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Antibacterial effect and immunomodulatory of culture leachate antigen for Candida albicans

Abstract:

This study was conducted to determine the antibacterial and immunomodulatory effect of the culture filtrate extract of *Candida albicans*.Male mice aged between 10 and 12 weeks were selected and placed in clean, sterile houses at temperature of (20_25) °C, with dark periods of approximately ten hours per day. They were provided with water and food, and their weights ranged between (19_35) grams. Before conducting the experiment, 6 groups were taken, each consisting of 6 mice. Bacterial doses were determined by measuring turbidity using a Densi check device, and the bacteria were diluted with normal saline solution at a turbidity of 3.5 for *Escherichia coli* and 4 for *Staphylococcus aureus*, and the dose was determined as 1.5 ml.

Group1(G)1 :normal group(Control group).

G2:Which was given the culture leacha antigen for *Candida albicans*.

G3:Which Was given *staphylococcus aureus*._

G4:Which was given *staphylococcus aureus* and treated with *candida albicans* antigen.

G5:Which was given *E.coli* and treated with candida albicans antigen.

G6:Which was given *E.coli.*

Then they were given *Candida albicans* extract after confirming their infection, and according to the weights of the mice used. The suspension was dissolved in 0.2 ml of distilled water. Blood was drawn again on the 14th day for immunological examinations.

The results showed that mice injected with *Staphylococcus aureus* bacteria and treated with the antigen had the highest levels of lymphocytes (173.6±2.19 pg/ml), followed by mice injected with *E. coli* bacteria and treated with the antigen (157.9±1.71 pg/ml). Compared to the control group (38.42±0.16 pg/ml), this increase indicates a strong immune response resulting from dual stimulation of bacteria and antigen.

Histological examination of the liver showed central vein, hepatocytes, and hepatic blood sinu. soids, with Kupffer cells.

التأثير الضد بكتيري والمعدل المناعي لمستخلص الراشح الزرعي للمبيضات البيض (Candida albicans)

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مستخلص:

اجريت هذه الدراسة لمعرفة التأثير الضد بكتيري والمعدل المناعي لمستخلص الراشح الزرعي للمبيضات البيض تم اختيار فئران ذكور بأعمار تتراوح بين 10 و12 أسبوعًا، ووُضعت في بيوت نظيفة ومعقمة بوجود الضوء ودرجة حرارة تتراوح بين 25–20 درجة مئوية، مع فترات مظلمة تبلغ حوالي 10 ساعات يوميًا. تم توفير الماء والغذاء لها، وتراوحت أوزانها بين 19 و35 جرامًا. قبل إجراء التجربة، تم عزل مجاميع الفئران بشكل منفصل، حيث تم أخذ 6 مجموعات تتألف كل منها من 6 فئران. وتم تحديد الجرعات حيث تم قياس العكورة باستخدام جهاز Densi check وتم تخفيف البكتيريا بمحلول ملحي عادي. العكورة بلغت 3.5 لبكتيريا E. Coli و4 لبكتيريا Staphylococcus aureus، وتم تحديد كمية الجرعة بـ 1.5 مل

المجموعة الاولى مجموعة السيطرة

المجموعة الثانية تم اعطائها مستضد الراشح الزرعي للمبيضات البيض

المجموعة الثالثة تمت اصابتها staphylococcus aureus

المجموعة الرابعة تمت اصابتها staphylococcus aureus ومعالجتها بمستضد الراشح الزرعي

المجموعة الخامسة تمت اصابتها ب e.coli ومعالجتها بمستضد الراشح الزرعي

المجموعة السادسة تمت اصابتها ب e. Coli

بعد إعطاء جميع مجموعات الفئران الجرعات المحددة من البكتيريا عن طريق الفم، تم إعطاؤها مستخلص المبيضات البيضاء بعد التأكد من إصابتها،وحسب أوزان الفئران المستخدمة. تم قياس الكمية بواسطة مايكرو بيبت وإذابة المعلق في 0.2 مل من الماء المقطر. تم سحب الدم مرة أخرى في اليوم الرابع عشر لإجراء الفحوصات المناعية.

