ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluate the Activity Antifungal of Aspirin In Mice Balb/C infected with *Candida albicans In vitro* and *In vivo*

Athraa A A Al-Hilfy and Najwa Mohammed Jameel Ali Abu-Mejdad*

Biology department, Science College, Basrah University

ABSTRACT

The present study was conducted to identify the types of *Candida* in the oral cavity using medium Chrom Agar Candida was diagnosed 5 types of *Candid* and *Candida albicans* was the most type dominance as the percentage of the occurrence of 80% Was selected as the number of isolates and test its ability to produce extracellular enzymes mycotoxins(aflatoxin) and adhesion test where chosen isolates with high efficiency in the production of enzymes , toxins and high affinity adhesion and tested their ability to infect mice so treatment it by aspirin. Evaluated the effectiveness of the drug aspirin in treatment infection *Candida albicans invivo, invitro* and histological study of its effects on the liver and kidney. This study proved that the for aspirin effectiveness of drug high to inhibit the *C.albicans in vitro*, which reach to 25 mm when using drug concentration 500 mg/ ml. Infection caused when animals injected yeast suspension Inside peritonea, changes in tissue livers of animals was the existence of an inflammatory response varying intensity, as shown by histological sections at the kidney level cases of hemorrhage and necrosis in the renal tubule,and found that the drug aspirin effect on in vivo infection has caused drug reduced histologic effects on the liver and kidney compared with the infected samples only.

Keywords: Aspirin, antifungal,candidiasis

February January

Corresponding author
Email:Najwa_22_4_1978@yahoo.com

ISSN: 0975-8585

INTRODUCTION

Candida albicans is considered to be most prevalent cause of mucosal and systemic infections compared to other types of the same genus where there are types of *Candida* in the oral cavity without signs or symptoms are present as mycobiota in the gut , but it when certain conditions such as immune dysfunction can invade the tissue in normal condition resist the incidence of these pathogens , such as the liver and kidneys [1]

Identified pathogenic yeasts based on biochemical tests and phenotypic characters Such as colonial colors on CHROMagar, show reliable methods for identification but not clear cut identification furthermore most of conventional approaches were time consuming (48 to 72 h) [2]

The virulence of *Candida* spp. is attributed to certain factors like adherence which is the first step in order to interfere yeast with the epithelium tissue and then invade the tissues, biofilm formation, and the production of tissue damaging extracellular hydrolytic enzymes[3] Extracellular hydrolytic enzymes like phospholipase and proteinase are important for colonization and invasion of host tissue [4]

The number of resistant fungal pathogenic and toxinogenic species is rising such as *Candida* which produce harmful secondary metabolites (mycotoxins). It was established from this yeast produce particularly harmful toxins such as aflatoxins [5] effect on The liver and kidneys are stressed by the increased responsibility of eliminating it .

These organs are not only important eliminative organs, but they also play a key role in the digestive process. When these organs become congested with *Candida* and mycotoxins, our digestion is further compromised. When normal digestive functions are disturbed.[6]

As a result of the emergence of resistance to many types of *Candida* drugs antifungal So attention turned towards the use of alternative treatments and the need for a new antifungal substances are becoming increasingly obvious. Led many researchers to investigate the antimicrobial effects of chemical compounds [7] .

including aspirin , a drug is a steroid often used alleviation of fever or anti-inflammation named Acetylsalicylic acid or acetosalicylic acid belong to class non-sterodial anti-inflammatory drugs Pathological fungi known to produce prostaglandins and then colonize and infection and aspirin inhibits the production and by following treatment *Candida* yeast infection and studies have been performed in this field[8]

Therefore, the current study aimed to isolate and diagnose some types of yeasts of patients suufered from oral candidiasis and causing experimental infection in mice and laboratory follow-up histological changes in the livers and kidneys it before and after treatment by drug aspirin and study some of the virulence factors of *Candida albicans*

ISSN: 0975-8585

MATERIALS AND METHODS

1- Collection and cultivation of specimens

During this study collected (100) swabs from oral cavity for patients suffered from oral candidiasis in Al-Zubair hospital /Basrah from period 1-4-2013 to 1-10-2013 took the swab from infected tongue surface which diagnosis by specialist doctor.

Including study 82 swab for male and 18 swab for female recorded for each patient information about (genus, age ,address ,job , using drugs and sociologist state) .

Also collected (100) swabs from oral cavity for healthy persons (50 male and 50 female) took swabs by sterile – cotton- swab) Cultured the swab on Sabourauds dextrose agar and incubated at 37 C for 48.

Occurrence percentage%= specimens number of yeast species /total number * 100

2- Examination an identification specimens

Using Chrom agar Candida medium[9] for identification yeasts isolates

Preparation Chrom agar Candida medium as follow :-

Chrom agar Candida medium (Himedia , India) 42.72 gm
D.W 1000 ml

Table (1):the colour variation of *Candida* sp on chrom agar candida medium

S	Colour on medium	Species
1	light green	*Candida albicans*
2	Metallic blue	*C.tropicalis*
3	Red-rose	*C.kruzi*
4	White	*C.parapsilosis*
5	Florescent violet	Other species

3- Aflatoxins production

Test ability of *Candida albicans* for production aflatoxins according to[10]

4- Extracellular enzymes production test

a- gelatinase production (protease production) [11]
b- lipase production [12]
c- phospholipase production

ISSN: 0975-8585

5- Adherence assay [13]

6-Antifungal activity of aspirin in vitro

Teast activity antifungal of *Candida albicans* according to[14]

