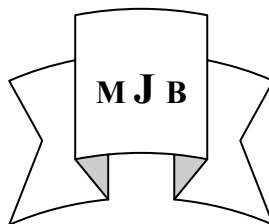


Treatment of Systemic Candidiasis in Rats with Water Extract of *Thymus Vulgaris* L.

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Abstract

Water extract of leaves from *Thymus vulgaris* L. was tested *in vitro* for its antifungal activity against pathogenic *Candida albicans*, using well diffusion method. Extract of *T. vulgaris* revealed a potent antifungal activity against *C. albicans* at 20% concentration. An experimentally – disseminated candidiasis was induced into a group of BALB/c rats by intravenous inoculum of *C. albicans* (0.2 ml of 3×10^5 cell / ml) and observations were made and recorded for a period of 30 days. Shivering, dullness, ataxia with a progressive decrease in body weight were observed in all the infected rats. White nodular abscesses were seen in kidney after 3 days of injection. Treatment of infected rats with the plant extract (0.5 ml administered orally for 14 days) caused an increase in the weights of the rats when compared with control animals.

الخلاصة

تمت دراسة فعالية المستخلص المائي لأوراق نبات الزعتر ضد خميرة *C. albicans* في الزجاج باستخدام طريقة الانتشار في الهلام (الحفر). أظهرت النتائج ان لمستخلص نبات الزعتر فعالية عالية ضد *C. albicans* وبتركيز ٢٠% حيث أدى إلى تثبيط نمو الخميرة. تم عمل تجربة للأصابة الجهازية بالمبيضة في الجرذان السويسرية من سلالة BALB/c أذ تم تلقيحها بالخميرة وبجرعة مقدارها ٠,٢ مل وبتركيز 3×10^5 خلية / مل بطريقة الحقن بالوريد، تركت الجرذان مدة ٣٠ يوم. تضمنت الأعراض المرضية ظهور حالة الارتعاش، قلة النشاط، خلجان، مع فقدان تدريجي في الوزن. كما أظهرت النتائج ظهور خراجات بيضاء عقدية في نسيج الكلية بعد مرور ٣ أيام من الحقن. أدت معاملة الجرذان المصابة بالمستخلص المائي لنبات الزعتر (٠,٥ مل معطاة عن طريق الفم لمدة ١٤ يوم) إلى زيادة واضحة في وزن الجرذان مقارنة مع حيوانات السيطرة (التي لم تعامل بالمستخلص).

Introduction

The prevalence of systemic fungal infections has increased significantly during the past decade and it's an important cause of morbidity and mortality particularly in immunocompromised patients [1]. This increase in part is due to more intensive antibiotic, corticosteroid and immunosuppressive therapy, invasive diagnostic and pressure monitoring devices and longer survival rates in critically ill patients [2].

C. albicans is a dimorphic fungus that exists as a commensal of warm-blooded animals including humans. It colonizes mucosal surfaces of the oral and vaginal cavities and the digestive tract and is also able to cause a variety of infections, depending on the nature of the underlying host defect. Therefore, *C. albicans* infections (candidiasis) are very infrequent in healthy individuals. Candidiasis may be divided into superficial (such oral and vaginal thrush and chronic

mucocutaneous candidiasis) and deep-seated (such as *Candida* due myocarditis and acute disseminated *Candida* septicemia) and represent a major clinical problem [3].

A great number of plant species contain various chemical substances exhibiting health benefit properties, antioxidative, anti-inflammatory and antimicrobial effects, their preventive and therapeutic use in animals is increasing [4].

Thyme (*Thymus vulgaris* L.) one of the plants which exhibit these properties. It's a member of Lauraceae family, a perennial labiate of the Mediterranean region and is now widely cultivated as tea, spice and herbal medicine [4,5]. Its leaves have been used as a stomachic, diuretic and urinary disinfectant. The anti-inflammatory effect of thyme has also been employed in traditional medicine. The main active ingredients of the thyme are thymol and carvacol with antioxidative, antimicrobial and antifungal effects [4,6]. Thymol also shows anti-aggregatory activity, strongly inhibiting platelet aggregation, which can be used in the prevention of thrombosis and arteriosclerosis [7].