أظهرت النتائج أن الفئران التي حقنت ببكتيريا Staphylococcus aureus وعولجت بالمستضد سجلت أعلى مستويات للخلايا الليمفاوية (173.6±2.19 pg/ml)، تلتها الفئران التي حقنت ببكتيريا E. coli وعولجت بالمستضد (157.9±1.71 pg/ml). مقارنة بمجموعة السيطرة (38.42±0.16 pg/ml)، تشير هذه الزيادة إلى استجابة مناعية قوية ناتجة عن تحفيز مزدوج للبكتيريا والمستضد. أظهر الفحص النسيجي لعضو الكبد الوريد المركزي وخلايا الكبد والجيوب الدموية الكبدية ، مع ظهور خلايا كوبفر .

Introduction

Escherichia coli is a gram-negative bacillus, motile by means of peritrichous flagella that surround the entire body and does not form spores. Its colonies are smooth, slightly convex, moist, not mucous or mucous when they have a capsule structure, with a complete sharp edge, and shiny pink. On MacConkey agar medium, green metallic sheen, on Eosin Methylene Blue (EMB) agar medium.(kozel *et al*., 2017).

One of the virulence factors of *Escherichia coli* is its possession of curli fibers, which possess physical and chemical properties that facilitate the process of biofilm production by the bacteria. The bacteria also possess vesicles located in the outer-membrane vesicles that bind with bacterial toxins, enzymes, and adhesion factors, as they act as A facilitation system that facilitates sending them to the host cells, and there are also outer-membrane proteins (OMPs), secreted toxins and secretions, all of which work to increase the ability of bacteria to infect. (Jensen *et al*., 2022) Fimbriae or Pilli are of three types (F- Fimbrial, S-fimbrial, P-fimbrial) and Type 1 Pilli, which is one of the most important factors that help bacteria adhere to the host's tissues, giving them the ability to form a biofilm and thus increasing their ability to acquire resistance to antibiotics (Vogel *et al*., 2023).

On the other hand, virulence factors play a pivotal role in enabling *Staphylococcus aureus* to invade host tissues, making it a serious health threat, especially in cases of food poisoning and skin infections. Exotoxins and selective adhesions are considered among the most important biological weapons used to attack body tissues, which makes understanding and diagnosing their mechanisms of action a necessary step in combating infection(Tong *et al*.,2015).

In this context, biological immunomodulators are substances that mediate immune system action mechanisms by stimulating immunity against a specific antigen or enhancing the effectiveness of a vaccine (Jensen *et al*., 2022).

Candida albicans is a type of yeast commonly found in the human body, especially in the digestive tract, mouth, and genital area. It acts as an opportunity pathogen, capable of causing infection in individuals with weakened immune systems or disruption of their

normal microbial flora. This duality of existence within the human body – as a harmless resident and as a potential threat – makes it a topic of great medical interest (Talapko *et al*., 2021).

Bacteria have developed many mechanisms to get rid of the effect of antibiotics. These mechanisms can be summarized according to what was stated in (Besch-Williford *et al*., 2007) and as follows:

1- Producing modified enzymes that inhibit the action of antibiotics, such as β-lactamase enzymes, which inhibit penicillin by cutting the β-lactam ring and thus stopping the effect of the antibiotic.

2- Reducing the permeability of the cell membrane and thus reducing the entrance of toxic substances and antibiotic .

3- Modifying the target site, where the bacteria work and create new targets in which the antibiotic work without affecting the bacterial cell.

4- Extrusion of antibiotics out of the cell using efflux pumps located in the cell membrane.

Materials and Methods

Immunological examination including ELISA technique in which

Rate Lymphocyte Function Associated antigen 3 (LFA-3)ELISA technique was used.

Screening principle:

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa strip plate provided in this kit is precoated with an antibody specific for LFA-3. Standards or samples are added to the wells of the appropriate Microelisa strip plate and conjugated to the specific antibodies. A Horseradish peroxidas (HRP)-conjugated antibody specific for LFA-3 is then added to each Microelisa strip plate well and incubated. Free components are washed off. TMB substrate solution is added to each well. Only those wells containing LFA-3 antibodies conjugated to LFA-3 and HRP will appear blue and then turn yellow after addition of stop solution. Optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the LFA-3 concentration. You can calculate the concentration of LFA-3 in samples by comparing the OD of the samples to the standard curve.