7-Antifungal activity of aspirin in vivo

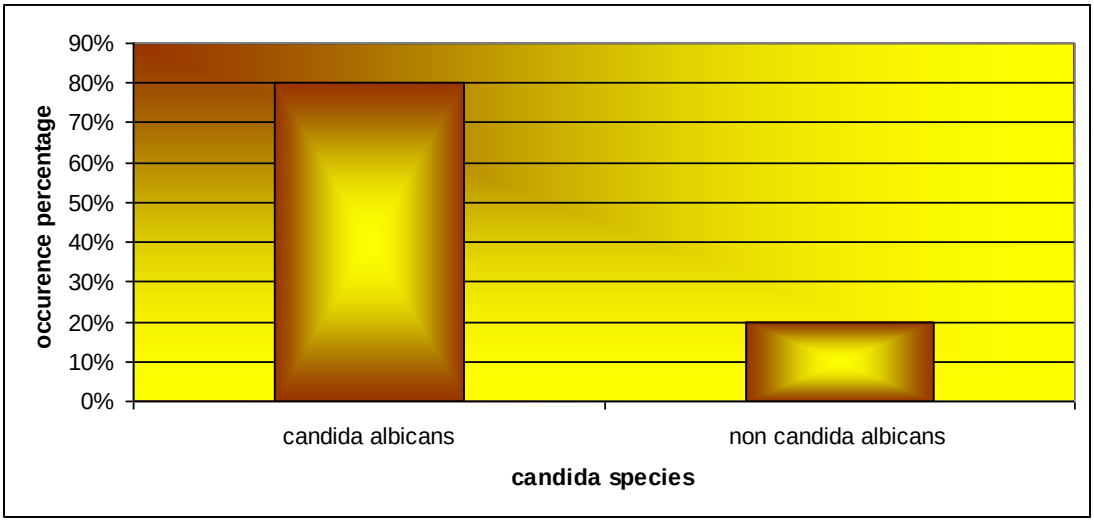
Preparation yeast inoculums

Inoculum was prepared in present study by transfer of part of the colony stimulating on Sabourauds agar center SDA For 48 hours of yeasts To 5 ml of sterile distilled water suspension strongly commentator and adjust the number of cells 3×10^{6} and 3×10^{8} cfu/ml according to MacFrland scale[15]

8-Using experimental mice

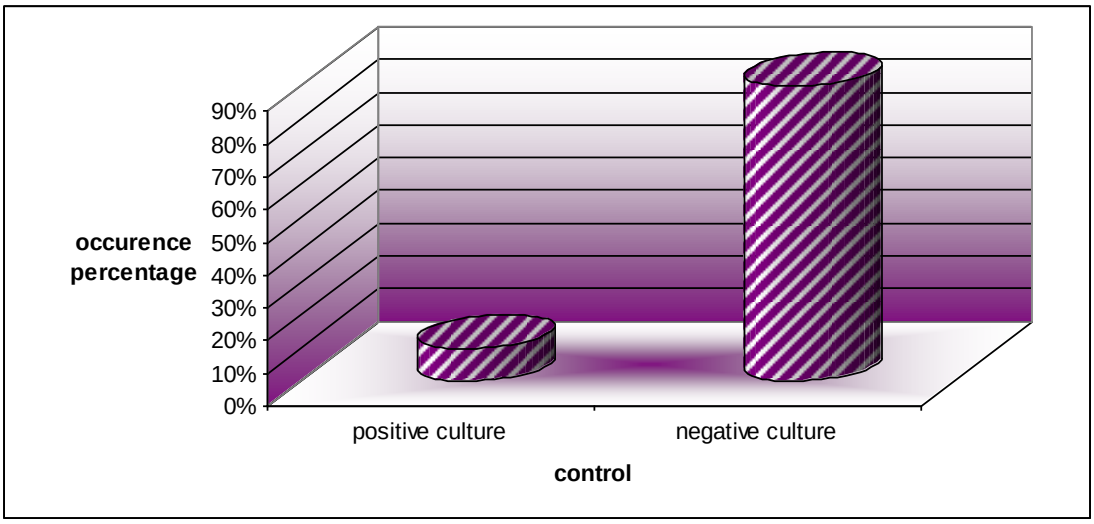
Using during study 16 from male mice strain Balb/c after 2 month . Divided experimental animals into 2 groups each group contain 8 mice .Infected first group $3*10^{8}$ cfu/ml yeast suspension by injection periton while second group infected $3* 10^{6}$ cfu/ml from yeast suspension and treated just 4 mice from second group by 500 mg /ml orally from Asprin drug after 3 days from infection so anatomy an infect experimental mice treatment and non treatment after 1 week from infection and taked parts from livers and kidnies and fixation 10 % Formaline for histological study Preparation liver and kidneys for histological study by using embedding in paraffin wax[16] and stained by Haematoxilin and Eosin stain[17]

