

***In vitro* and *in vivo* studies on the antifungal activity of probiotics and Seaweed extract (*Ascophyllum nodosum*).**

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Abstract

These study included *In vitro* and *in vivo* studies on the antifungal activity of probiotics and Seaweed extract(*Ascophyllum nodosum*).The *in vitro* study using the probiotics with concentrations 0.0%,1.5% (w/v),1%(w/v) ,0.75%(w/v) 0.5%(w/v) and0.25%(w/v), inhibited the growth of *Trichophyton mentagrophty* with mean inhibition percentage 76%,79%,82.8,86% and 87%,while of *Candida albicans* is 41.7%,58.3%,66.6%,66.6% and 75% respectively compactable with control (0%).Whereas the inhibition percentage of *T. mentagrophty* in 1%,3%, 5%,7% and 9% concentration of seaweed(*A.nodosum*) 45%,55%,63%,66% and 70%,while of *C. albicans*

is 33.3%,50%,58.3%,53.3% and 66.6% respectively compactable with control (0%). *In vivo* antifungal activity studies on candidiasis in mice treated with the probiotics (2.5g/kg body weight) , showed that spleen had showing fibrosis and enlargement in white palp ,The liver showed hemorrhage and hydropic degeneration ,while the tissues of kidney showed as a normal and no changes occur. while mice treated with Seaweed extract (2.5ml/kg body weight, spleen had showing increase in number of megakaryocyte ,The liver showed mild hemorrhage and hydropic degeneration ,while the tissues of kidney showed no changes occur as a normal tissue ,compatible with negative control(not treatment).

Key word: probiotics, *Ascophyllum nodosum*, antifungal activity, Seaweed extract

INTRODUCTION

Mycoses are diseases caused by fungi with the increase in the immunosuppressive patients . fungal infections caused various diseases that can be local' superficial infections ' or systemic. The systemic infections are particularly serious and potentially life-threatening (1). Small group of fungi cause relatively mild infections of the skin e.g. Dermatophytes and *Malassezia* species,While fungi that cause severe cutaneous infections e.g.*Sporrix schenk* and fungi that cause life threatening systemic infections e.g.*Aspergillus fumigatus* ,*Cryptococcus neoformans* and *Candida albicans* (2,3)

C. albicans is dimorphic fungi that causes variety opportunistic infections in humans , it colonizes mucosal surfaces of the oral and vaginal cavities and the digestive tract and is also able to cause a severe of infections, depending on the underling host defect such as oral thrush and chronic mucocutaneous candidiasis ; acute disseminated candida septicemia and candida due myocarditis (4).*C. albicans* is frequently in patients undergoing

chemotherapy for cancer ,prolonged antibiotic therapy ,immunosuppressive therapy and ets. (5,6,7)

probiotics are live microorganisms that confer beneficial on the health of the host when administered in appropriate amounts (8,9) microorganism involved in the composition of probiotics must to possess antimicrobial activity against pathogenic microorganism, and to produce antimicrobial substances including acidic products , bacteriocin like substances and hydrogen peroxide(H₂O₂)(10,11).

probiotics ;such as lactic acid bacteria 'widely used in the food industry 'are proved to be beneficial to the patients of digestive diseases and prevent the gastrointestinal bacterial infection (12,13,14) lactobacilli have been used as probiotics for a number of disease applications and are generally regarded as safe for human use ' making them an ideal candidate for the development of a therapeutic(15,16) , one of the beneficial effect of probiotic is a high antimicrobial activit

against *C.albicans*, Several studies have assessed the efficacy of probiotics for A well-known problems that long term exposure to anti-fungal agents promotes acquired resistance(20),So that the aim of this study was to test the inhibitory effect of probiotics an *in vitro* against *C. albicans* and *Trichophytone mentagrophyte*. Also study the inhibitory effect of probiotics an *in vivo* against *Candida albicans* .

2-MATERIAL AND METHODS:

Isolates

Candida albicans, and *Trichophytone mentagrophyte* isolates were obtained from mycology laboratory, Department of biology of the sciences college /Al-mustansyria university. This isolates was maintained on slants of sabourauds dextrose agar (SDA) until used.