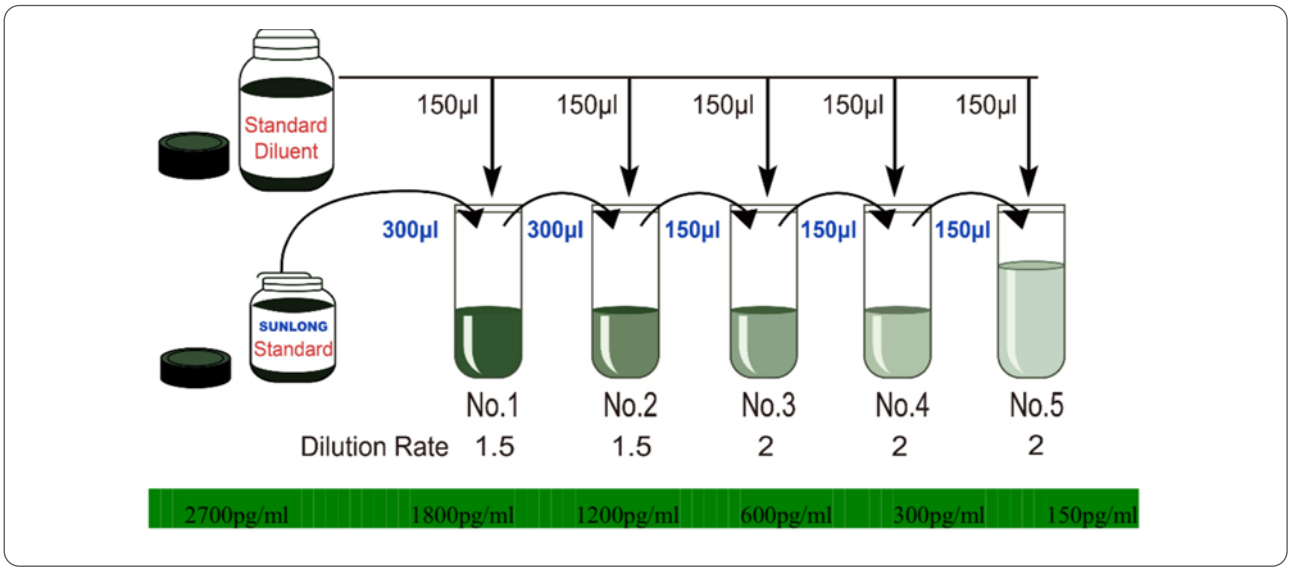
Sample preparation

Serum preparation: After collecting whole blood, allow the blood to clot by leaved it undisturbed at room temperature. taked 10 to 20 minutes. Removed

the clot by centrifugation at 2000-3000 rpm for 20 minutes.

Inspection method:

1. Prepare the dilutions: We diluted the standard with small tubes first, then pipeted a 50ul volume from each tube into a small well, each tube using two wells, for a total of ten wells.



2. In the Microelisa strip plate, leaved an empty well as a blank control. In the sample wells, 40 µl of sample dilution buffer and 10 µl sample are added (dilution factor is 5). Samples should be loaded to the bottom without touching the wall of the well. Mixed well with gentle shaking.

3. Incubation: Incubated for 30 minutes at 37°C after closed with a sealing plate membrane.

4. Dilution: Diluted the concentrated wash buffer with distilled water.

5. Washing: Carefully removed the sealing plate membrane, blew it out and refilled it with washing solution. Discarded the wash solution after resting for 30 seconds. Repeated washing for 5 times.

6. Added 50 µl HRP-Conjugate reagent to each well except the blank control well.

7. Incubated as described in Step 3.

8. Washed as described in step 5.

9. Staining: Added 50 µl chromogen solution A and 50 µl chromogen solution B to each well, mixed with gentle shaking and incubated at 37°C for 15 minutes..

10. Termination: Added 50 µl stop solution to each well to terminate the reaction. The color in the well changed from blue to yellow.

11. Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero.

Preparation of tissue sections

Organs were taken from rats and placed in 10% formalin solution until the tissue sections were prepared. The tissue sections were prepared according to the method mentioned in Al-Hajj (2010) in the Life Sciences Laboratory, College of Science, Tikrit University as follows:

Fixation: The targeted organs were fixed immediately after dissection of the rats with 10% formalin fixative (90 ml of water + 10 ml of 40% formaldehyde) for 24 hours.

Washing: The samples were washed with running water to remove the fixative residue for half an hour.

Dehydration: The samples were passed through increasing concentrations of ethyl alcohol (30-50-70-80-95-100)% in order to gradually remove water from them, and the duration of each pass was 30 minutes. The absolute alcohol step (100%) was repeated twice to complete the complete removal of water.

Clearing: The samples were placed in xylene for 30 minutes in two stages to make the tissue more transparent. Infiltration: The specimens were placed in a mixture of xylene and paraffin wax melted at 58°C (1:1) for 15 minutes, then passed through melted wax for half an hour with three passes to ensure even penetration of the wax.

Embedding: The specimens were quietly embedded in melted wax inside an L-shaped iron mold, and the information (group name and organ type) was written and stuck to one side of the mold using forceps. A hot needle was passed near the model to eliminate any bubbles that might be present around the sample, then the sample was left to cool and solidify after which it was separated from the molds.

Trimming and Sectioning: The wax molds containing the specimens were trimmed with a sharp blade, then placed on a holder fixed to the rotary microtome, and cut at a thickness of 5 micrometers. The strips containing the tissue sections were transferred to a water bath at 40°C to spread the tissue and prevent the cells from accumulating on top of each other. The sections were carried on glass slides marked with a diamond pen (organ type The group name), after wiping it with the loading medium (Mayer's albumen)

prepared by adding 1 ml of egg white and 1 ml of glycerol. The components were mixed in a flask, then the mixture was filtered several times using several layers of medical gauze, and the filtrate was collected in a tightly capped bottle placed in the refrigerator until use after adding 1 g of thymol crystals to prevent mold. The slides were placed on a hot plate at 40°C and left to dry.

Staining: Harris Hematoxylin & Eosin was used according to the method of Luna (1968), and the tissue sections were stained according to the method of Humason (1997) as follows:

The glass slides were passed through xylol twice for 3 minutes each time to remove the wax.

Then they were passed through a series of decreasing concentrations of ethyl alcohol (100-30)% for 3 minutes for each concentration.

Sections were stained with hematoxylin solution for 5 minutes, then washed with running water for 3 minutes to distinguish the color and remove excess dye.

Stained with eosin for 15 seconds, then washed with water in dishes for 5 seconds to distinguish the color.

Passed with a series of ascending concentrations of ethyl alcohol (30-100)% for 2 minutes for each concentration.

Placed in dishes on xylol for 5 minutes for the purpose of clarification.

Mounting: The slides were covered with the mounting medium (Dibutylphthalate Polystyrene Xylene - D.P.X.) for the purpose of mounting, then a glass cover was placed on them and left on a hot plate at a temperature of 40ºC to accelerate drying, then stored in their special boxes.

Results and discussion of immunological test

The results of the statistical analysis are shown in Table (4-1). An increase in the Lymphocyte level is observed at a level of $P<0.05$ in the blood serum of the group of mice that were injected with *Staphylocccus aureus* bacteria and treated with the antigen, followed by the group that was injected with *E coli* bacteria and treated with the antigen, which amounted to (173.6±2.19 pg/ml).) and (157.9±1.71 pg/ml) compared to the rest of the groups and the control group, which amounted to (38.42±0.16 pg/ml).

Table (1_4) lymphocyte concentration level in blood serum

Mean ± SD			groups
38.42	±	d 0.16	Control group
102.1	±	c 1.00	Candida albicans antigen
125.6	±	b 1.85	*Staphylocccus aureus*
173.6	±	a 2.19	*Staphylocccus aureus* treated with candida albicans antigen
108.9	±	c 1.38	*E. coli*
157.9	±	a 1.71	*E.coli* treated with candida albicans antigen

Different letters vertically indicate significant differences at $P \leq 0.05$.

