EFFECT OF ASPIRIN AS ANTIFUNGAL DRUG AGAINST SOME OPPORTUNISTIC FUNGI

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ABSTRACT

The effect of aspirin(non steroidal anti-inflammtory drug a cyclooxygenase inhibitor) as antifungal has been studied against some opportunistic fungi : *Aspergillus flavus , A. niger , A. terreus ,Cryptococcus neoformans, Penicillium sp* . and *Trichoderma sp.*

Aspirin was showed a potent activity against all tested fungi *in vitro* . Aspirin gives the greatest effects in a concentration of 1000 µg , 2000 µg and 3000 µg causing 100% inhibition .

INTRODUCTION

Aspirin is one the world's oldest and most common drugs , which was widely used in alleviation of fever , an excellent pain killer , anti – inflammatory drug . The chemical name of aspirin is acetylsalicylic acid or acetosalicylic acid(ASA) , a white crystalline compound that belongs to the class of ($NSAID_s$) non-steroidal anti – inflammatory drugs (1) .

Oxylipins are oxygenated lipids , divided to many groups , oxylipins in mammals is the eicosanoids , which include prostaglandins and leukotrienes (2) these products are potant modulators of host immune responses , also oxylipins and eicosanoid produced by eukaryote (plant , fungi , parasites)organisms (3) .

Pathogenic fungi were known as producing prostaglandins and may play an important role in fungal colonization and a topic disease development (3) .

The correlation between oxylipin production and fungal pathogenecity was explained (4). Fungal oxylipin plays an important role in the alteration the ratio of asexual to sexual sporulation of filamentous fungi especially *Aspergillus spp.*(5,6,7) . oxylipin was necessary to facilitate Co-flocculation in yeast (8) . The products have been found to be widely distributed in fungi (9,10,11).

The presence of aspirin sensitive – 3 hydroxy fatty acids (3- OH oxylipins) in yeasts were uncovered in 1991(12).

The effect of aspirin on vaginal isolates of *Candida albicans* from patients with recurrent candidiasis was studied (13).

Many opportunistic fungi (*Absidia corymbifeara , Aspergillus fumigatus , Blastomyces dermatitis , Fusarium dimerun , Penecillium spp , , Rhizopus spp.* and *Sporothrix schenckii*) have the ability to produce eicosanoid (subset of oxylipins) both from simple metabolites and from arachidonic acid(14) .

The biofilm formation in *Candida albicans* was inhibited by aspirin(15). Acetylsalicylic acid (aspirin) as anti fungal in *Eremothecium* and other yeasts was used (16).

Although the anti – *Aspergillus* activity . Mortality remains un acceptably and less susceptible to anti fungal against Aspergillosis and other fungal diseases began emerge (17) .

This study conducted to use aspirin as antifungal drug against some filamentous fungi and yeasts .

MATERIAL AND METHODS

The susptibility testing of aspirin against single isolate of six fungal species namely (*Aspergillus flavus* , *A. niger* , *A. terreus* , *Cryptoccocus neoformans* , *Penicillium sp.* and *Trichoderma sp.*) has been used .

Fungal inoculum

Fungal species were activated on sabouraud's dextrose agar(SDA) for 5-7 days for filamentous fungi and for 2-5 days for *Cryptoccocus neoformans* . The fungal spores and (few colonies for *C. neoformans)* were harvested and transferred to 5 ml of sterilized distilled water and shacked genetally then the numbers of fungal cells was counted by using Neubauer counting chamber , then adjusted to concentration of 10^6 cells / ml (18).

Antifungal Drug

Commercial sample of aspirin drug produced by Sammara drugs, Iraq (SDI) was used . Two tablets of aspirin (each one contain 300 mg of acetylsalicyclic acid) were powdered , then dissolved in 10 ml of ethyl acetate and shacked vigorously for 2 minutes . The mixture was filtrated by filter paper Whatman No. 1 then the filtrate was left to dry in Petri dish at room temperature in the dark until dry then the melting point was determined by using melting point apparatus to insure the purity of the compound (1) .

Preparation of Stock solution

Three hundred mg of pure acetyl salicylic acid as powder was dissolved in 10 ml of the organic solvent dimethylsulphoxide (100%DMSO), the final concentration of stock solution should be (30000µg/ ml) left at room temperature for 30 minutes for autosterilization .

Preparation of control medium

Control medium was prepared by adding 3 ml of Sabouraud′s dextrose broth (SD) to two glass vial each one contain 27 ml of SDA medium , which put in water bath at 50-52 ◌̊ C mixed well , poured in sterilized Petri dishes (19) .

