Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

*Available online at*www.jcbsc.org

Section B: Biological Sciences

CODEN (USA): JCBPAT Research Article

The physiological effect of *Cryptococcus neoformans* infection on laboratory mice

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Received: 04 June 2015; **Revised:** 18 June 2015; **Accepted:** 23 June 2015

Abstract: *Cryptococcus neoformans* is one of the opportunistic yeast that found naturally in the soil, plants and food industrial products. The study represents the effect of this yeast species on immunocompetent laboratory mice. Balb/c mice (2-4 months age, 20-25 gm weight) in three groups A,B,C (6 each: F & M) were intra peritoneal injection with 0.1 ml of identified yeast suspension in three concentrations (1×10^{4} , 1×10^{6} and 1×10^{8} cell/ ml^{3} respectively) for two weeks (once/ week), while the fourth group D was control (injected with 0.1 ml Distilled water). All groups monitored for 70 days, after that period animals were sacrificed. During the experimental period the infected animals showed unstable behavior with necrosis in their front limbs after 7 and 17 days of injection. The blood tests showed significant decreasing in the Hb concentration especially in group B (3.033 mg/100ml). The total leukocytes were increased insignificantly especially in group B while the percentage of neutrophils and basophiles were increased significantly in this group (44% and 2% respectively). The group C showed significant increasing in monocytes percentage (21.8%). The biochemical parameters showed significant increasing in AST in group C (139.67 IU/L) and ALT levels in group B (60.33 IU/L) which are indicator of liver damage.

Keywords: *Cryptococcus neoformans*, albino mice, intra- peritoneal injection, blood parameters.

INTRODUCTION

The *Cryptococcus neoformans* is one of the opportunistic yeast that infects immuno compromised patients and increased their infection in AIDS patient to 5-13 % [1]. The yeast classified as true

pathogen that caused death in immunocompetent hosts [2, 3, 4, 5 and 6]. This type of yeast was found naturally in soil, fruits, vegetables, diary and juice products also in wood and eucalyptus residues. The infection with *C. neoformans* was through inhalation that entered the body and spread by lymph or blood [4,7,8,9] and caused infection in human lungs and central nervous system [(10)]. The yeast characterized by oval- spherical capsule with 1-30 nm in thickness and 4-10 nm in diameter and uni-multi cell [5, 9, 11]. The inflammation response to *C. neoformans* infection varies in the hosts depending on the yeast virulence [12] that correlated with capsule formation including glucuronoxylomannan, galactoxylomannan and manno-proteins which in turn stimulate antibodies information and suppressed the phagocytosis and inherent factor including melanin [13] . The pathogenicity is depending on capsule size that can be induced laboratory by increasing carbon dioxide level and decreased the iron concentration, suggesting the size is an important factor in host infection especially in mammals [1,14]. The aim of this study to represent the pathogenesis of *Cryptococcus neoformans* infection after intra- peritoneal injection in mice to investigate their effects on blood parameters and liver enzymes.

MATERALS AND METHOD

Animals: female and male Balb/c albino mice aged 2-4 months and weight 20-25 gm was used in the study in four groups A,B,C and D (6 mice:3 F & 3 M for each group).

Yeast: *Cryptococcus neoformans* sample was isolated and identified in Mycology laboratory / College of Science / Basrah University. The yeast grow on sabouraud –dextrose agar (SDA) in 25 C for 4 days. The suspension prepared according to the method [15]. Three concentrations were used 1×10^4 , 1×10^6, 1×10^8 cell/ ml^3 .

Experimental infection: Three groups of animals (A, B and C) were intra-peritoneal injection with 0.1 ml of yeast suspension of each concentration for two weeks (once/week) while the fourth group (D control) injected in distilled water all groups were monitored for 70 days, after that the animals were sacrificed after anesthetized. Blood samples were obtained from heart puncture and collected in EDTA- tubes for blood parameters [16,17]. Liver alanine transamianse (ALT) and aspartate transaminase (AST) were measured by enzymatic method using diagnostic kit from Biolabo companies (France). The organs liver and alimentary canal were obtained in all groups also to isolated and identify the yeast capsules after squashing the organs with Indian ink or cultured samples of infected livers on SDA medium.