Candida species are commonly found as part of the normal microbiota in humans, colonizing various mucosal surfaces and skin (Mendoza-Reyes *et al*., 2022). However, under certain conditions, such as immunosuppression or disruption of normal microbial homeostasis, *Candida* can become an opportunistic pathogen and cause a range of infections, from localized mucosal candidiasis to life-threatening systemic candidiasis (Talapko *et al*., 2021).

Recent research has shed light on the ability of *Candida* to exert antibacterial effects, which may have significant implications for the management of bacterial infections. One of the main factors contributing to the antibacterial properties of *Candida* is biofilm formation (Ramage *et al*., 2012).

Gram-positive bacterial infections, such as those caused by *Staphylococcus aureus*, are commonly observed in research mice (Besch-Williford & LFRANKLIN, 2007). This infection can lead to a range of clinical manifestations, from localized skin and eye infections to life-threatening sepsis (Williford & LFRANKLIN, 2007).

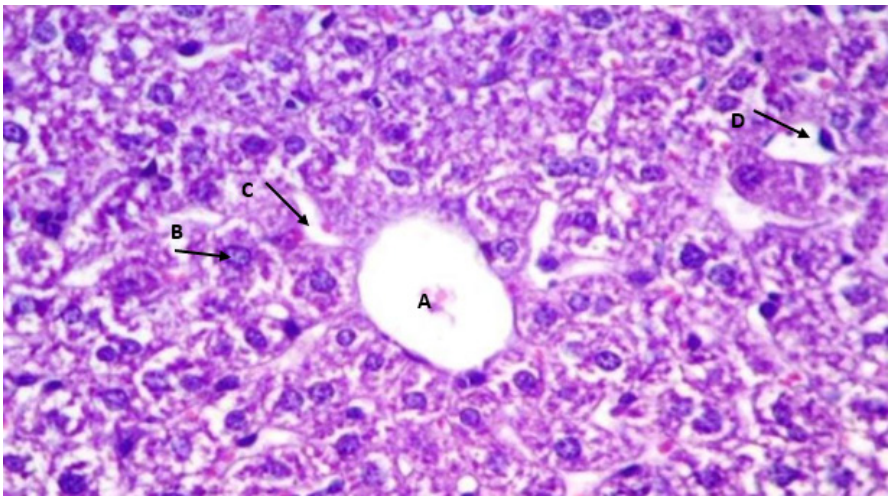
In the current study, we investigated the effect of *S. aureus* infection on the levels of lymphocytes, a key component of the adaptive immune system, in the blood serum of a group of mice. Our results showed a significant increase in the level of lymphocytes in the blood serum of the Candida group of mice injected with *S. aureus* bacteria, compared to the uninfected control

group, and these results were similar to both (Tong *et al*., 2015) and (Thomer et al., 2016).

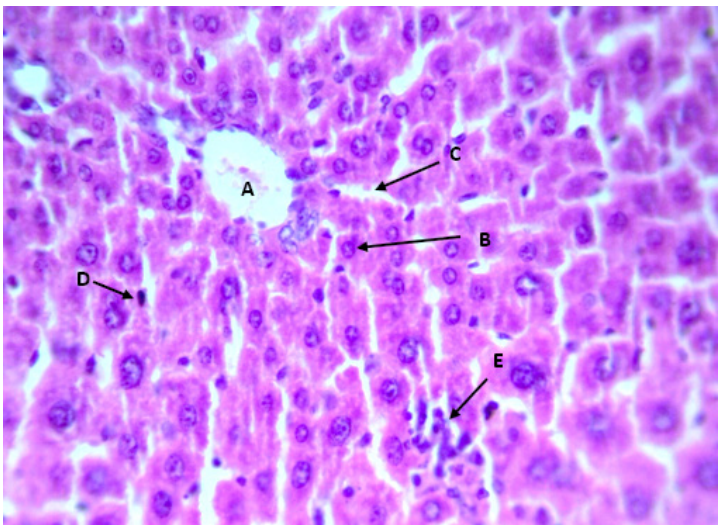
This finding is consistent with the marked T-cell activation and dysregulated inflammation that can occur during *S. aureus* infection (Vogel *et al*., 2023). Infiltration of inflammatory cells, including lymphocytes, into infected tissue is a hallmark of the host immune response to bacterial infection (Hu *et al*., 2020).