Preparation of probiotics and Seaweed extract

We used probiotic that posed in the markets under the trade name (Iraqi probiotic) product locally in the College of Agriculture / Baghdad University, each gram of it contain the following microorganism :

Lactobacillus acidophilus (10^8)cfu /g ;*Bacillus subtilis* (10^9)cfu/g ; *Lactobacillus spp.* (10^8)cfu /g and *Saccharomyces cervisiae* (10^9) cfu/g .The probiotic was diluted into different concentration 0.0%,1.5% (w/v),1%(w/v) ,0.75%(w/v) 0.5%(w/v) and0.25%(w/v)using sterile normal saline. Seaweed extract of brown algae *Ascophyllum nodosum* is obtained as a commercial foreign product from College of Agriculture Baghdad university. seaweed extract were (1, 3, 5, 7, 9) v/v using sterile normal saline.

Determining antifungal activities of probiotic and Seaweed extract

Prior to the experiment, *C. albicans* isolate were cultured at 37°C for 48h on sabourauds dextrose agar ,while *Trichophytone mentagrophyte* was cultured at 28°C for 7days on sabourauds dextrose agar. 20 ml of Sabouraud Dextrose Agar culture medium with 5 ml each of the above concentrations of the probiotics and Seaweed extract, mixed and then poured in sterile petri plates and allowed to solidify. Discs of 5 mm diameter were cut from the periphery of the test organisms were an aseptically inoculated upside down on the surface of the SDA medium. Inoculated petri plates were incubated at 25°C ± 2°C and the diameter of the fungal growth was measured on 7th day, it was determined in millimeters, the antifungal activity was calculated according to the following formula(21).

%growth inhibition= (Colony diameter in control – colony diameter in treated)/ Colony diameter in control×100

prophylaxis and therapy of *C.albicans* infections(17,18,19).

In vivo anticandida activates of probiotic and Seaweed extract

- Laboratory animals

Young male Swiss albino mice their age among 8-12 weeks and weighing between 23 and 25were used for this study. The mice were obtained from the animal house in college of medicine Baghdad University. The cages with the mice were placed in room (temperature 26 +2°C), water and food were provided to animals.

- Suspension preparation

C. albicans colonies were suspended in 5ml of 0.85% normal saline, suspension was mixed for 15 second with a vortex, then its concentration was adjusted to 1.5×10^8 cfu/ml based on a standard 0.5 mcfarland (22).

-Antifungal assay

C.albicans used in this study was 1.5×10^8 cfu/ml PBS. Animal were divided into three groups of 5 mice each and received in treatment as described in flowing:

Group1 (Negative control): The animals of this group administrated orally by 0.1ml of *C.albicans* , 48h gap followed by treatment with phosphate buffer solution (PBS) orally ,once daily for 7 days.

Group2 (Treatment): The animals of this group administrated orally by 0.1ml of *C.albicans* , 48h gap followed by treatment with 2.5g/kg body weight of probiotic orally ,once daily for 7 days.

Group3 (Treatment): The animals of this group administrated orally by 0.1ml of *C.albicans* , 48h gap followed by treatment with 2.5ml/kg body weight of seaweed extract orally ,once daily for 7 days.

All mice were killed by cervical dislocation on day 8 after orally *C.albicans* inoculation, the liver ,kidneys and spleen of each animal were removed aseptically and placed in 10% formalin solution then ,dehydrated with 100% ethanol solution and embedded in paraffin wax .Paraffin wax sectioned in to 4µm thickness ,then stained with hematoxylin-eosin stain and observed under a photomicroscope(22,23).