RESULTS



Figure(1):occurrence percentage of *Candida* species in patients

ISSN: 0975-8585



Figure(2):occurrence percentage of *Candida* species in control

ISSN: 0975-8585

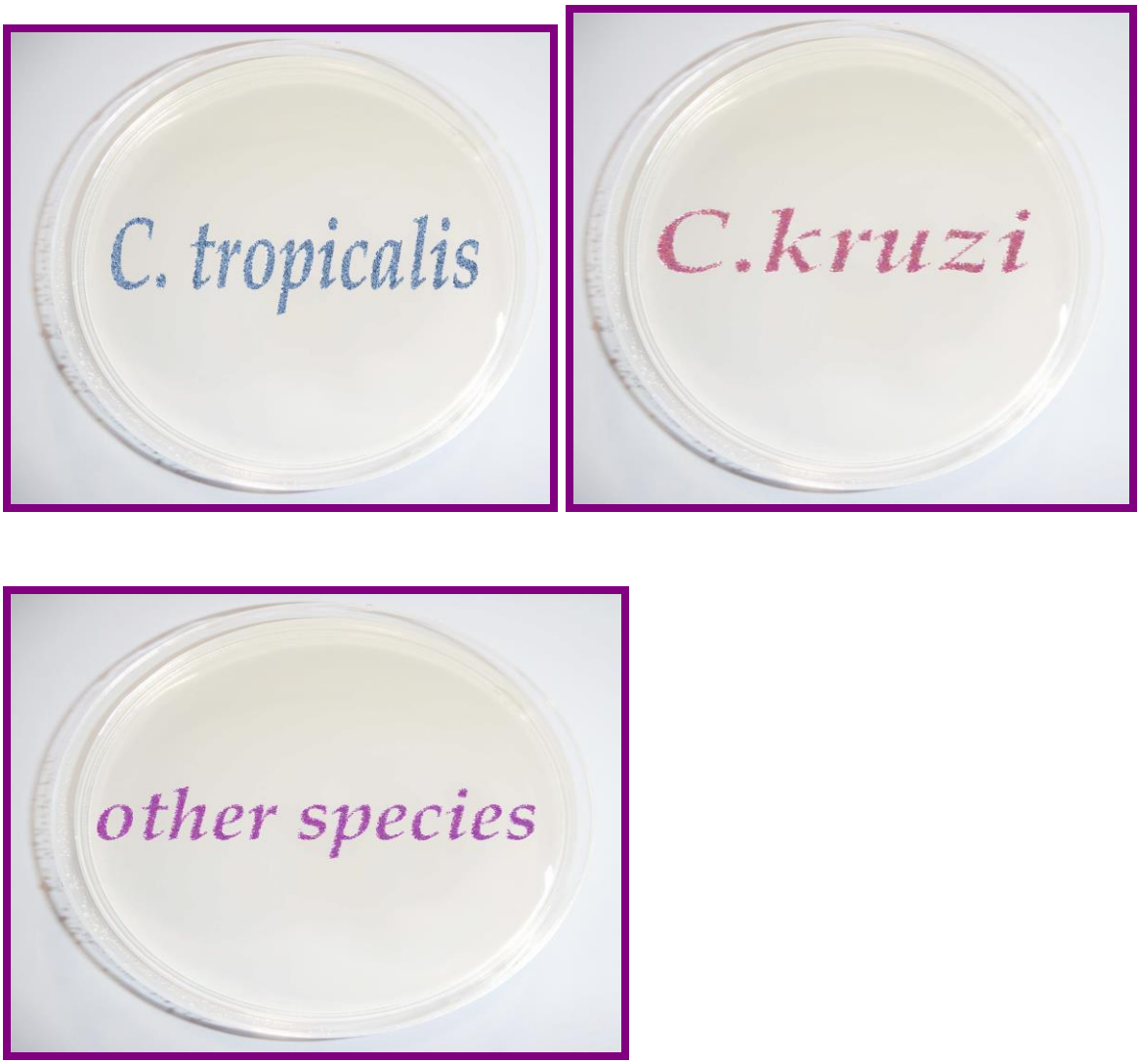


Plate (1):colour variation for species of *Candida* on chrom agar *Candida*

Toxicity test

Showed the result ability of *Candida albicans* is produce aflatoxins by change the colour of coconut agar from white to rose after exposer for ammonia evaporation

Table(2) :positive results of toxicity test

Yeast isolate	Toxicity test
Candida albicans	+

Table (3) Enzymatic activity of *Candida albicans*

Yeast isolate	Enzymatic activity		
Candida albicans	Protease	Phospholipase	Lipase
	+	+	+

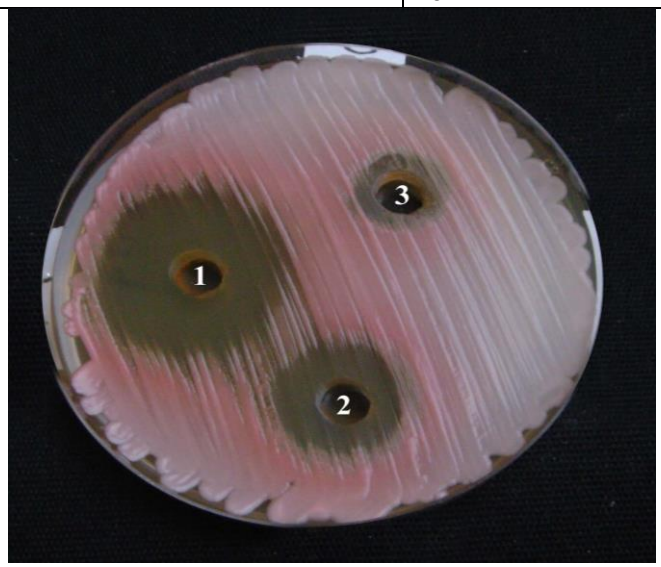
ISSN: 0975-8585

Table (4) Adhesion test of *Candida albicans*

Yeast isolate	Adhesion test
Candida albicans	+

Table (5) effect drug asprin on *Candida albicans in vitro*.