The objective of this study is to evaluate the anti-fungal activity of water extract of *T. vulgaris* against pathogenic *Candida albicans* on Sabouraud Dextrose Agar (SDA) and also determine the effect of extract on *C. albicans* in rats.

Materials and Methods

Plant material

Dried leaves of *Thymus vulgaris* were purchased from a local market in Babylon Province, Hilla City, and identified at Babylon University Herbarium.

Preparation of water extracts (H₂O).

The leaves of *T. vulgaris* were grounded into powder using a grinder. One hundred g of fine-powder were infused in 200 ml distilled water until complete exhaustion and left for 30 min. The extract was then filtered using Whatman No.1 filter paper, after that the filtrate was centrifuged at 3000 round /min for 10 minutes and dried in oven at 60°C. The final dried material was stored in labeled sterile bottles [8,9].

Fungal culture

C. albicans was used to test the activity of the extract it was isolated from women suffering from urinary tract infection attending urine unit at Maternity and Pediatric Hospital in Hilla, Babylon province, 2008. The Urine sample was performed according to the procedure described by McGinniz [10].

Yeast cells were cultured on Sabouraud Dextrose Agar (Oxoid, USA) supplemented with chloramphenicol, and incubated at 37°C for 72 h, yeast isolate was identified following the scheme of Buckley [11] and Ellis [12] including germ tube test, production of chlamydospores and physiological and biochemical tests.

Antifungal susceptibility testing

For Antifungal susceptibility test, well diffusion method was used, 1 ml (containing approximately 10⁵ cell/ml) of *C. albicans* was poured on sterile Petri dishes, then a thin layer of SDA was displaced into these plates. The plates were incubated at 37°C for 24 h.

A cork borer was used to make pores (4×4 mm in diameter) on the surface of SDA. 50 µL of 20% (w/v) water extract of *T. vulgaris* was inoculated into each pore. Plates were incubated at 37°C for 24 h. The inhibitory activity of *T. vulgaris* against *C. albicans* was measured by clear zone appeared around each pore [13,14].

Experimental infection in rats

C. albicans was grown in Sabouraud dextrose broth culture at 25°C for 24 h, then washed 3 times in sterile normal saline and adjusted to a concentration of 3×10^5 cell/ml on a haemocytometer.

20 white BALB/c rats, of either sex (10) weeks old, weighing between 65-75 g were purchased from the Animal House of College of Medicine, University of Babylon. Rats of either sex were intravenously inoculated with a 0.2 ml of 3×10^5 cell/ml saline suspension of *C.albicans*. The animals were observed regularly for clinical signs, as well as their feed and water intake, for a period of 30 days, the body weights of all the animals from the control and infected groups were recorded. Some animals were anaesthetized by chlorophorm and scarified at 2,3,4 day after infection for detecting the site of infection [15,16].

Testing of water extract of *T. vulgaris* on rats.

Rats were divided into two groups: experimental and control. The experimental group was given 0.5 ml of extract orally. The control group was given tap water. Each rat was weighed every day. The Duration of the test was 14 days [17,18].

Statistical analysis

F test was used to analyze the quantitative data, *P*-values < 0.05 were considered significant.

Results

Antifungal susceptibility testing

The *in vitro* test on antifungal activity revealed that the water extract of leaves of *T. vulgaris* with high inhibitory activity inhibited the growth of *C. albicans* on SDA (zone of inhibition 23 mm) using the extract concentration of 20%. Fig-1.

Experimental infection in rats

The control rats did not manifest any abnormal signs during the study period, however the treated rats showed, shivering, dullness and ataxia. Two animals died, on the 2nd day and other on the 3th. day. The body weight of the *C. albicans* inoculated rats progressively decreased in comparison with the control rats. Small multiple white nodular abscesses were seen in kidneys after 3 days of infection. Fig-2.

