Short Communication

Anti-Fungal Activity of Thymoquinone and Amphotericine B Against Aspergillus Niger

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Abstract:
Activity of Nigella sativa oil, ether extract and some of its active principles have been reported in the literature against a number of bacteria and Candida albicans. In the present study the effect of thymoquinone, an active principle of N. sativa and amphotericine B was determined against Aspergillus niger ATCC 16404. The organism was grown on dermasel agar containing 0.062, 0.125, 0.25, 0.5, 1.0 & 2.0 mg/ml of thymoquinone and dermasel agar alone as a control. There was 16.7, 36.2, 47.3, 67.8, 90.6 & 100% inhibition of growth of Aspergillus niger with these concentrations after 96 hours of incubation. Growth on the control plate after 96 hours was considered as 100%. Similarly there was 52.3, 65.1, 76.7, 81.6, 84.7, 85.6, 90.7, 92 and 93.8% inhibition of growth with 0.007, 0.015, 0.031, 0.062, 0.125, 0.25, 0.5, 1.0 and 2.0mg/ml of amphotericin B.

Key words: Aspergillus niger, Nigella sativa, thymoquinone, amphotericin B and mycoses
Introduction:

*Nigella sativa*, called as Habbah Al-Sauda in Arabic countries, is commonly used as a natural remedy for many ailments over 2000 years and is frequently added to bread and prickle as a flavouring agent\(^{(1)}\). Many active principles including thymoquinone, have been isolated from *N. sativa* seed\(^{(2-4)}\). Activity of *N. sativa* oil, ether extract and its active principles has been reported in the literature against a number of bacteria (including *Staphylococcus aureus*, *Pseudomonas aeruginosa* & *Escherichia coli*) and a yeast, like *Candida albicans*\(^{(5-8)}\). More recently ether extract of *N. sativa* and thymoquinone have been reported to possess antifungal activity against dermatophytes\(^{(9)}\).

*Aspergillus* species are the most common mold causing severe infections\(^{(10-12)}\). *Aspergillus niger* is a filamentous mold. Even though filamentous molds are ubiquitous in the environment, only over the past two decades have such saprophytic fungi emerged as a major threat in patients with compromised host defenses, such as those with hematologic malignancies, bone marrow transplant recipients and HIV infection and is reported to cause cutaneous infection, paranasal aspergilloma and osteitis of middle ear in such patients\(^{(13-16)}\). *Aspergillus niger* is also reported to cause endocarditis after heart surgery and infection of exenterated orbit even in immunocompetent patients\(^{(17, 18)}\).

In this paper we report the findings of the study of antifungal activity of thymoquinone, an active principle of *Nigella sativa*, and amphotericin B against *Aspergillus niger*.

Materials and Methods

a. Growth and identification of *Aspergillus niger*

A standard strain of *Aspergillus niger*, ATCC 16404, was cultured on dermasel agar (Oxoid). The plates were incubated at 30°C for 96 hours. The growth was identified as *Aspergillus niger* by colonial morphology and by microscopy after staining with lactophenol cotton blue.

b. Preparation of Reagents & Media

Thymoquinone (Aldrich, USA) was dissolved in 5 ml of ethanol and then mixed with sufficient amount of pre-sterilized dermasel agar to obtain serial dilutions containing 2.0, 1.0, 0.5, 0.25, 0.125, & 0.062 mg/ml of thymoquinone. Similarly amphotericin B (SIGMA, USA) was dissolved in 5
ml of DMSO and then serially diluted in dermasel agar to give final concentrations of 2.0, 1.0, 0.5, 0.25, 0.125, 0.062, 0.031, 0.015 & 0.0075 mg/ml. Four plates were used for each concentration of thymoquinone and amphotericin B. Four plates of dermasel agar alone were prepared as a control containing corresponding amounts of solvents.

C. Susceptibility Testing

Susceptibility testing was carried out as described Ali-Shtayeh (19). A 5 mm in diameter mycelial disc of *Aspergillus niger*, cut from the periphery of 48-72 hours old culture in dermasel agar was aseptically inoculated onto each set of above mentioned plates. The inoculated plates were incubated at 30°C for 96 hours. The growth was examined after 48 & 96 hours of inoculation and results were interpreted by measurement of the mean diameter of the growth. The percentage inhibition of *Aspergillus niger* with different concentrations of thymoquinone and amphotericin B was then calculated by taking its growth on non-drug dermasel agar as 100%. Minimum inhibitory concentration (MIC) of thymoquinone and amphotericin B was determined as their minimum concentration showing ≥90% inhibition of growth of the fungus.