3-RESULTS AND DISCUSSION

The results of antifungal activities of probiotic in Table – 1 showed the ability of probiotic in inhibition of growth *Trichophytone mentagrophyte* and *Candida albicans*, the growth of both fungus is inhibited by probiotic and this inhibition rate increases with the increase of concentrations. Thus, Mean inhibition percentage of *T. mentagrophyte* in 0.25%,0.5%, 0.75%,1% and 1.5% concentration of probiotic

76%,79%,82.8,86% and 87%,while of *C. albicans* is 41.7%,58.3%,66.6%,66.6% and 75% respectively compactable with control (0%).

Table -1- Mean inhibition percentage of *Trichophytone mentagrophy* and *Candida albicans* at different concentrations of probiotic

Inhibition percentage%	Concentrations%				
	0.25	0.5	0.75	1	1.5
Types of fungi					
<i>Trichophytone mentagrophy</i>	76	79	82.8	86	87
<i>Candida albicans</i>	41.7	58.3	66.6	66.6	75

The inhibition percentage of seaweed extract (*Ascophyllum nodosum*) against *T. mentagrophy* and *C. albicans* are shown in table 2,the inhibition percentage were varied in fifth concentration of seaweed extract compared with control ,the inhibition percentage of *T. mentagrophy* in 1%,3%, 5%,7% and 9% concentration of seaweed 45%,55%,63%,66% and 70%,while of *C. albicans* is 33.3%,50%,58.3%,53.3% and 66.6% respectively compactable with control (0%).

Table -2- Mean inhibition percentage of *Trichophytone mentagrophy* and *Candida albicans* at different concentrations of seaweed extract

Inhibition percentage(%)	Concentrations%				
	1	3	5	7	9
Types of fungi					
<i>Trichophytone mentagrophy</i>	45	55	63	66	70
<i>Candida albicans</i>	33.3	50	58.3	58.3	66.6

The beneficial effects of probiotic have improvement of the health in gastro- intestinal infection, reduction of the serum cholesterol

levels, anticancer properties, protection of the immune system, antidiarrhoeal properties , restoration of the microflora in the stomach and intestines after antibiotic treatment, ,antimutagenic effect and

etc(24,25). *In vitro* ,some substances produced by specific probiotics strain have been found to exert an inhibitory effect upon *C.albicans* (26,27,28).

Furthermore , Norerrb (29) have found that *in vitro* assays *Lactobacillus* spp can significantly inhibit *C. albican* germ tube formation. While, Savage (30) found that the ability of *Lactobacillus* to displace *Candeda* from the epithelial layer of the stomach, inhibit hyphal invasion . Tang *et al* .(18) suggest that *Lactobacillus rhamnosus* LGG , *Bacillus subtilis* , *L.plantarum* and *L.johnsonii* *B.longum* , *B.lich* maybe potential strains to use antifungal drugs ,nd could in hibit the switch of *C.albicans* from bud to filament *in vitro*.

Laboratory animal studies also suggest that probiotics may be helpful for the presentation of candidiasis. mice immunesuppressed with corticoid drug recovered more quickly from orogastric candidiasis when they fed cultures of *L.acidophilus* ,*L. casei* and *L. delbrueckii* prior to oral *C. albicans* challenge (31). Mohammed *et al.*, (32) studiesThe inhibition activity of seaweed extract against phytopathogenic fungi *Pythium ultimum* and *Rhizotonia solani* using of *Cladophora glomerata* ,while Al-Hameri(33).Studied the antifungal activities of seaweed extract *Ascophyllum nodosum* against *Fusarium oxysporium* caused wilt fusarium of tomato .

Jayaraman *et al*(34) examined the effects of Stimplex™, a marine plant extract formulation from *Ascophyllum nodosum*, on some common cucumber fungal pathogens including *Alternaria cucumerinum*, *Didymella applanata*, *Fusarium oxysporum*, and *Botrytis*

cinerea.. Plants treated with Stimplex™ showed enhanced activities of various defense-related enzymes including chitinase, β-1,3- glucanase, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, and lipoxygenase. Cucumber plants treated with Stimplex™ also accumulated higher level of phenolics compared to water controls. These results suggest that seaweed extracts enhance disease resistance in cucumber probably through induction of defense genes or enzyme.