Concentration mg/ml	Inhibition zone(mm) for aspirin after three days
500 mg/ml	25
250mg/ml	15
125 mg/ml	10



Figure(3):Antifungal activity of aspirin against *candida albicans* in vitro Inhibition zone scale by mm(1): 25 mm,(2):15 mm,(3):10 mm

ISSN: 0975-8585

Figure(4): infected liver by *Candida albicans*

Histological study

Liver

showed tissue sections of the livers animals infected with *Candida albicans* severe changes in liver was wide sinusoids hepatic and irregular platelets and nuclei lysis and necrosis was observed yeast cells aggregation between hepatocytes (Figure7.8) .

The results showed histological examination of the livers of infected animals and treatment with aspirin during the experiment period and the presence of liver cells regenerate and nuclei clear with the regularity of platelets and sinusoids hepatic (Figure 9) .

ISSN: 0975-8585

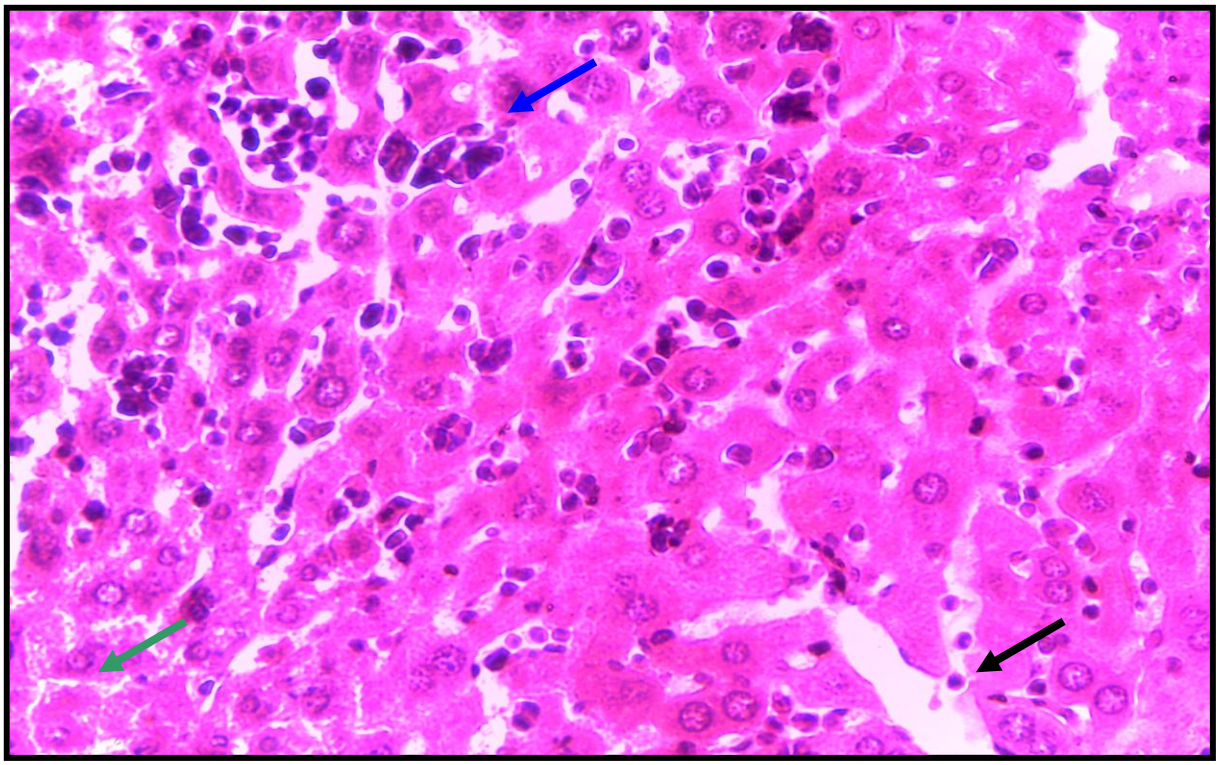


Figure (5): Section in mice liver infected by *Candida albicans* showed wide sinusoids hepatic ( )nuclei lysis ( ) with aggregation yeast cells( ) stains(H.E) 330 X