Preparation of DMSO control

We added0.65 ml of 100% DMSO to 7 ml of Sabouraud′s dextrose broth (SD) medium and mixed 3 ml from mixture were added to two glass vial each one contains 27 ml of SDA medium were put in water bath at 50-52 ◌̊ C final mixture for each vial poured in sterilized Petri dishes to solidify (19) .

Testing of Biological activity of aspirin as antifungal

The agar diffusion method(20) was used as follows :-

A 0.2 ml of fungal inoculum (1×10^6 cells / ml) was seeded in Petri dishes contain SDA by sterilized pipette size 1 ml and spreaded by sterilized L- shap glass spreader . Left for 30 minutes for adsorption of fungal spores. Made wells by sterilized cork borer size 6 mm , then 0.1 ml was transferred to each well , then , incubated at 25 ±2 ◌̊ C for 4-5 days for filamentous fungi, 2 days for yeast. , then they were examined to observed the clear zone around the wells and these are measured by millimeters . The Minimal Inhibitory Concentration (MIC) was used(20) were determined by using SDA at the following concentrations : 3000 µg / ml , 2000 µg / ml , 1000 µg / ml , 900 µg / ml , 800 µg / ml , 700 µg / ml , 600 µg / ml , 500 µg / ml all these were inoculated with 0.01 ml of fungal inoculum of the testing fungi by micropipette . Two replicates were done for each concentration . The MIC was determine as the least concentration showed no growth .

RESULTS

In this study investigate the aspirin in 3000 µg / ml had active effect in fungi isolates Fig. 1. The diameter of inhibition zone ranged from 14 mm (*Aspergillus terreus*) to 3 mm (*Cryptococcus neoformans*)