The histomorphological study in G1(negative control),in which mice infected with *C.albicans*, showed that spleen had showing necrosis in spleen cells ,fibrosis and cell of *Candida albicans* found in tissues of spleen(figure1),The live showed hemorrhage, and sinusoided sinuses (figure2), while kidney showed

hemorrhage ,necrosis and *C.albicans* cell appearance in their tissue (figure 3)

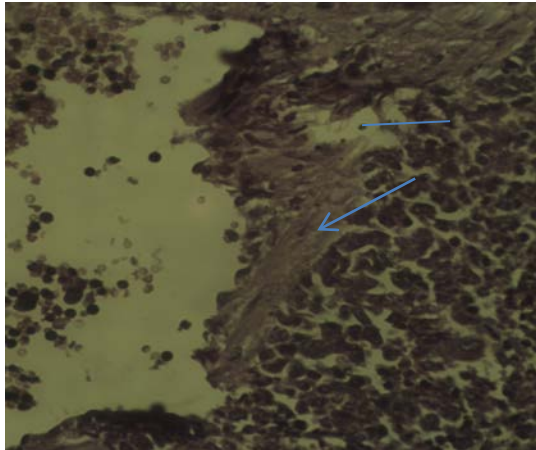


Figure1:G1, showing necrosis in spleen cells ,fibrosis (→) and cells of *Candida albicans* (—) found in tissues of spleen.40x(H and E).

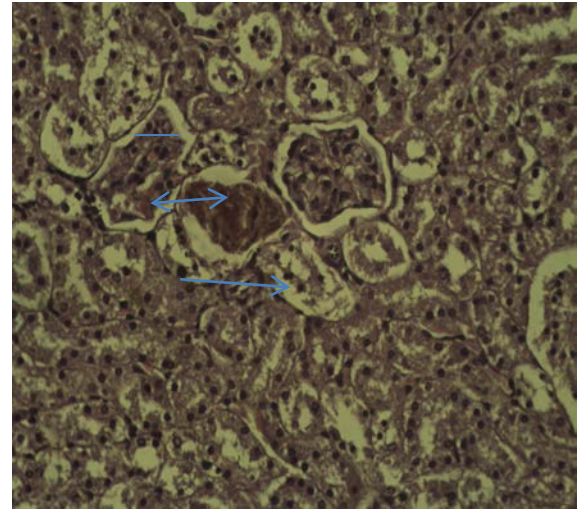


Figure 3:Group1, showed hemorrhage(↔), necrosis(→) and *C.albicans* cells (—) appearance in section of kidney. 40 X(H and E).

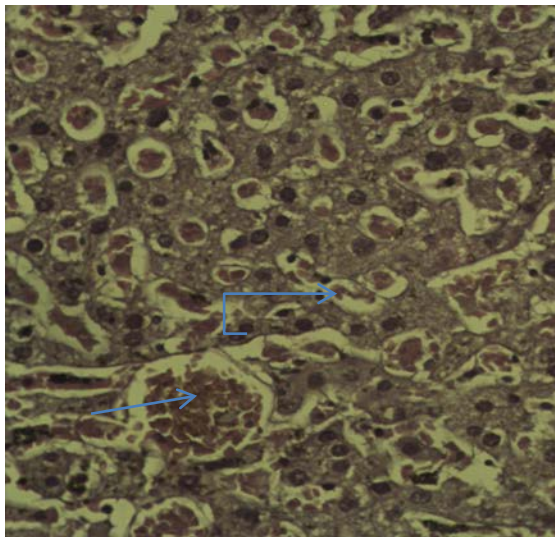


Figure 2: Group 1, showed hemorrhage, (→) and sinusoidal sinuses in section of liver, (↙) 40 X(H and E).