Testing of water extract on rats

During the course of treatment, treated rats show no signs of abnormal behavior, however their eating habit seems to increase with concentration of treatment (20%). The weight of rats treated with the plant extract was higher (68-110g) and significantly different (*P*<0.05) in comparison with the control group (60-44g). Fig-3.

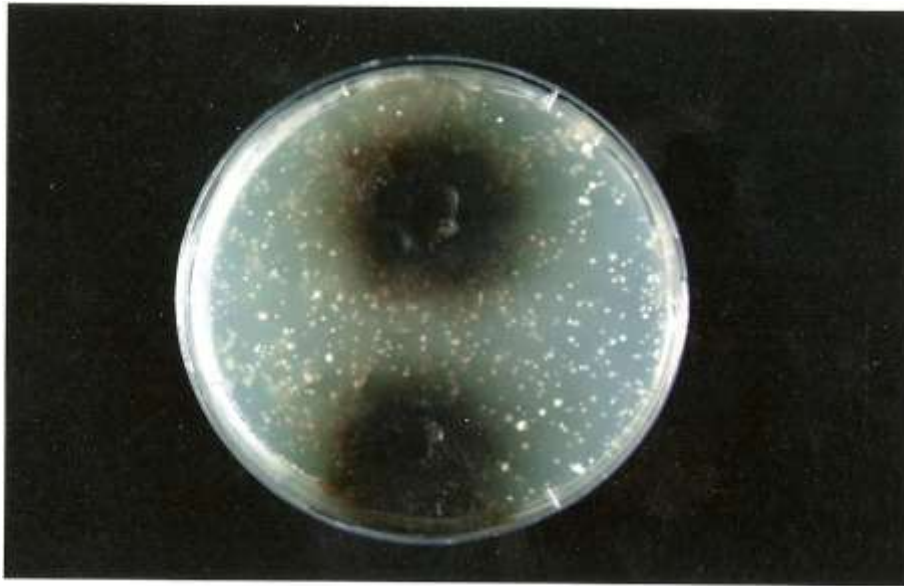


Figure1 Inhibition zone of water extract of *Thymus vulgaris* on *Candida albicans* on SDA .



Figure 2 Kidney of rat 3 days after intravenous inoculation with *C. albicans* . Multiple white abscesses are present .

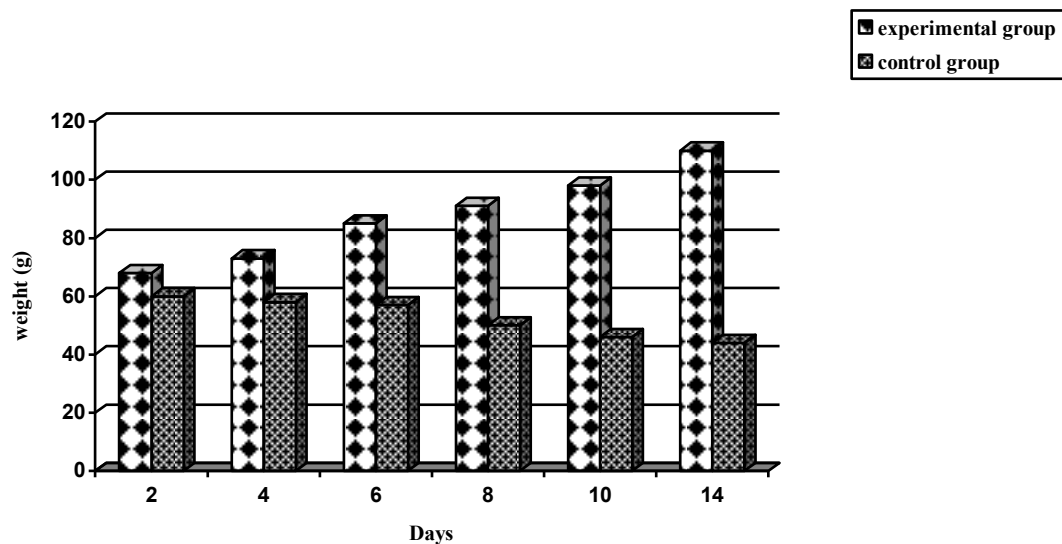


Figure 3 Body weight of BALB/c rats treated orally with water extract of *T.vulgaris*.