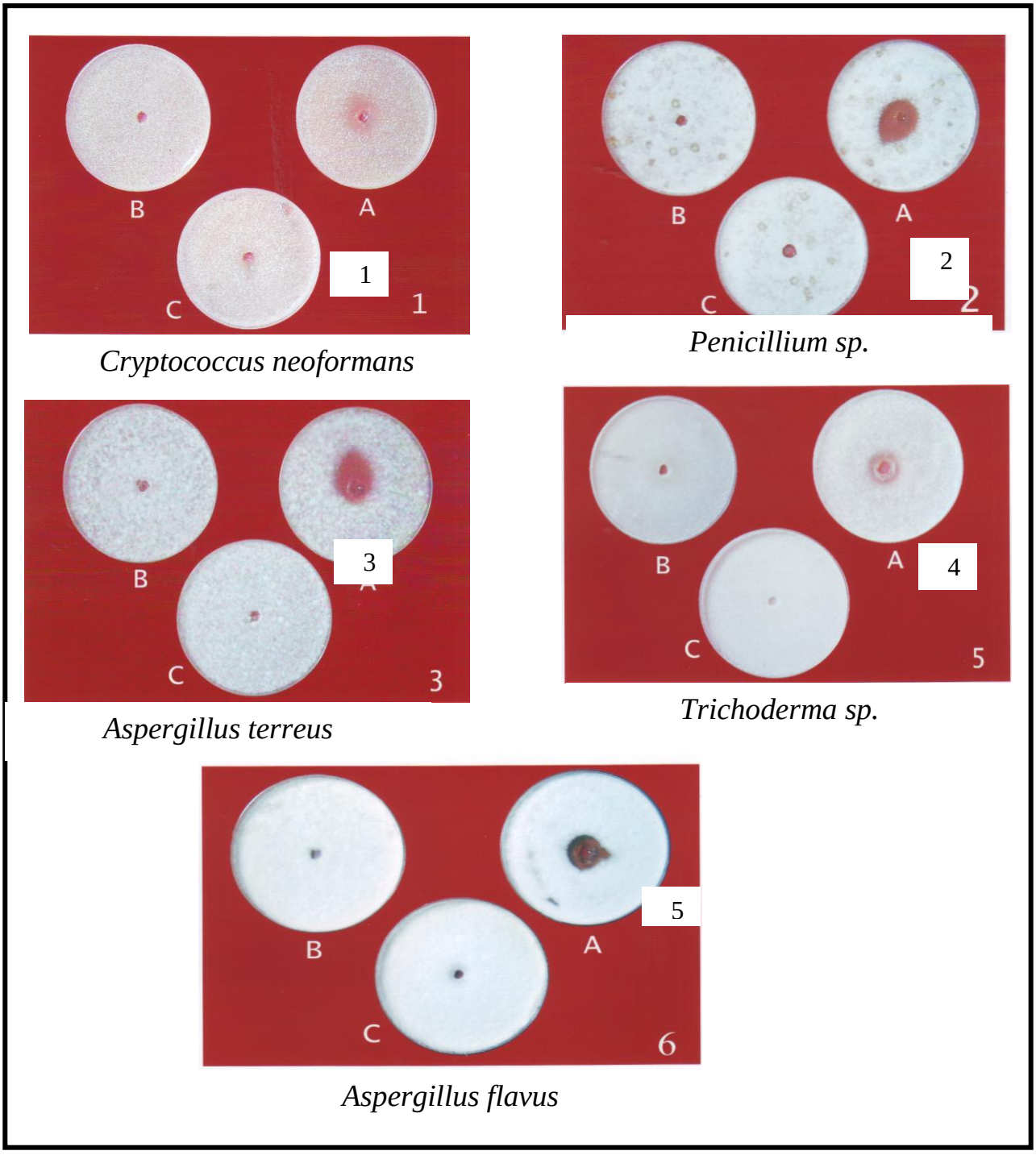

Figure (1) : Effect of stock solution of drug on growth oftesting fungi . A : treatment B: Control C: Control DMSO

Results of MIC observed complete inhibition 100 % at 1000 µg / ml , 2000 µg / ml , 3000 µg / ml for all testing fungi Table.1 and Fig.2 In concentrations 500-900 µg / ml were appeared no effect on testing isolates.

Table (1) : Effect of different concentrations of drug on growth of fungal isolates

Fungal isolates	Aspirin							
	Minimal Inhibitory Concentration (MIC) µg / ml							
	3000	2000	1000	900	800	700	600	500
Aspergillus flavus	-	-	-	+	+	+	+	+
A. niger	-	-	-	+	+	+	+	+
A. terreus	-	-	-	+	+	+	+	+
Cryptococcus neoformans	-	-	-	+	+	+	+	+
Penicillium sp.	-	-	-	+	+	+	+	+
Trichoderma sp.	-	-	-	+	+	+	+	+

