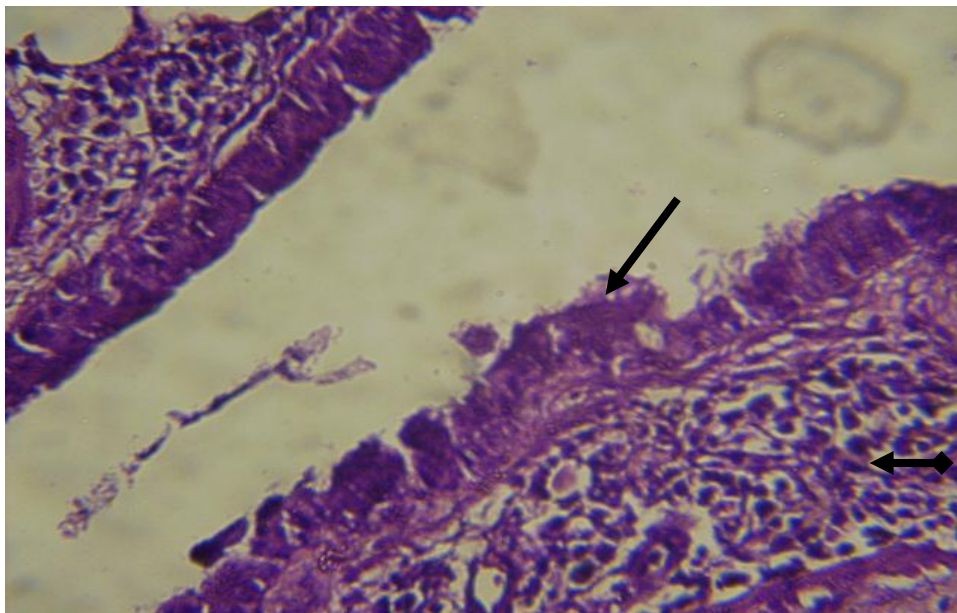


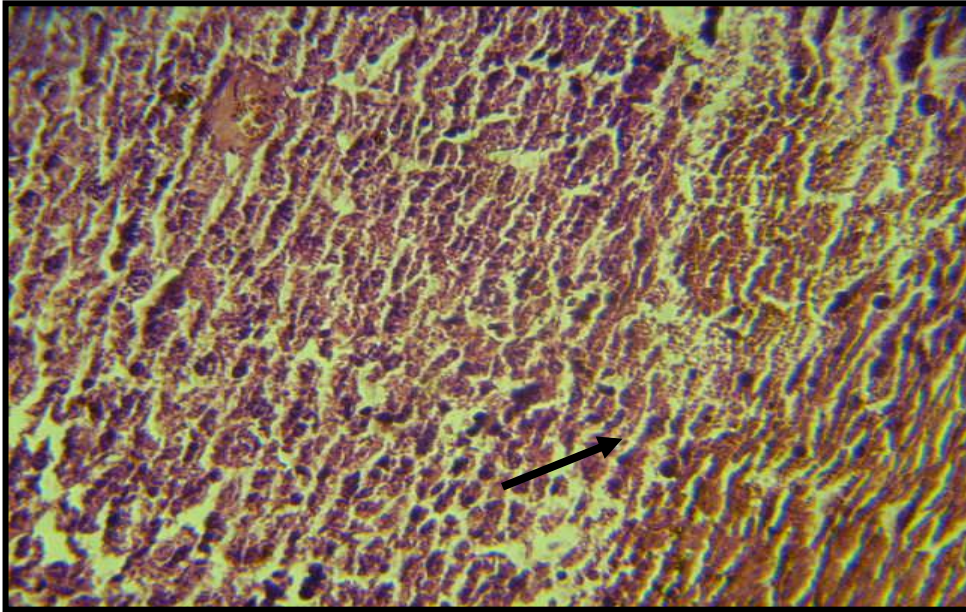
Fig 4: histological section in the liver of animals infected with *C. albicans* showed abscess adhered with stomach → (H&EX20).