Statistical analysis: Data were analyzed by one- way or two way ANOVA using a general liner model procedure using SPSS version 15 statistic program. Comparisons between means were made using least significant differences (LSD). Differences were considered to be significant at $p<0.05$.

RESULTS

The intra peritoneal injection of *Cryptococcus neoformans* caused infection in all animal groups A,B and C and that notes from necrotic lesions in the front limbs in the infected animals after 7 days in group A (pic 1) and 17 days in group (pic 2) also caused mortality after 33 and 53 days of infection in group B.

The animals in the experimental groups were observed to increase their activity and instability in their behavior compared to normal activity in the control group.

The sacrificed animals showed infections that observed in many parts of alimentary canal especially in group C with high dosage that characterized by granular abscess, inflammatory swelling , granular nodules and liver fibrosis (pic 3 and 4) related to yeast capsules (pic 5 and 6).

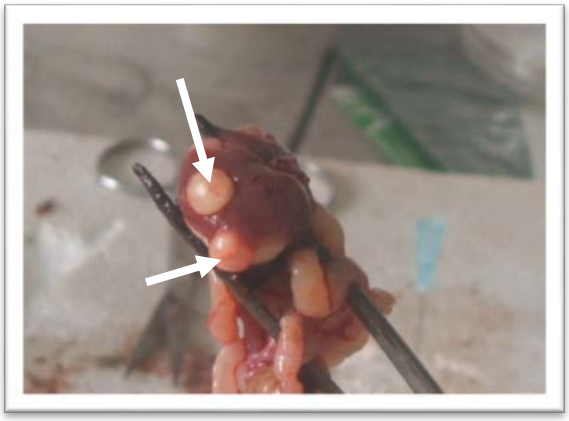
The statistical analysis of blood parameters showed significant ($p<0.05$) diminished in HB levels in all experimental groups and the least value in group C (3.033 gm/ml) compared to control group (9.167 gm /ml) (fig 1) while there is no significant increasing in leukocyte number in experimental groups especially in group B (5.285×10^3 cell/ml) (fig 2).

The results referred to significant ($p< 0.05$) increasing in neutrophils and basophils number in group B (44% and 2 % respectively) compared to control group D (28% and 0.5% respectively) (fig 3) while the monocytes increased significantly ($p< 0.05$) in group C (21.8%) compared to control group (16.83%). The group B showed significant decreasing in lymphocyte number 37% compared to control 52.6% (fig 4).

The biochemical parameters results showed significant ($p< 0.05$) elevation in AST values especially in group C (139.67 IU/L) (fig 5) while the elevation of ALT value was insignificant compared to control group and the higher level in group B (60.33 IU/L) (fig 6).

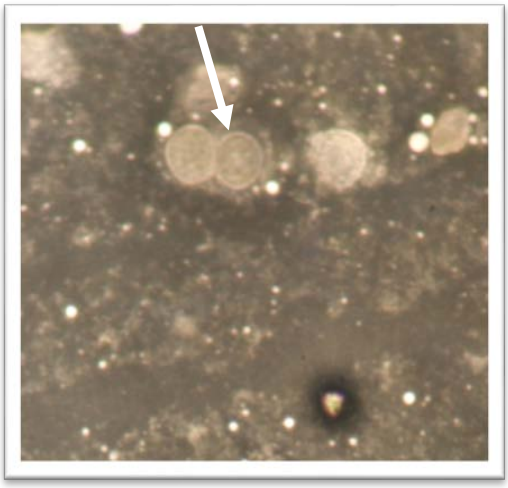
Picture 1: necrotic lesions in front limb of infected mouse with *C.neoformans* in group A after 7 days of injection

Picture 2: necrotic lesions in front limb of infected mouse with *C.neoformans* in group B after 17 days of injection time.