Results and discussion of tissue sections:

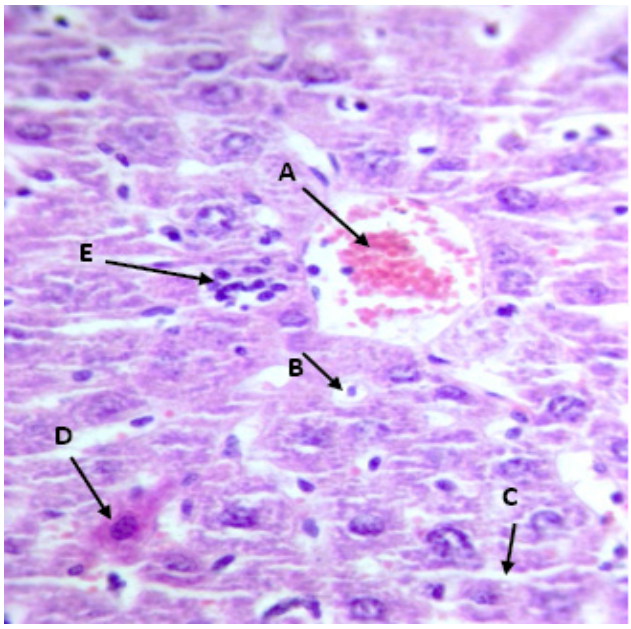
Histological examination of the liver section:



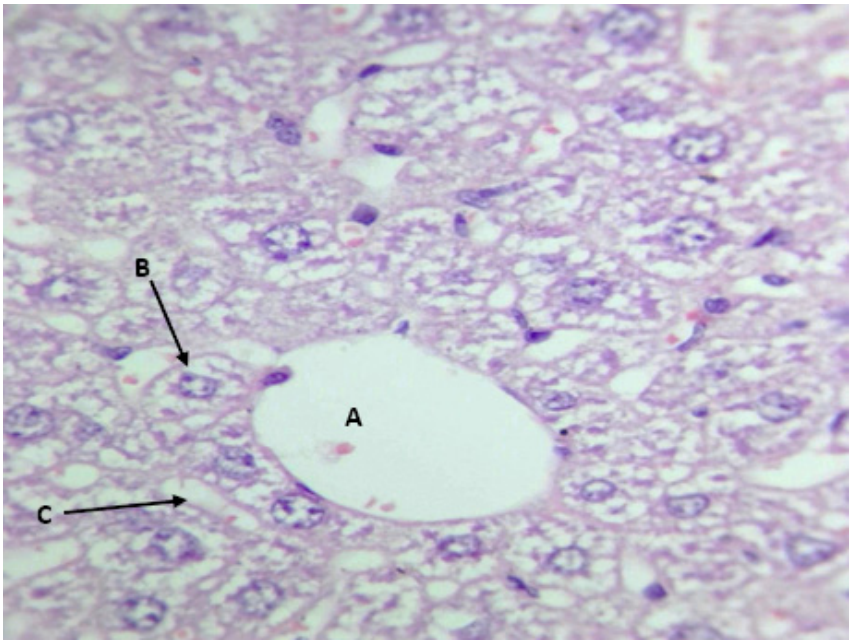
Figure(1)Cross_section of the liver tissue of the control group showing (A)the central vien (B)hepatocytes(C)hepatic sinusoids(D)kupffer cells(H&E)