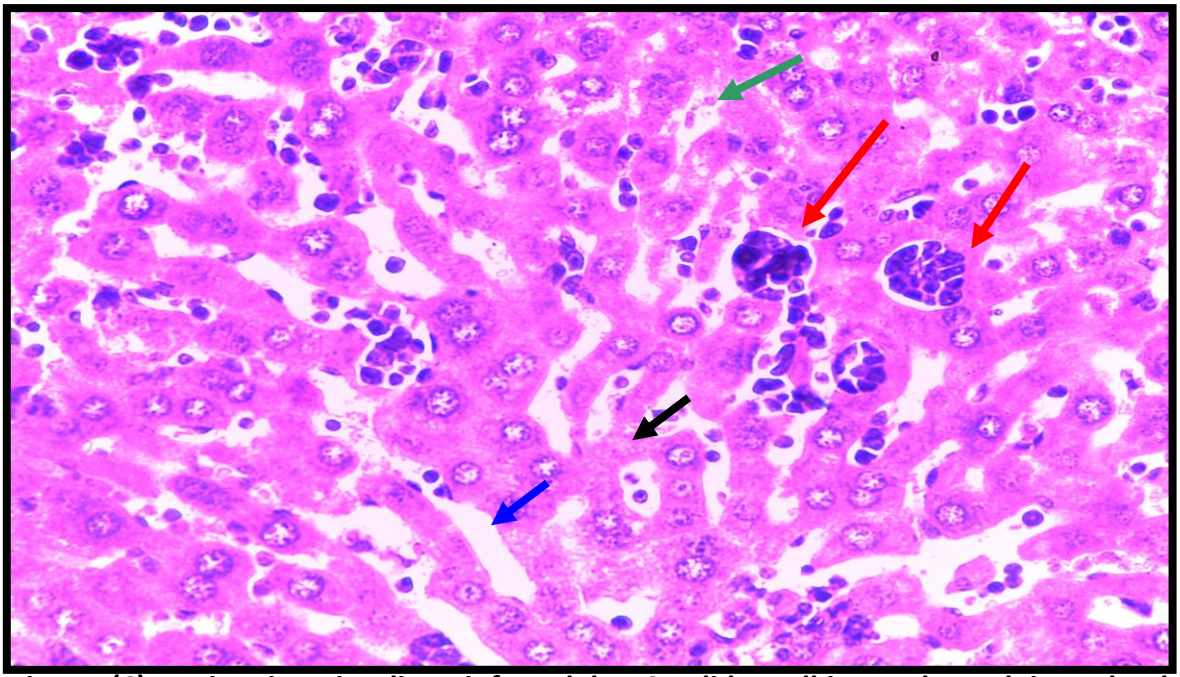


Figure (6):section in mice liver infected by *Candida albicans* showed irregular hepatic platelets ( ) nuclei necrosis( ) and wide blood sinusoids ( ) with aggregation yeast cells as mass between hepatocyte ( ) stains(H.E) 330 X

ISSN: 0975-8585

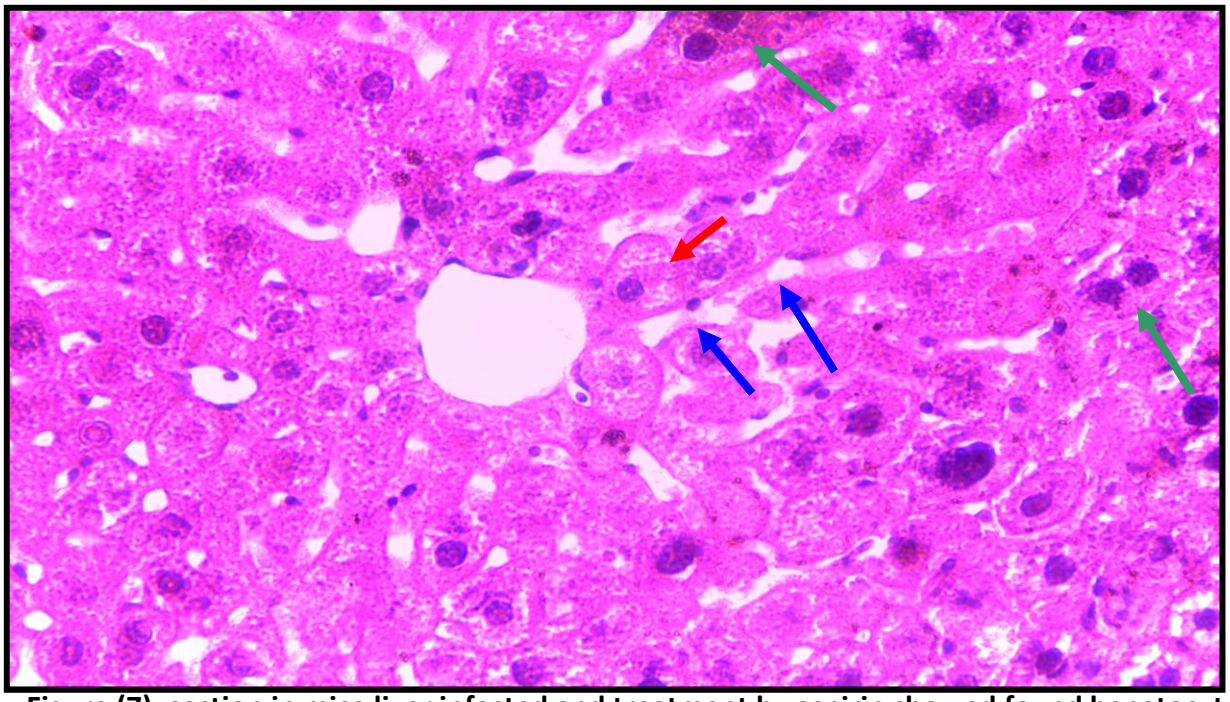


Figure (7): section in mice liver infected and treatment by aspirin showed found hepatocyte regeneration and nuclei clear (→) with the regularity of hepatic platelets and blood sinusoids (→)still cytoplasm in liver cell showed arrangement rough granules stains(H.E) 330 X

kidney

The results of a microscopic examination of the kidneys of animals infected with yeast during the period of the experiment and the presence of pathological changes consisted lysis of renal tubule and heavy defense cell with bleeding and atrophy of the glomeruli and some vascular congestion (Figure 10.11) explained histological sections of the kidneys of infected animals and drug treatment renal tubules near and far lined cubic epithelial tissue (Figure 12)

