The effect of aspirin as antifungal drug against some medical important fungi in in vitro

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Abstract

Aims: To evaluate the antimicrobial activities of aspirin against, Aspergillus flavus, Cryptococcus neoformans and Candida albicans. H

Aspirin was showed an antifungal 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, anti – inflammatory drug . it was first marketed in 1899, it has been widely used for the treatment of pains, fever and colds. In the meantime, their antifungal activity has been evaluated in the laboratory so as to find new fungicides with high efficacy and low toxicity(1). The chemical name of aspirin is acetylsalicylic acid or acetosalicylic acid(ASA) , a white crystalline compound that belongs to the class of (NSAIDs) non-steroidal anti – inflammatory drugs, Salicylic acid also has anti-fungal properties which can be used to eliminate tinea, a fungus involved in various types of skin infections. Salicylic acid can eliminate tinea versicolor, an infection of the top layer of the skin that causes scaly, discolored patches; tinea pedis (athlete's foot); tinea cruris (jock itch); tinea corporis (ringworm of the body) and tinea capitis (ringworm of the scalp) (1,2) . Oxylipins are oxygenated lipids, divided to many groups, oxylipins in mammals is the eicosanoids, which include prostaglandins and leukotrienes (2) these products are potent 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 pathogenicity 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 flocculation in yeast (8) . The products have been found to be widely distributed in fungi (9-11). 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 ermatitis, 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). Aspirin (15) inhibited the biofilm formation in Candida albicans. 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 susceptibility testing of aspirin against single isolate of Aspergillus flavus, Cryptococcus neoformans and Candida albicans has been used.

Fungal species were activated on sabouraud‘s dextrose agar (SDA) for 5-7 days for filamentous fungi and for 2-5 days for Cryptococcus neoformans. The fungal spores and (few colonies for C. neoformans and Candida albicans) were harvested and transferred to 5 ml of sterilized distilled water and shacked

well then the numbers of fungal cells was counted by using Neubauer counting chamber, then adjusted to concentration of 106 cells/ml and using procedure of germ tube and characteristics of Candida albicans for definitive diagnosis of its isolate (18).

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) .

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 (30000 µg/ml) left at room temperature for 30 minutes for auto sterilization .

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 ºC mixed well , poured in sterilized Petri dishes (19) .

Exactly 0.65 ml of 100% DMSO was added 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 ºC 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 (1×106 cells / ml) was seeded in Petri dishes contain SDA by sterilized pipette size 1 ml and spread by sterilized L-shaped 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 ºC 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 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 (20).

RESULTS

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

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 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). In support of the above observations, Alem & Douglas (2004) demonstrated that biofilms formed by Ca. albicans can be inhibited as much as 95% by aspirin (15).

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). 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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استعمال الاسبرين كمضاد فطري ضد بعض الفطريات الطبية خارجياً

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الملخص

الهدف: لتقييم فعالية الاسبرين كمضاد فطري ضد

أعطى الأسبرين فعالية كمضاد فطري خارجياً وكانت التراكيز: ميكروغرام 1000، 2000، ميكروغرام 3000 ميكروغرام قد أعطت فعالية بنسبة 100%

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

Minimal Inhibitory Concentration (MIC) µg / ml	Fungal isolates		
	Aspergillus flavus	*Cryptococcus neoformans*	*Candida albicans*
3000	-	-	-
2000	-	-	-
1000	-	-	-
900	+	+	+
800	+	+	+
700	+	+	+
600	+	+	+
500	+	+	+

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