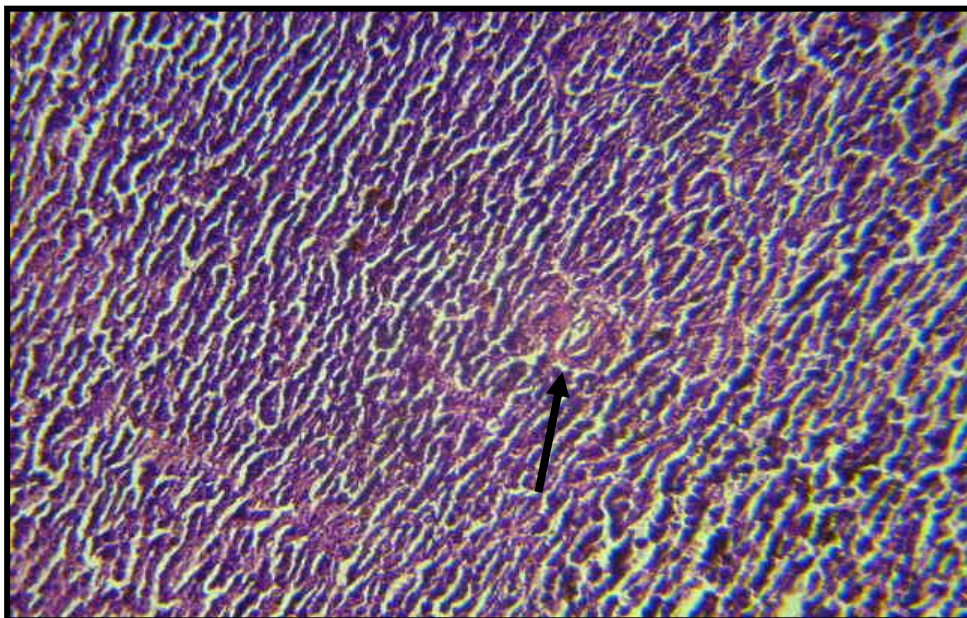
**Fig 5: histological section in the liver of animals infected with *C. albicans* revealed abscess**  
→ **encapsulated by thick fibrous connective tissue adhered to esophagus**  
**(H&EX20).**



**Fig 6: Histological section of the lung of animals infected with *C. albicans* showed**  
**hyperplasia of the epithelial lining of mucosa of the bronchioles** ← **with**  
**inflammatory cells infiltration in the subepithelial layer** ←● **((H&EX40).**