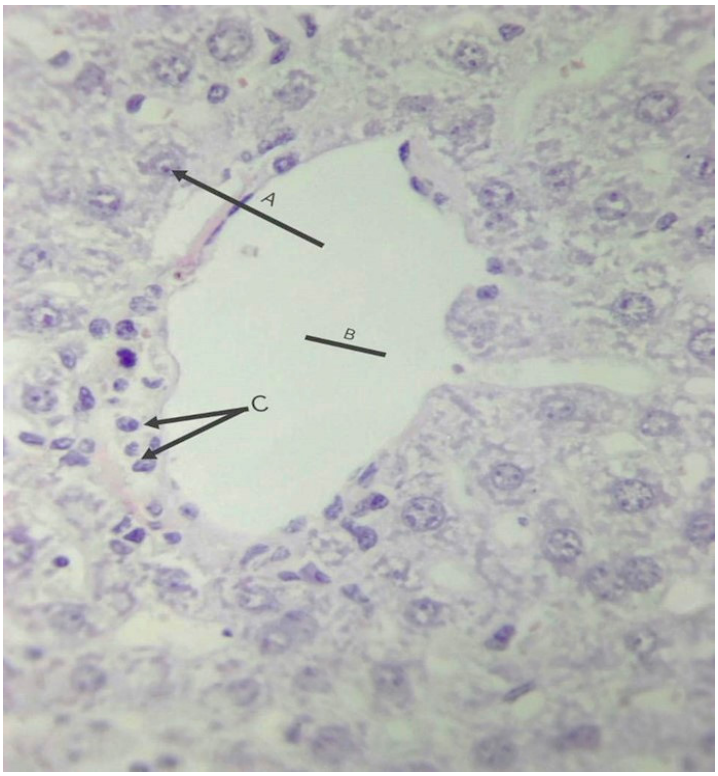
Figure(2)Cross_section of liver tissue of group 2 that was given *candida albicans* antigen showing (A)central vien(B)hepatocytes(C)hepatic sinusoids (D)kupffer cells(E)inflammatory lymphocyte infiltration(H&E)



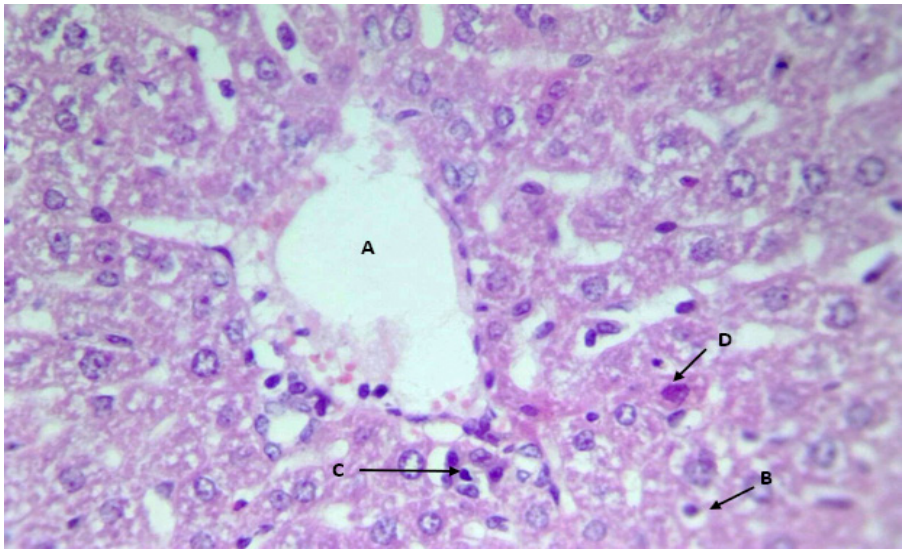
Figure(3)Cross_section of liver tissue of group 3 infected with *staphylococcus aureus* showing (A)central vien hemorrhage (B)hepatocyte necrosis(C)disorganization of sinusoids(D)presence of macrophages(E) infiltration of inflammatory lymphocytes(H&E)



Figure(4)Cross_section of liver tissue of group 4 infected with *staphylococcus areus* and treated with *candida albicans* antigen (A)the central vien appears normal (B)The hepatocyte appear active (C)The sinusoidal reorganization(H&E)



Figure(5)Cross_section of liver tissue of group 5 infected with *E.coli* and treated with *candida albicans* antigen (A)heopatocytes (B)central vien (C)inflammatory cells.



Figure(6)Cross_section of liver tissu of group 6 infected with e.coli (A)central vien (B)necrosis of some cells (C)infiltration of inflammatory lymphocytes (D)presence of macrophages

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