Results

There was 16.7, 36.2, 47.3, 67.8, 90.6 & 100% inhibition of growth of *Aspergillus niger* with 0.062, 0.125, 0.25, 0.5, 1.0 & 2.0 mg/ml of thymoquinone, giving MIC of 1.0 mg /ml when growth recorded after 96 hours. The growth on the control plates after 96 hours was considered as 100%. Similarly, there was 52.3, 65.1, 76.7, 81.6, 84.7, 85.6, 90.7, 92 & 93.8% inhibition of growth with 0.007, 0.015, 0.031, 0.062, 0.125, 0.25, 0.5, 1.0 & 2.0 mg/ml of amphotericin B, giving MIC of 0.5mg/ml. Results are shown in table 1.

Table (1)

<table>
<thead>
<tr>
<th>Concentrations of drugs used (mg/ml)</th>
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<tbody>
<tr>
<td>2.0</td>
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<tr>
<td>Thymoquinone</td>
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<tr>
<td>Amphotericin B</td>
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</table>

*NT: Not tested* (Virtually no inhibition observed in initial experiments).
Discussion

Aspergillus species are the most common mold causing severe invasive infections in immunocompromized individuals (10-12). Fluconazole and ketoconazole are inactive against Aspergillus (20-22). Currently amphotericin B is most widely used against aspergillus infection, but failure of ampoterricin B treatment against invasive aspergillosis has also been reported. Overall, the response to amphotericin B remains poor, with a favourable outcome in only 30–40% of treated patients (23, 24). So a newer effective drug is required for invasive aspergillosis.

Successful validation of amphotericin B testing against Aspergillus species has been problematic (24). In the present study more than 80% inhibition was obtained with amphotericine B at a concentration of 0.062 mg/ml and more than 50% inhibition was shown by 0.0075 mg/ml, which also seems very high.

In the present study we observed a dose related anti-aspergillus effect of thymoquinone. Previous reports also showed concentration-dependent inhibition of growth of Gram-positive & Gram-negative bacteria, and yeasts by N. sativa seed and hexane-extracted N. sativa oil (7, 25). Thymoquinone showed comparable activity against Aspergillus niger although in relatively higher concentrations. However, thymoquinine at lower concentrations did not show comparable activity.

Possibly our study will promote further investigations to determine usefulness of thymoquinone and related compounds against aspergillosis and other opportunistic mold infections.
References


leukemica patient complicated with chronic otitis media due to *Aspergillus niger* and yeast-like fungi caused by superinfection. Kansenshogaku Zasshi, 1999, 73: 618-622
(بحث مفصل)
نشاط الليموفكتينون والأمفوتروسين (ب)
المضاد للنوع الأساسي للسيرجيلوس نيجير

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المفصل:
أصبح استعمال زيت النيجلا سافينا (والسمة بالحبة السوداء باللغة العربية) خطأً
ل كثير من الأسماك في بلدان الشرق الأوسط شائعاً. فكثيراً ما يتم إضافتها إلى الخبز و
المخللات لمكحول الطعام والرائحة. وقد سجلت الدوريات الطبية نشاط زيت النيجلا
سافينا المستخلص أو الشق النشط منه ضد عدد من البكتيريا وفطريات المكابديدا
البيكينيز (مكابديدا البيضاء).

ومن ثم كانت فكرة البحث لتتركز على تقديم فائدة زيت النيجلا سافينا أو أحد
مشتقاتها النشطة على العدوى من الطحالب العابرة حكم الأسيرجيلوس نيجير (الأسود) والتي
قد تحدث في الأشخاص ذوي الاضطراب المناعية. قُمت دراسة نشاط الليموفكتينون
والأمفوتروسين (ب) ضد الأسيرجيلوس نيجير (الأسود) ATCC 16404

و لقد أثبتت الدراسة زيادة تصاعدية لوقف نمو الأسيرجيلوس الأسود النوع
ATCC 16404 وATCC 2610 (62.7 و 76.6 و 80.7 و 81.3 و 92 و 96 و 70.7 و 75.7 و 77.6 و 79.5 و 80.6 و 81.7 و 90.8 و 93.8 و 96.2 و 100) مع التركيزات المتزايدة
للليموفكتينون (26.2 و 30.7 و 32.6 و 35.3 و 37.7 و 40.2 و 42.7 و 45.1 و 47.6 و 50.1 و 52.6 و 55.1 و 57.6 و 59.1 و 61.6 و 64.2 و 67.6 و 69.6 و 72.5 و 75.6 و 78.6 و 81.7 و 84.7 و 87.6 و 90.7 و 93.8 و 96.2 و 100) مع التركيز
المتزايدة لأمفوتروسين (ب) (70.7 و 76.2 و 82.1 و 88.0 و 94.0 و 100) وعند تأخير
البحث عن العدوى من الطحالب الضارة بعد 48 ساعة محسناً النمو 10% عياراً.

كما أثبتت الدراسة زيادة تصاعدية لوقف نمو الأسيرجيلوس نيجير (الأسود)
النوع ATCC 16404

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