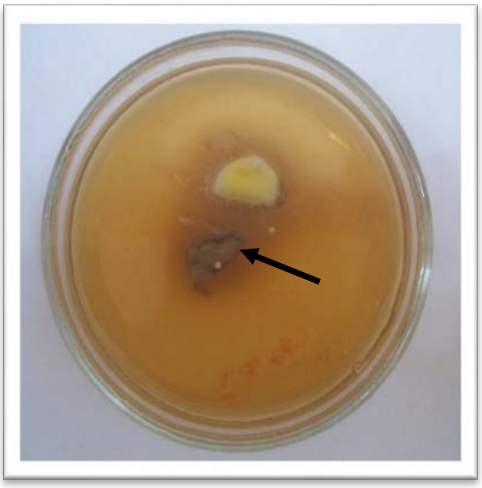


Picture 3: abscess and inflammatory swelling in liver of infected mice after 70 days of injection time.

Picture 4: abscess and inflammatory swelling in alimentary canal of infected mice after 70 days of injection time



Picture 5: direct examination of *C.neoformans* capsules from infected orangs using Indian ink.



Picture 6: *C.neoformans* isolated from infected liver of injected mice.

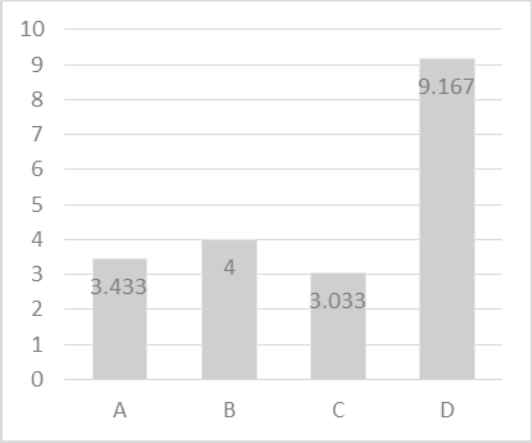


Figure 1: effect of intra peritoneal injection of *C.neoformans* on hemoglobin levels in experimental groups(A, B and C injected, D control), gm/100 ml.

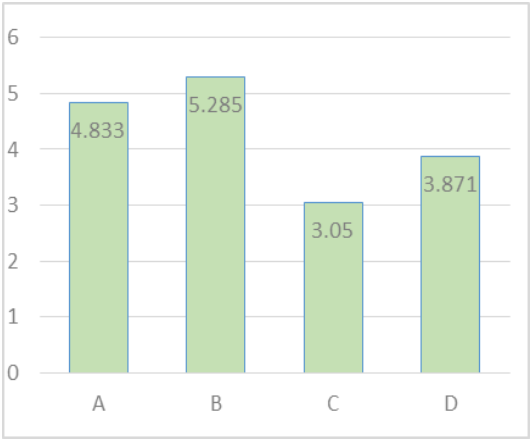


Figure 2: effect of intra peritoneal injection of *C.neoformans* on total leukocytes numbers in experimental groups(A, B and C injected, D control

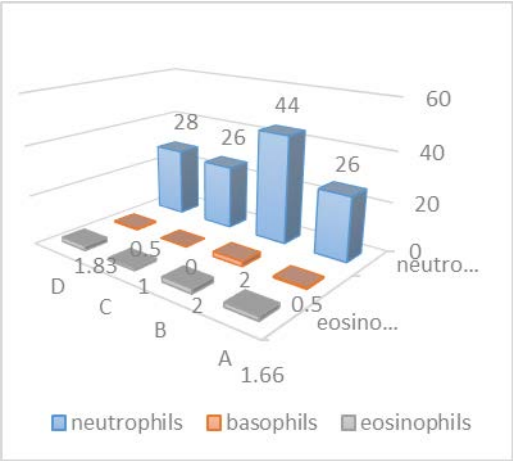


Figure 3: effect of intra peritoneal injection of *C.neoformans* on granular leukocytes numbers in experimental groups (A, B and C injected, D control)

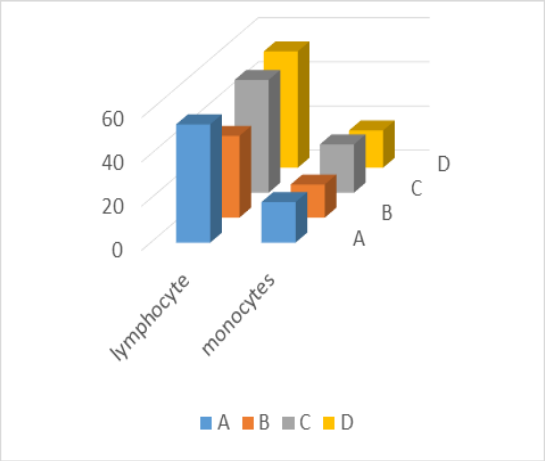


Figure 4: effect of intra peritoneal injection of *C.neoformans* on lymphocyte and monocyte numbers in experimental groups(A, B and C injected , D control) .