ISSN: 0975-8585

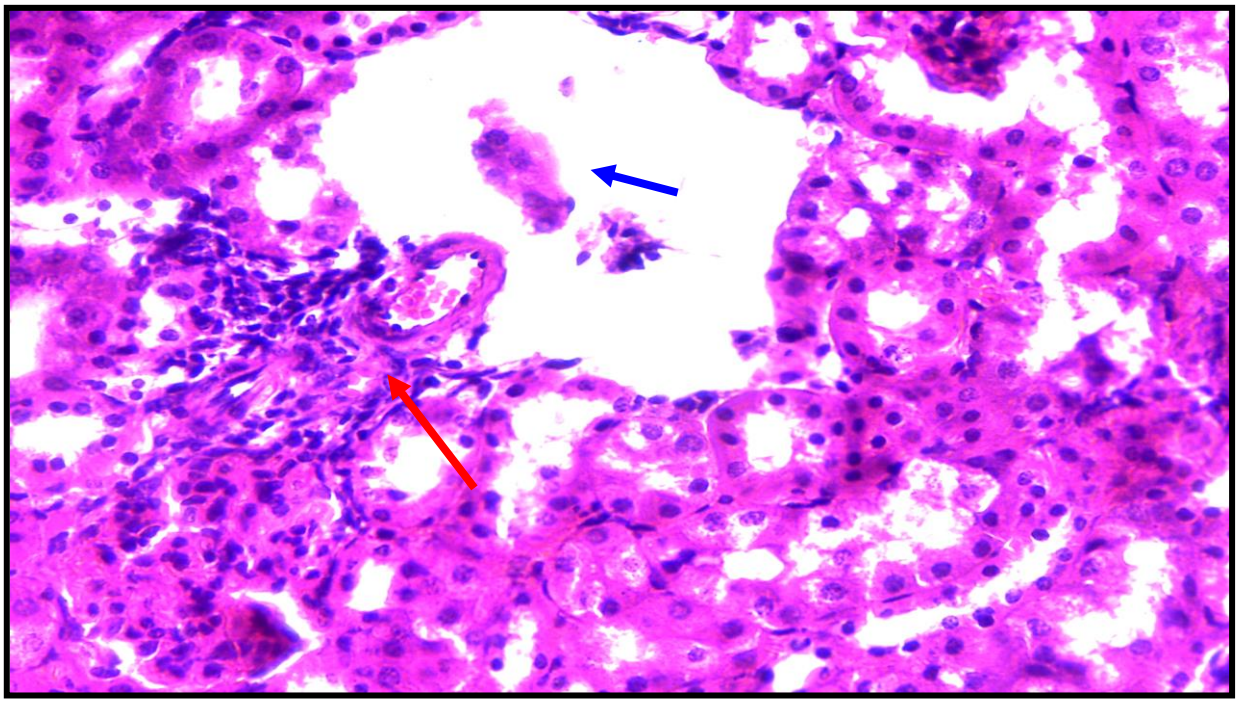


Figure (8):section in mice kidney infected by *Candida albicans* showed lysis of renal tubules (→) and heavy defense cells (→) stains(H.E) 330 X

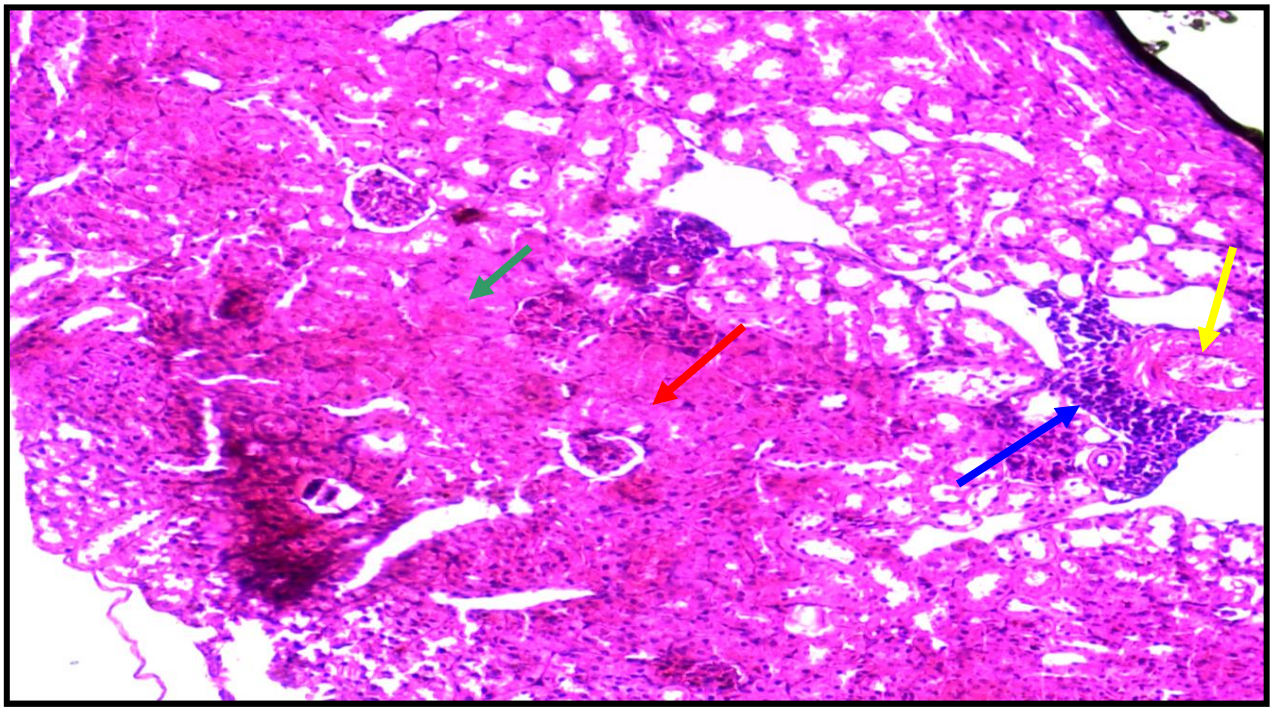


Figure (9): section in mice kidney infected by *Candida albicans* showed sever bleeding in some region (→) and atrophy of the glomeruli (→)heavy defens cells (→)and some vascular congestion.(→) stains(H.E)150 X