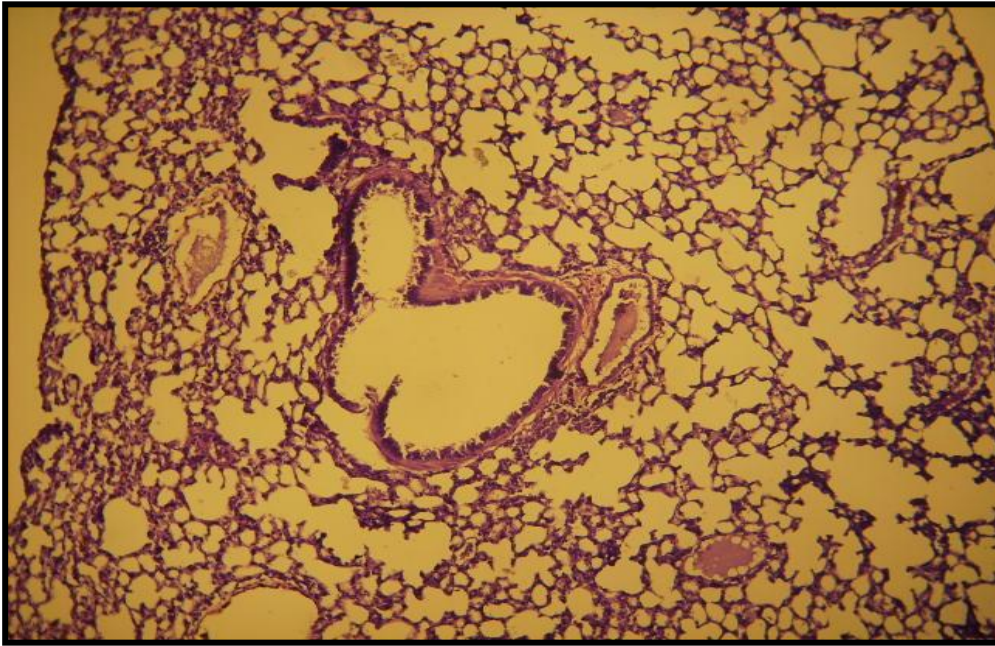


**Fig7: Histological section of the spleen of animals infected with *C. albicans* revealed inflammatory cell infiltration with hemorrhage in the red pulp → (H&EX40).**

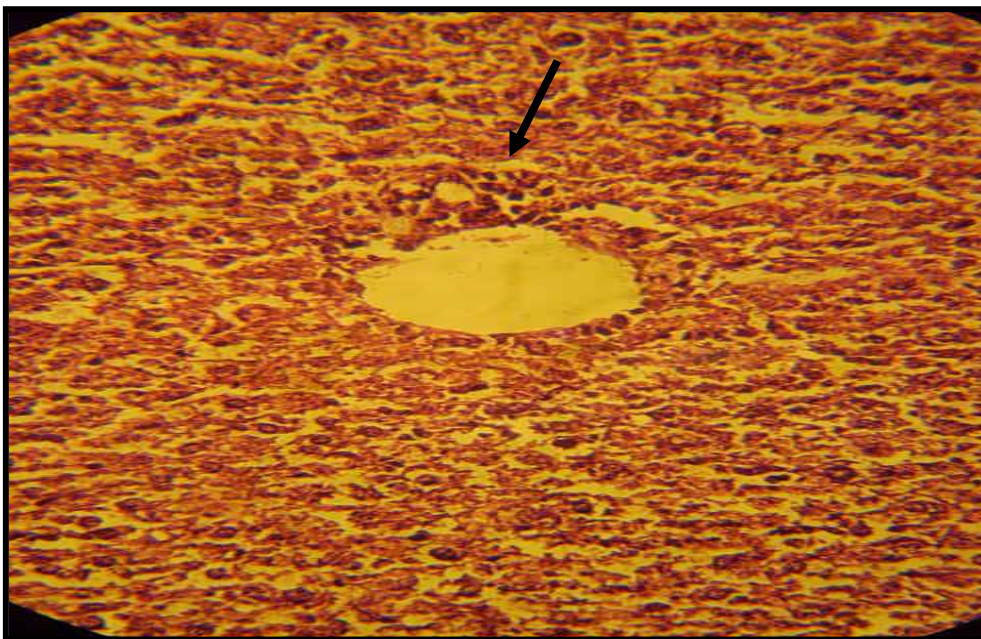


**Fig 8: histological section in the spleen of immunized animals at 20 days post-challenge showed hyperplasia of white pulp → (H&EX40).**





**Fig 9: : histological section in the lung of immunized animals at 20 days post-challenge showed no pathological lesions (H&EX20).**



**Fig 10: histological section in the liver of immunized animals at 20 days post-challenge revealed aggregation of mononuclear cells around the central vein ← . (H&EX40).**

## تأثير التمنيع بالمستضد المتكسر لجرثومة البروسيلة المجهضة على الإصابة التجريبية للفنران بالكانديدا

\*محمد جويد علوان\*\* سرهد سعد عبود العبيدي\*أبراهيم عبد الحسين الزبيدي  
\*وحدة الأمراض المشتركة، كلية الطب البيطري، جامعة بغداد، بغداد، العراق  
\*\* فرع الأمراض والدواجن، كلية الطب البيطري، جامعة بغداد، بغداد، العراق