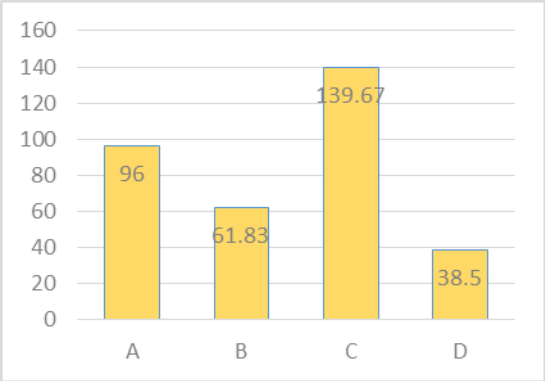


Figure 5: effect of intra peritoneal injection of *C.neoformans* on AST enzyme in experimental groups(A, B and C injected, D control). IU/L

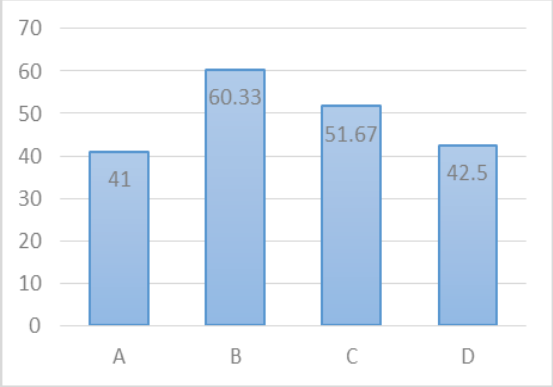


Figure 6: effect of intra peritoneal injection of *C.neoformans* on ALT enzyme in experimental groups (A, B and C injected, D control). IU/L

DISCUSSION

The instability behavior that observed in infected mice with *Cryptococcus neoformans* yeast were agreed with some observations recorded by other researchers including irritation in central nervous system, instable movements and necrosis in front limbs in addition to signs of inflammations [18,20].

The virulence of yeast infection was depending on type of laboratory animals, and in this study the infection of albino mice may related to be more sensitive than other animal types like rabbits which represent high resistance to yeast infection and need to depress their immunity for infection [19].

The diminished of Hb value in the infected animals (groups A,B and C) may related to the ability of *C. neoformans* to used iron for growing therefore the yeast will compete with the host to use iron by destroying protein – containing iron like hemoglobin [20] by converting ferric ion to ferrous more suitable form of iron to use by the yeast [21].

The increasing of leukocyte number (neutrophils and monocytes) in infected mice may related to be the first line of defense against infection but their relation with yeast infection was not clear may be returned to yeast ability to prevent the phagocytosis by intracellular or extracellular existing or related to capsules formation [22].

The elevation in AST and ALT enzymes levels resulted to the damage the cells of the infected livers as these enzymes highly accumulated in liver, heart, kidney and muscles of the body compared to the low levels in blood stream so their values increased during liver infection with yeast when hepatocytes undergoing destroying and showed necrosis [23].

CONCLUSION

This study elevated the physiological effectiveness of *Cryptococcus neoformans* infection on laboratory mice, the results demonstrated that infection caused irritation in central nervous system with inflammatory symptoms (necrosis) and damage of liver by elevated in AST and ALT levels in addition to anemia by diminished blood Hb also induced immune system by increasing leukocytes numbers.

ACKNOWLEDGEMENT

The authors thank Assistant professor Shereen J. Al- Ali for help in this work also to Biology Department- College of Science- University of Basrah to carry out the research work.

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