ISSN: 0975-8585

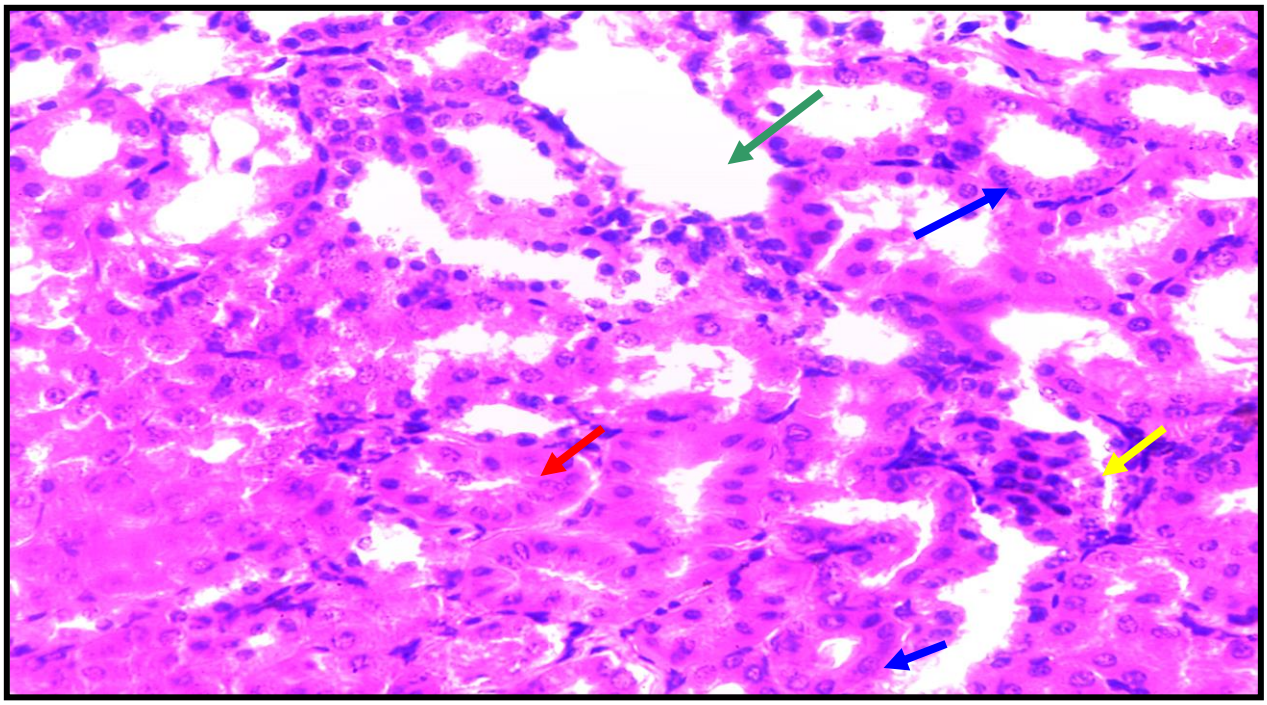


Figure (10): section in mice kidney infected by *Candida albicans* and treatment by aspirin showed found proximal renal tubules (→)distal renal tubules (→) lined cubic epithelial tissue (→)with occurrence few defense cells (→) stains(H.E) 330 X

DISCUSSION

This study showed that the type of *Candida albicans* is more species presence in the oral cavity of patients and a control group Figure (1,2) This is consistent with the study of [18]

Oral candidiasis is the most common diseases that widely prevalent in most sex and age's groups of patients that required for rapid presumptive identification by reliable and precise methods. CHROMagar was developed for presumptive identification of important *Candida* spp [19] The identification of *Candida* species based on colony color on CHROMagar was showed excellence results in this study plate (1)Table(1)

The results of the current study, the injection yeast inoculum Comparative tube Mackfrlande A second concentration 3* 10^8cfu/ml led to the early death of the mice, and this is consistent with the study of [20]

More ever the greater severity of the lesions induced by the *Candida albicans* may be due to the adhesive capacity of the organism Table (4)this coordinate with[21]

When examined the livers of infected mice after 1 week from infection it were appearance contain large numbers of yeast colonies which noticed as white spots including whole livers figure (4)

ISSN: 0975-8585

showed tissue sections of the livers animals infected with *Candida albicans* severe changes in liver was wide sinusoids hepatic and irregular platelets and nuclei lysis and necrosis was observed yeast cells aggregation between hepatocytes (figure .5.6.7) .

This may be due to the toxic effects of the toxins produced by the yeast *Candida albicans* a table (2) that demonstrates the ability of yeast to produce the toxin aflatoxin, which led to the aforementioned variations[10]

Or cause hepatic variations may be due to the effectiveness enzyme which is characterized by the yeast tested Table (3) as it demonstrates its ability to produce enzymes and phospholipase,lipase,protease, which is working on the decomposition of the plasma membranes of the cells of liver and damage it [22]

May be caused by variations occurring in kidney figure (8.9.10) due to high toxicity of the toxin produced by the yeast and not to the possibility of kidney to do the basic functions through the filtration process and subtract for Toxic Substances and deposition and then accumulate in large quantities within glomerulonephritis and tubules Moreover longer kidney organ rich in fat and mycotoxins affinity with high penetration of fatty tissue and this is consistent with what he referred to [23]

The main role of mycotoxins is breaking down fat and proteins in tissue, leading to concentration and then the inability of cells to divide and by following her death

showed the results activity of aspirin in vitro Table (5) figure (3) beside of the effect it on the infected mice in-vivo may cause the drug to reduce histological effects on the liver and kidney, compared with only infected samples. Aspirin work in two direction Inhibition the fungal prostaglandins which prevents fungal colonization and Candida chronic infection[8]