*(+) : growth
*(-) : no growth

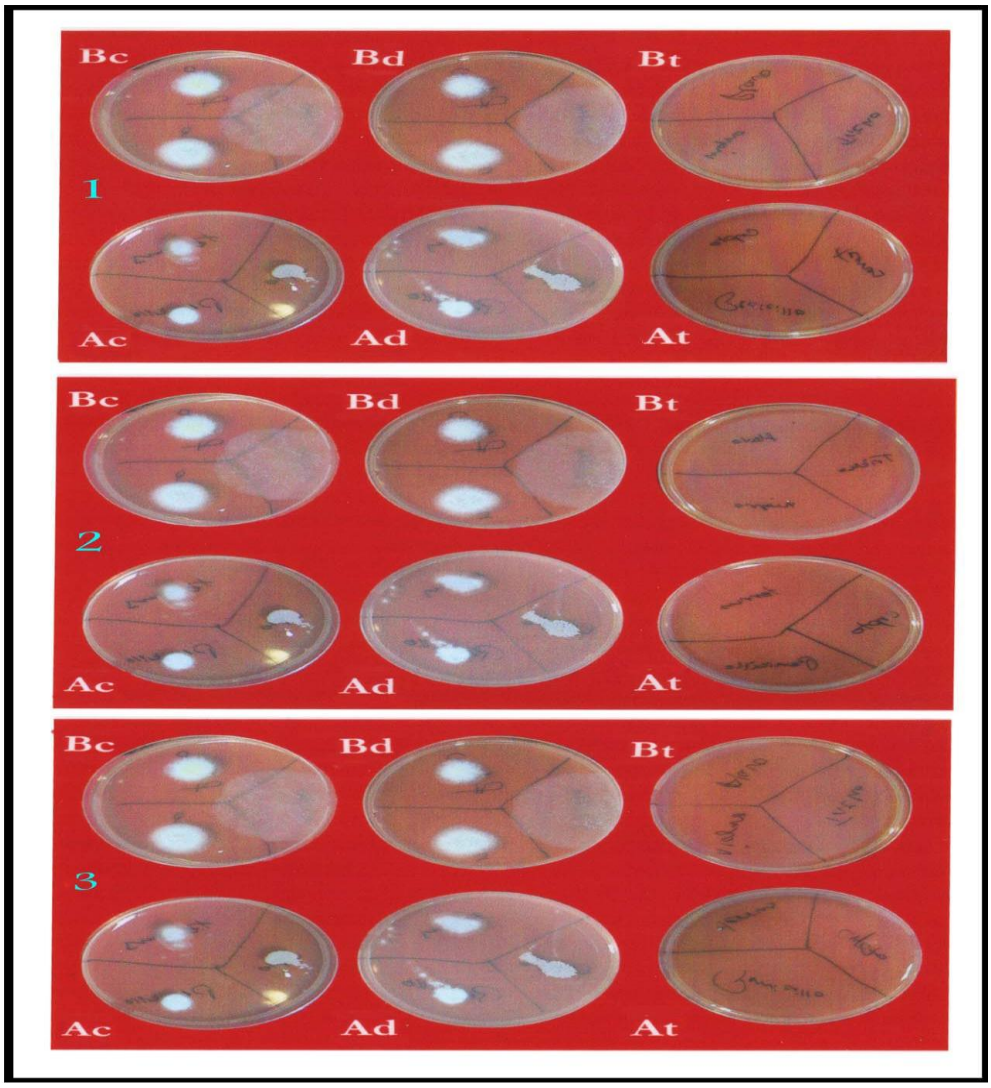

Fig .2 : effect of concentrations of aspirin on growth rate of fungi
(1 : 1000µg , 2: 2000µg , 3:3000µg)
Bt :*Aspergillus flavus, A . niger , Trichoderma sp* , treatment
Bd : Control
Bc : DMSO control
At : *Aspergillus terreus , Cryptococcus neoformans , penicillium sp* . treatment
Ad ;Control
Ac : DMSO control

DISCUSSION

The incidence of infection caused by opportunistic fungi had increased markedly with increasing in frequently of organ transplantation , cancer chemotherapy human immunodeficiency virus infection (21).

Resistance to a range of antifungal agents in clinical use were emerged so researchers try to create and develop new drugs (17) .

In Vitro antifungal activity of aspirin against opportunistic fungi were shown in this study Table 1 Fig. 1. the results agreed with study of Mohammad and Douglas (15) who found that aspirin causing up 95% inhibition in growth of *Candida albicans.* Also agreed with (23) who found strongly suppressed of aspirin against *Candida albicans* .In addition agreed with results shown by other workers(22,24).

Several studies demonstrated that oxylipins and eicosanoid were produce by eukaryotic microbes(3,23).These compound represent a potential class of novel virulence factors (4,14) however , fungal exposure to action of aspirin , which effects on their growth and colonization by inhibition of oxylipin production which was take place in mitochondrial β- oxidation (11).The present study observed the fungal isolates completely inhibited when they exposed to aspirin action Fig. 2 which indicate that these fungi produce the prostaglandins.This is agreed with reported (14,23,). Fungal prostaglandins may represent signaling molecules of similar type (3- R) – Hydroxy oxylipins (prostaglandins) which are derived from arachinoidic acid (14,22) .

The synthesis of these compound appears to take place in hyphae and suppressed by aspirin (13,22).

The role for oxylipins in directing the meiospore – metaspore balance emerged from studies by (6) which identified an *Aspergillus nidulans* enzymes (dioxygenase) required for biosynthesis of the factor component localized in lipid bodies of conidiophores , Hülle cells and cleistothecia .

Tsitigiannis *et al* .(24) mentioned that the fungicides such as aspirin was targeting the oxylipin biosynthesis enzymes , this component could lead to novel control strategies of mycopathogens.

The fungal infection are most chronicity and can used aspirin to offsetting the negative effects of fungi also as antifungal .

Aspirin work in two direction :-

1- suppresses the activity of prostaglandins in host , this reaction can offsetting effects of immunoresponse in addition prevent the fungus to use it .
2- Inhibition the fungal prostaglandin which prevents fungal colonization and chronic infection (14,25).

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تأثير الأسبرين كمضاد فطري ضد بعض الفطريات الانتهازية

علياء احمد البدر

قسم علوم الحياة ، كلية العلوم ، جامعة البصرة ، البصرة ، العراق

الخلاصة

درس اثر الأسبرين (عقار غير ستيرويدي ، مضاد للالتهاب ومثبط لانزيم السايكلوجينيز) كمضاد فطري ضد بعض الفطريات الانتهازية *Aspergillus flavus , A. niger , A. terreus ,Cryptococcus neoformans,* *Penicillium sp* . and *Trichoderma sp.*

وجد ان الاسبرين ذو فعالية قوية ضد جميع الفطريات المدروسة مختبريا واعطى اكبر تاثير عند التراكيز 1000 µg/ml , 2000 µg/ml and 3000 µg/ml اذ ادت هذه التراكيز الى حدوث تثبيط كامل للنمو الفطري .

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