Discussion

Thyme (*Thymus vulgaris*) essential oil is known to possess antimicrobial activities against a wide range of microorganisms including fungi however, their antimicrobial activities were found higher against fungi than against bacteria [19].

The results of the present study revealed that water extract of *T. vulgaris* has antifungal properties against pathogenic *C.albicans* at 20% concentration. Our results are similar to those given by Brr and Mahmoud [20], they have reported that plant extracts of cinnamon, clove and thyme at 20% concentration inhibited many types of yeasts including *C.albicans*, *Debaromyces hansenii* and *Saccharomyces cerevisiae*, over all, thyme extract was the most potent extract. Nzeako and Al-Lawati[14] also described the effect of oil extract of *T.vulgaris* against *C.albicans* *in vitro*.

The antifungal effect of *T.vulgaris* on *C.albicans* can be attributed to thyme oil which has high thymol content strongly inhibited fungal growth [5,14]. Thymol is an amphipatic monoterpene, which

suggests that it affects cell membrane structure by generating asymmetries and membrane tensions, this confirmed by the fact that terpenes alter cell permeability by entering between the fatty acyl chains making up the membrane lipid bilayers, disrupting lipid packing, and changing membrane fluidity. The damage of *C.albicans* envelope becomes greater at increasing thymol concentrations and longer time of incubations, increasing the number of flattened cells with surface folds, cells with holes, collapsed cells and ghosts. Terpenes also inhibit *Candida* respiration and this suggest adverse mitochondrial effects [21]. Pina-Vaz *et al.*, [22] reported that thymol oil produced lesions in the plasma membrane of fungi there by allowing the organism to die by the efflux of the contents of the plasma membrane. They also showed that concentration below the minmum inhibitory concentration values of the plant significantly inhibited germ tube formation, an important virulence factor.

Disseminated candidiasis was induced in rats following intravenous inoculations of *C. albicans*, 3×10^5

yeast cell /ml and observations were recorded for a period of 30 days . Clinical signs observed in the infected rats were initially characterized by shivering, dullness, ataxia with a progressive decrease in body weight were observed in the infected rats. These findings can be attributed to severe stress due to systemic fungal infections leading to anorexia associated with pyrexia , reduced water intake and are in agreement with the earlier observations with experimental candidiasis in guinea pigs [23] and in rabbits [16].

The results revealed also the development of multiple white abscess in kidneys after 3 days of infection .Papdimitriou and Ashman[15] reported that though significant blastospore deposition of *C.albicans* occurs in the lung,spleen and liver, the only histological evidence of colonization by the fungus,with a mild transient infection, is observed in the liver.In the same study, animals received a lower inoculum developed moderately severe myocarditis with foci of fungal replication being in the heart rather than the spleen and liver, these lesions gradually subsided while the lesions in the brain and kidney continued to develop into abscesses. Other workers [24] observed that the blastospore deposition of *C.albicans* occurs in the kidney and brain but the highest colony counts occurs in kidney, the last being least susceptible. Odds *et al.*, [25] noted that in mice inoculated intravenously with *C.albicans* , the kidneys were the organs with the highest burdens of the yeast, throughout the observation period.

The reason for the kidney is the susceptible site for infection in the present study related to differences in population of tissue phagocytes, or in access of effector cells to the various site of infection and may also be of importance [14], however there is

certainly evidence that *Candida* blastospores germinate more readily in extracts of brain or kidney than extracts of liver, heart, spleen or lung [26].

The study also showed that there was a positive effect of *T.vulgaris* extract on the weight status of the test rats. The weight of rats treated with plant extract were higher (68-110g) in contrast to the control group (60-44g). This indicates that the physical status of treated rats were better. Lee *et al.*, [27] demonstrated that essential oil of thyme may stimulate the digestion process and effect affect growth performance. Other study [28] reported a positive effect of essential oils on growth performance by increasing weight gain and feed intake.

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