In G2,in which mice infected with *C.albicans* and treated with probiotic ,showed that spleen had showing fibrosis and enlargement in white palp (figure4),The live showed hemorrhage and hydropic degeneration (figure5), while the tissues of kidney showed as a normal and no changes occur (figure 6)

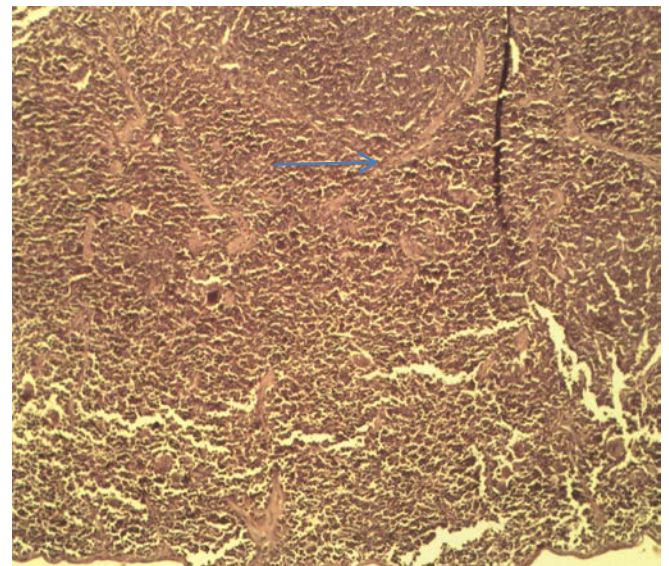


Figure4: Group2, showing fibrosis (→) and enlargement in white pulp in section of spleen , 250 X. (H and E).

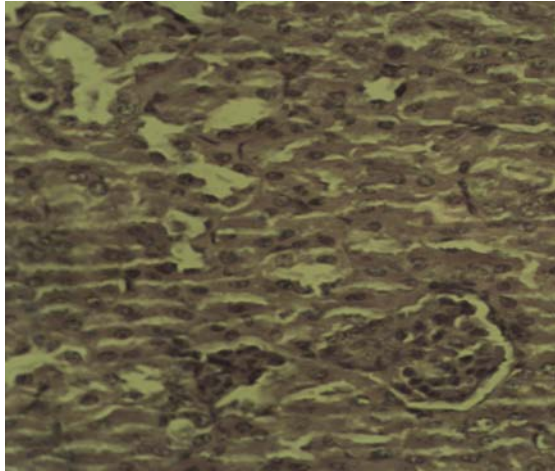


Figure5: Group2, showed as a normal and no changes occur in section of kidney,X250(H and E).

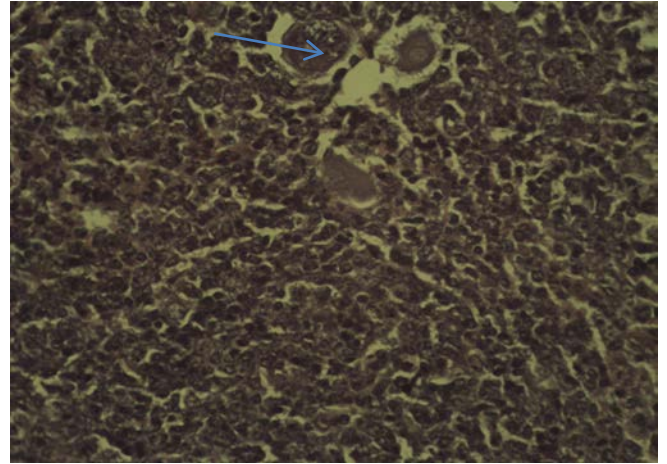


Figure7 : G3 showing increase in number of megakaryocyte → in spleen section250 X. (H and E).

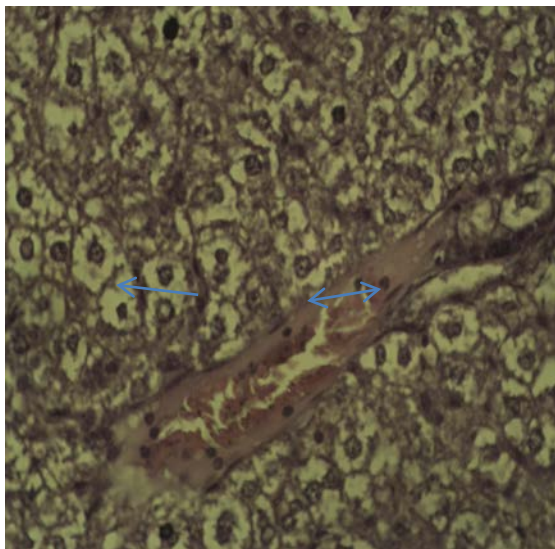


Figure 6: G2: showed hemorrhage(↔)and hydropic degeneration(→)in section of liver. 250 X. (H and E).