CONCLUSION

This study concluded that identification of *Candida* spp. by phenotypic assays such CHROMagar very excellance and the aspirin can be used as antifungal instesd of drugs which yeast resistance it

REFERENCES

[1] Aher, C.S. Species distribution, virulence factors and antifungal susceptibility profile of *Candida* isolated from Oropharyngeal lesions of HIV infected patients. .2014. *Int.J.Curr.Microbiol.App.Sci* 3(1): 453-460

[2] Zaidan Khlaif Imran and Al-Ghalibi, H. Genotyping identification of Candida spp. Isolated from onychocandidiasis patients by phenotypic methods , PCR and rapid –PCR . .2014.American Medical Journal 5 (1): 1-7

[3] Sardi, J., Scorzoni, L., Bernardi, T., Fusco- Almeida, A., and Mendes Giannini, M *Candida* species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. J Med Microbiol. 2013. 62:10-24.

[4] Sachin, C. D., Ruchi, K., and Santosh, S. In-vitro evalution of proteinase, phospholipase and haemolysin activities of *Candida* species isolated from clinical specimens. Int J Med Biomed Res. 2012. 1:153-157.

[5] Paškevičius, A.; Švedienė . J .; Levinskaitė1,L.; Repečkienė1,J.; Raudonienė1,V.and ; Melvydas,V. The effect of bacteria and essential oils on mycotoxin isolated from feed of plant origin .2014. Vet. Med Zoot. T.J. 65 (87):1-10.

[6] Tari Lee Cornish, N.C. Yeast buster to the rescue .3 rd ed. Printed in Canada. .20141-50.

[7] Císarová , M.; Kačinová,J and Tančinová , D.. Antifunal activity of selected essential oils against the fngal species of the genus Eurotium contact vapour.2014. J Microbiol Biotech Food Sci / Císarová: 3 (special issue 1) 202-205

[8] Al-Bader , A.A. Effect of aspirin as antifungal drug against some opportunistic fungi.2008. Bas.J.Vet.,Res.,7(2):101-107 .

[9] Odds ,F.C. and Bernaerts, R.. Chrom agar Candida anew differential isolation medium for presumptive identification of clinically important *Candida* species .1994.J.Clin.Microbiol.,32:1923-1929.

[10] Al- Ghalibi, Haider Habib Hattit . The negative effects of secondary metabolic products of the fungus Aspergillus flavus on certain criteria blood in white rats,2008 .Science Journal Qadisiyah g Pure. 13(3):61-68.

[11] Bisson , J.W. and Cabelli, V.J. Membrane filter enumeration method for *Clostridium perfringens* 1979.. *Appl.Envir.Microb.J.* 37:55-66.

[12] Sierra,G. A simple method for detection of lipolytic activity of micro-organisms and some observations on the influence of the components between cells and fatty substrates.1957 .Ned.J.Hyg . 23 : 15-22.

[13] Al-Abeid ,H.M.; Abu-Elteen ,K.H. ; Elkarmi ,A.Z. and Hamad, M.A.Isolation and characterization of *Candida spp.* In Jordan cancer patients:Prevalence , Pathogenic ,Determination and antifungal sensivity.2004 .Jpn.J.Infect.Dis., 27:269-284.

[14] NCCLS National Committee for Clinical Laboratory Standards. .Refrence method for broth dilution anti fungal susceptibility pesting of conidium forming filamentous fungi :1998. proposed standard M38-P.Wayne . PA , USA .

[15] Collee, J. ; Fraser, A. ; Marmion, B. & Simon, A. Makie and McCartney practical medical microbiology.1996. 14 th ed. Churchill Liverstone. New York .978.

[16] Luna , L. Manual of histological staining methods of the armed forced institute of pathology .1969. 3 rd ed . McGraw-PHH Bwklo , London .

[17] Drury , R.A.B and Wallington , E.A. Experimental aspergillosis in Japanes quils (*Coturnix coturinx japonica)*. Clinical signs and haematological change . Mycopathol.1967 .102:179-184.

[18] Abdul-Raheem , B.S. Astudy on occurrence of *Candida* species in the oral cavity of diabetic patients in Basrah .2012. Master thesis .college of science Basrah university . 104.

[19] Chang, C., N. Leaw, H. Huang, L. Wu and C. Chang . Rapid identification of yeasts in positive blood cultures by multiplex PCR method.2001. J. Clin. Microbiol., 39: 3466-3471. DOI: 10.1128/JCM.39.10.3466-3471.

[20] Khosravi , A.R. ; Shokri , H.; Nikaein , D.; Erfanmanesh ; Fathinia , M and Hilan , J.A . Evaluation of the pathogenicity of *Candida zeylanoides I in* Balb / c mice .2013. Turk.J.Vet.Anim.Sci.37:408-413.

[21] Calderon, R.A. and Braun ,P.C. Adherence and receptor relationships of *Candida albicans.* 1991. Microbiol.Rev.J. 55:1-20.

[22] Nahla, A.G. and Ahmed , R. Pathological studies on experimental systemic candidiasis induced by *Candida albicans* isolated from different animals in immunosuppressed mice2011.J.of American Science . 7(3):97-107.

[23] Blyth , W. and Stewart , E. Systemic candidiasis in mice treated with prednisolone and Amphotericin B .2. Ultrastructure and evidence for fungal toxin .1978. Mycopathol .J. 66 : 51-57.