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First report of Antifungal activity of Tamoxifen against Filamentous Fungi

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Abstract: **We tested the activity of anticancerous compound tamoxifen (TAM) using the standard CLSI guidelines against filamentous fungi including *Aspergillus spp, P. marneffei, Mucor* and *Rhizopus* in a microplate based assay. MIC/MEC endpoint was calculated against drugs fluconazole, itraconazole, caspofungin (commercially known as Cancidas) and TAM. The endpoint of TAM was found to be 16-64µg/ml after 24-48 hrs with reduced filamentation at sub MIC concentrations. Our results imply that TAM affects the growth of various filamentous fungi tested, including *Aspergillus* (sp.), *Penicillium, Mucor* and *Rhizopus.* To our knowledge, this is the first report of TAM with antifungal activity against filamentous fungi.**

Keywords: **fungi, filamentous, yeast, drug resistance, *Aspergillus.***

1. INTRODUCTION

Fungal pathogens are generally opportunistic and dimorphic. Different fungi including yeast and filamentous are often associated with significant morbidity and mortality in humans. Being a eukaryote, limited number of antifungal are available for the treatment of infections and this problem is further aggravated with resistance generation against available antifungal dues to various reasons like expulsion of drugs out of cell, target mutation or duplication etc. Additionally, number of immunosuppressed and immunocompromised patients are also on the rise and these individuals are at higher risk for acquiring severe microbial infections. Thus, there is a need to look for newer alternatives as antifungal beyond conventional options.

Recent reports suggested evolutionary relatedness between yeasts, filamentous fungi to mammals. [1] Thus, it's natural to observe many natural-product toxins with antimicrobial activity are also toxic to mammalian cells. [2] Interestingly, although it makes difficult to have antifungal agents without toxic side effects, on the other hand many drugs administered to humans do have activity against infecting microbes too. [3] In this study, we have found the antineoplasmic agent tamoxifen (TAM) having antifungal activity against different filamentous fungi.

TAM is used in breast cancer therapy as it is antagonist of the estrogen receptor (structure in figure 1A). Sometimes breast cancer cells (hormone receptor-positive) requires estrogen for growth, as it binds on estrogen receptor in these cells and activate them to proliferate.[4] It is also reported to have antifungal activity against yeasts including *Candida albicans* and non-*albicans*, *Saccharomyces cerevisiae* and *Cryptococcus neoformans* with minimum inhibitory concentration (MIC) between 6-8 µg/mL.[4] In this paper, we are first time reporting TAM to be active against different filamentous fungi including different species of *Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus*.

2. MATERIALS AND METHODS

Materials

Media chemicals were obtained from Difco (BD Biosciences) and HiMedia (Mumbai, India). The drugs fluconazole, itraconazole, and tamoxifen (TAM) were obtained from Sigma (St Louis, Mo, USA). Caspofungin® (CAS) was procured from Merck (Merck and Co., USA).

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Fungal strains and culture

Strains and clinical isolates were maintained on Potato Dextrose agar (PDA) at 35ºC. The conidial suspension was prepared by scrapping the surface of the agar slants with 1 ml of sterile 0.9% saline containing 0.05% Tween 80 (NST). [5] The strains used in this study are listed in Figure 1B. Filamentous fungi included in this study are: *A. flavus*, *A. fumigatus*, *A. niger*, *A. terrus*, *Penicillium marneffei*, *Mucor* and *Rhizopus.*

Drug Susceptibility assay:

Drug susceptibilities of fungal strains were performed with a broth micro dilution method in accordance with CLSI document M38(A) for filamentous fungi. The 2X drug dilutions were prepared with RPMI 1640, and dispensed into rows two to 11 of standard 96-well plates in 100 µl volumes. Row one contained 200 µl of drug-free medium (sterility control) and the wells of row 12 were used as growth controls. An inoculum of 1×10^3 CFU in 100µl was added in all wells except sterility control and incubated at 35°C.[6] The minimum inhibitory concentration (MIC) end point was taken after 48 hrs, is defined as the lowest drug concentration in which no detectable growth was visible (24 h for *Mucor* and *Rhizopus*) whereas minimal effective concentration (MEC) was the lowest drug concentration resulting in aberrant hyphal growth.

Germ tube Inhibition assay- A conidiospores suspension ($1X10^3$) was incubated in RPMI medium at 35˚C with (2 µg/ml) or without TAM for 15h under static condition in microtiter wells. A spore is considered germinated when the length of the germ tube is twice or more the size of a spore.

3. RESULTS

Activity of tamoxifen aganist filamentous fungi-

The activity of tamoxifen was tested against ATCC and clinical cultures of different filamentous fungi including *Aspergillus sp., Penicillium marneffei*, *Mucor* and *Rhizopus* with TAM and standard drugs including azole and caspofungin (CAS) as given in figure 1(B). It was reverified that FLC is not the drug of choice aganist filamentous fungi and inactivity was seen till 64 µg/ml in *A. flavus, A. fumigatus, A. niger, A. terrus, P. marneffei, Mucor* and *Rhizopus* whereas CAS showed the endpoint of 64µg/ml for *P. marneffei, Mucor, Rhizopus* and *Aspergillus sp* except *A. terrus* where it came out to be >64 µg/ml. ITR showed best activity between ≤0.03to 0.5 µg/ml among the standard drugs tested.

TAM was found to have good activity in the range of 16-64 µg/ml aganist *A. flavus, A. fumigatus, A. niger, A. terrus and P. marneffei*. *Mucor* cultures showed best activity at 8µg/ml. Thus, TAM was found to be active against all cultures tested.

Conidia germination-

The cultures of *A. fumigatus* and *A. niger* were also tested for their retardation in conidia germination at sub inhibitory concentration of TAM and seen under the microscope. As can be seen in the figure 2A that the conidia of the fungi have reduced filamentation with TAM. It suggests that TAM affects the growth of different filamentous fungi by reducing their conidia germination. *A. fumigatus* and *A. niger* not only had reduced germination of conidia but their length was also found to be reduced compared to control samples grown without TAM (figure 2B).

4. DISCUSSION

In this study, we are reporting the activity on filamentous fungi including, *A. flavus, A. fumigatus, A. niger, A. terrus, P. marneffei, Mucor* and *Rhizopus* with a known antineoplastic compound TAM. TAM is a well known inhibitor of calmodulin in mammalians and yeasts as well.[4] Earlier, studies suggested that TAM-treated yeast show decreased calmodulin function, including lysis, disrupted actin polarization, and decreased germ tube formation.[4] Thus it indicated that TAM reduces filamentation by suppressing the Ca^{2+}-calcineurin pathway and also suggest towards potential of microorganisms as screening tools to elucidate the mechanisms of action of novel pharmacological agents.

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