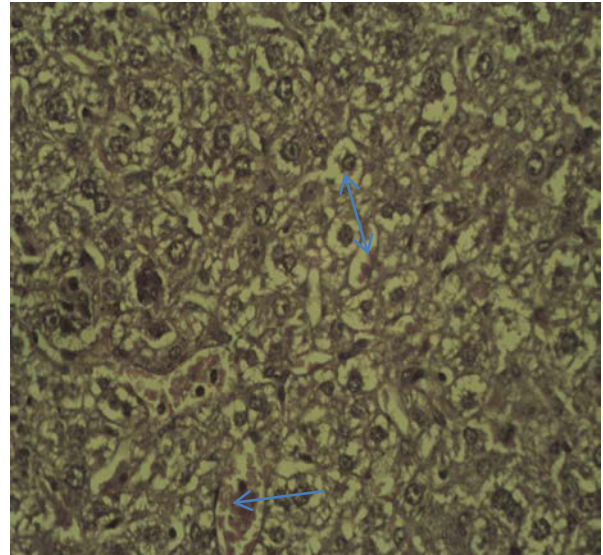


Figure 8, G3 showed mild hemorrhage(→) and hydropic degeneration (↔) in liver section. 250 X. (H and E).

In G3, in which mice infected with *C.albicans* and treated with Seaweed extract of brown algae *Ascophyllum nodosum*, spleen had showing increase in number of megakaryocyte (figure7), The liver showed mild hemorrhage and hydropic degeneration (figure8), while the tissues of kidney showed no changes occur as a normal tissue (figure 9)

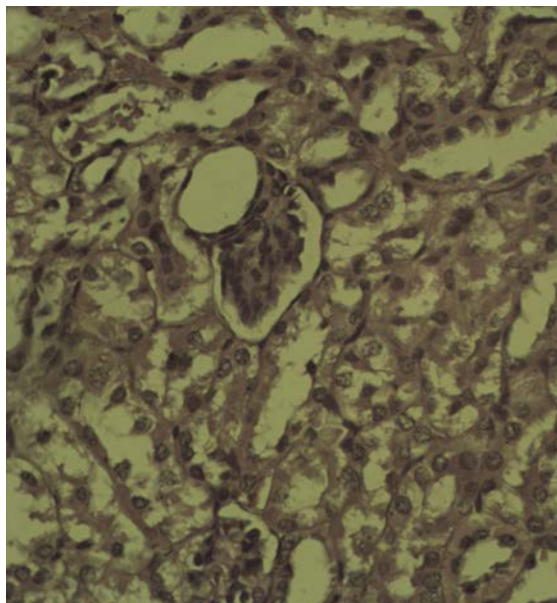


Figure 9,G3 showed no changes occur as a normal tissue in kidney section. 250 X. (H and E).

The effect of probiotic bacteria to reduce candidiasis infection in mice study by Doug *et al.* (35), found that the prolonged survival of mice, decreased severity of mucosal and systemic candidiasis, modulation of immune responses, decreased number of *C. albicans* in the alimentary tract also that the probiotic bacteria produced biotherapeutic effects by inhibition of *C. albicans* growth, stimulation of the mucosal and systemic immune systems and possibly by nutritional and competitive means.

4- CONCLUSION

The probiotics and Seaweed extract (*Ascophyllum nodosum*) possesses significant *in vitro* and *in vivo* antifungal activity against *Candida albicans* and therefore it could be used in the treatment of *C. albicans* infection.

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