### الخلاصة

تهدف هذه الدراسة لتقييم فعالية التمنيع باستخدام المستضد الكلي الناتج من تكسير جرثومة البروسيلة المجهضة على الفنران المصابة تجريبيا بفطر ( المبيضات البيضاء) حيث تم استخدام 21 من الفنران البيضاء من كلا الجنسين تتراوح أوزانها بين 20-24 غم قسمت عشوائيا الى ثلاثة مجاميع و تم إعطاء المجموعة الاولى المستضد المتكسر لجرثومة البروسيلة المجهضة 0.5 مل (1 ملغ بروتين) وبجرعتين بين كل واحدة وأخرى 10 أيام. وأجري الفحص الجلدي بعد 27 يوما من التمنيع بالجرعة الاولى. أما المجموعتين الثانية والثالثة فأعتبرت مجموعتي سيطرة موجبة وسالبة على التوالي. تم بعد 30 يوما من التمنيع بالجرعة الاولى للمستضد إعطاء جرعة تحدي بفطر (المبيضات البيضاء) مقدارها 0.5 مل من محلول تركيزه 10 x 1 خميرة \مل عن طريق الحقن داخل التجويف البريتوني (IP). بينما أعطيت المجموعة الثالثة 0.5 مل من المحلول الفسلجي المتعادل المعقم داخل التجويف اللبريتوني. أظهرت النتائج أن الحيوانات الممنعة أعطت أسجابة مناعية خلوية ولم يتم عزل الفطر من الحيوانات في هذه المجموعة مع وجود تغيرات مرضية طفيفة مقارنة مع ما اظهرته مجموعة السيطرة الموجبة (المجموعة الثانية) والتي أظهرت أفات مرضية شديدة تمثلت بأرتشاح الخلايا المتعددة الانوية و بؤر متعددة من الأورام الحبيبية مع عزل أعداد كثيرة من الفطر من الاعضاء الداخلية لحيوانات هذه المجموعة.

### REFERENCES

1. Edward, JD. Invasive Candida infections: evolution of a fungal pathogen. N. Engl. J. Med. 324:1060-1062.1991.
2. Connor, MI and Sobel, JD. Epidemiology of recurrent vulvo-vaginal candidiasis; identification and strain differentiation of Candida albicans. J. Infect. Dis. 15:358-362.
3. Kasperm, LH, and Buzoni, GD. Opportunism and AID: walking the lightrope. Parasitol. Tody.15:145-156,1999.
4. Calderone, RA. Molecule interaction at the interface of *Candida albicans* and host cells. Arch. Med. Res. 24:275-279.1998.
5. Martin, SC, and Bemhard, HH. Virulence factors that promote invasion of *Candida albicans*. Infect. Immunol. 45:234-240.1998..
6. Fidel, PA. and Soel, JD. Immunopathogenesis of recurrent vulvo-vaginal candidiasis. Clin. Microbiol. Rev. 9:335-0340 2004.
7. Romani, L. Innate and adaptive immunity in *Candida albicans* infections and saprophytism. J. Leukoc. Biol. 68:175-179.2005.
8. Vazquez, TA. and Balish, E. Macrophages in resistance to candidiasis. Microbiol. Mol. Biol. Rev. 61:170-192.1999.

9. Omani, OL.; Bistoni, F. and Puccetti, P. Initiation of T helper cell immunity to *Candida albicans* by IL-12: the role of neutrophils. Chem. Immunol. 68:110-135.2004.
10. Romani, L.; Mencacci, A; Tonnetti, L.; Spaccapelo, R; Cenci, E. and Bistoni, F. Interleukin-12 but not interferon- $\gamma$  production correlates with induction of Thelper type-1 phenotype in murine candidiasis. Eur. J. Immunol. 24:909-915.1994.
11. Mencacci, A; Saccapelo, R.; Dej, G.; Enssle, K.; Cassone, A.; Bistoni, F and Romani, L. (2006) CD4+T-helper cell responses in mice with low level *Candida albicans* infection. Infect. Immun. 64:4907-4915.
12. Odds, F C. *Candida* and candidiasis. 2<sup>nd</sup> ed London. Bailliere-Tindall. PP.68.1988.
13. Diamond, RD. *Cryptococcus neoformans*.In Mandell ,GL.;Douglas,RG.;Bennett,JE.ed.In principles and practice of infectious diseases. New York, NY. Churchill Livingstone. 2331-2340.1990.
14. Kobayashi, SD, and Cutler,JF.*Candida albicans* hyphal formation and virulence :is there a clearly defined role?.Trends Microbiol.6:2-4 2005.
15. Romani,L.;Mencacci,A;Cenci,E.;Delsero,G.and Puccetti, P. An immunoregulatory role for neutrophils in CD4+ T helper subset selection in mice with candidiasis.J.Immunol, 158:2356-2362.1999.
16. Romani, L.Immunity to *Candida albicans*: Th1, Th2 and beyond.Curr.Opin.Microbiol.2:363-367.1999.
17. Lodder, J.The yeast,A taxonomic study 2<sup>nd</sup>.Revised and enlarged edition 1974.Edited by :J.Lodder.1974.
18. Hiallibarton,BL.and Blazkovec,AA.Delayed hypersensitivity and acquired cellular resistance in guinea pigs infected with *Listeria monocytogenes*.Infect Immun.11:1-7.1975.
19. Luna,LG.Manual of histologic staining methods of the armed forces institute of pathology. 3<sup>rd</sup>.Ed. Mcgraw-Hill, New York.
20. Smith,R.;Adam,B.;Sowa,AG. And Ficht,TA.Induction of lymphocyte responsiveness by the outer membrane-peptidoglycan complex of rough strain of *Brucella abortus*, .Vet. Immunol, Immunopathol. 26:31-48.1990.
21. Dzata, GK.; Wyckoff, JD. And Confer, AW. Immunopotential of cattle vaccinated with a soluble *Brucella abortus* antigen with low LPS content:an analysis of cellular and humoral immune response.Vet.Microbiol.29:15-261991.
22. Vazquez,TA. And Balish,E.Macrophages in resistance to candidiasis. Microbiol. Mol. BIOL. Rev. 61:170-192.2006.
23. Friedman, H.Stress and microbial infection. In: Psychoneuroimmunology, stress and infection, edited by Friedman,H.,KleinmTW. And riedman, AL. New York:rcr,1996, P.64-78.
24. Cristana,FE;Ginseppe,DS;Angela,B.;ClaudiamM; Antonio,S and Romani,L.Dendritic cells discriminate between yeast and hyphae of the fungus *Candida albicans*:Implication of initiation of T helper cells immunity in vitro and in vivo.J.Exp.Med.191:1661-1674.2007.
25. Hsieh, CS; Macatonia, SE.; Tripp, CS.; Wolf,AO. And Murphy,KM.Development of Th1 CD4+ Tcells through IL-12 produced by *Listeria* -induced macrophages. SCIENCE. 260:547-549.1993.
26. Kaufmann, SH.Immunity to intracellular bacteria. Annu. Rev. Immunol. 11-129-163.1997.
27. Yifan,Z;Yang,JI. And Christina,C.Cytokine response of T.cell subsets from *Brucella abortus* infected mice to soluble Brucella protein.Infec.Immun.61:2481-2847.1993.
28. Blasi,E.;Pitzurra,L;Bartoli,A.;Puliti,M. and Bistoni, E.Tumor necrosis factor as an autocrine and paracrine signal controlling the macrophage secretory response to *Candida albicans*. Infect. Immun. 62:1199-1206 .1994.

29. Maria,CG.;Claudia,S.;Maria,EC.;Beatriz,SR.and Silvia,C.Immunocompetence of macrophages in rat exposed to *Candida albicans* infection and stress. Am. J. Physiol. Cell Physiol.284:111-118.2005
30. Rittig, MG.; Burmester,GR. And Krause, A.Coilin phagocytosis:when the zipper jams,the cup is deformed. Trends Microbiol. 6:381-388.1998.
31. Beno,DW;Stover,g. and Mathews,HL.Growth inhibition of *Candida albicans* hyphae by CD8+ lymphocytes. J. Immunol. 154:5273-5281.1998.
32. Ghannoum, MA.Secreted fungal phospholipase C facilitates *Candida albicans* dissemination.ASM conference on Candida and candidiasis.12P12-15.2003.
33. Cossar,P.Host/pathogen interaction:Subversion of mammalian cell cytoskeleton by invasive bacteria. J. Clin. Invest.99:2307-2311 1997.