Correlation between Dermatophytosis and caffeine- containing beverages

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Abstract

Dermatophytosis is the most common skin diseases of humans. In order to determine any possible correlation between dermatophytosis and beverages containing caffeine (coffee, tea, cacao, and cola drink), an investigation was done for potential antidermatophytic effects of these beverages against three species with two variants of *Trichophyton*; *Trichophyton mentagrophytes var. mentagrophytes, Trichophyton mentagrophytes var. interdigitale, Trichophyton rubrum and Trichophyton simii*.

On the other hand, a survey among dermatophytosis patients was performed to detect the effects of caffeine consuming on development of dermatophytic disease.

The results revealed that there was no activities of beverages against dermatophytes, as well as no relationship between tea consuming and dermatophytosis development. Whereas, pure caffeine had been exhibited a well antifungal activities against dermatophytes.

الخلاصة

تعتبر الإصابة بالفطريات الجلدية من أكثر الأمراض التي تصيب جلد الإنسان ، ولأجل التحري عن العلاقة بين هذا النوع من الأمراض و المشروبات الحاوية على الكافئين (القهوة والشاي وشراب الكولا) ومدى التأثير المضاد لهذه المشروبات على ثلاثة أنواع من الفطريات الجلدية *Trichophyton mentagrophytes var. mentagrophytes, Trichophyton* مع ضربين لأحدهما والتي شملت الفطريات:

mentagrophytes var. interdigitale, Trichophyton rubrum and Trichophyton simii..

ومن جهة أخرى فقد اجري مسح لأشخاص مصابين بالفطريات الجلدية لغرض معرفة مدى تأثير تناولهم للشاي الحاوي على الكافئين على تطور الإصابة لديهم. وقد بينت النتائج عدم وجود تأثير للمشروبات المفحوصة وكذلك عدم وجود تأثير لتناول الشاي على نشوء وتطور المرض مقارنة مع مركب الكافئين النقي والذي اظهر فعالية تثبيطية واضحة ضد الفطريات المنتخبة.

Introduction

Caffeine, 1,3,7-trimethylxanthin, naturally occurs in the leaves, seeds or fruits of more than 63 species of plants worldwide. The most common plant sources for caffeine are coffee and cocoa beans, cola nuts and tea leaves (1). High concentrations of caffeine can be detected in margin of young leaves of coffee (2). Biosynthesis of caffeine in leaves of coffee plant is reduced remarkably in both fully developed mature and aged leaves (3). Caffeine synthase is an enzyme that is responsible to form caffeine in coffee and tea plants from 7-methylxanthine compounds (4).

Human beings consume large amount of beverages containing various concentrations of caffeine daily, such as

coffee, tea, cacao and cola all over the world. Analysis the content of common coffee cup size of caffeine at homes indicates that medium cup size for coffee or tea is involved about 225 ml of coffee with 115 to 175 mg of caffeine per cup. Tea in general is lower than coffee in caffeine per serving. Both coca-cola and diet coke broth contain 45.6 mg of caffeine per 12-oz can, while chocolate bar ranges from 6 to 26 mg of caffeine per oz (2).

Caffeine has more than one important physiologic effect on central nervous system (CNS) representing mainly by stimulation functional jobs of this system to be more efficiency. Consuming of coffee increases arousal and cognition of a person who normally consume no more than 500 mg of caffeine per week (5). Davis *et al.* (2003) (6) demonstrated that blockade of adenosine receptors by caffeine seemed to be the most likely mechanism of CNS stimulation and delayed fatigue. Caffeine also has a potential activity to treat asthma (7). However, the reasons for adding caffeine to some soft drinks is for its flavor characteristic (1) and for its ability to improve the process of information and counteracts periods when the body's circadian rhythm or 'body clock' is at a low ebb, such as in the middle of the night or after lunch (8).

Naturally, caffeine is regarded to be very toxic compound for many types of micro-organisms when occurs in its forming plants (9). The chemical defense theory proposed that caffeine in young leaves, fruits and flower buds acts to protect soft tissues from predators such as insects (1). Caffeine toxicity encouraged biological researchers to assay its ability to inhibit growth, *in vitro* and *in vivo*, of different variety of micro-organisms, especially those have pathogenic effects.

A large number of fungi have the ability to cause severity diseases in humans. Dermatophytosis is one of common infectious disease on human's skin. It is caused by invasion of the stratum corneum by dermatophytic fungi, including three main genera, *Trichophyton*, *Microsporum* and *Epidermophyton* (10).

Antifungal activities of caffeine, either alone or in complexes with other chemical compounds, were frequently investigated by many studies. Caffeine in the Mg (Π) complex structure exhibited antifungal effect against *Microsporum gypseum*, one of dermatophytic fungi, and caused morphological changes in other species of fungi, such as *Botrytis cinerea*, (11). Furthermore, morphological changes of *Sporothrix schenckii* from yeast to mycelia structure was particularly blocked by caffeine with prevented of conidia germination from the same fungi (12).

Plants containing caffeine also prevented growth of many types of pathogenic fungi. Opportunistic fungi on human's skin, *Pityrosporum orbiculare* and *Pityrosporum ovale*, that cause tinea versicolor disease result in changing in skin color was hindered by alcoholic and aqueous extract of coffee seeds and tea leaves due to their contents of caffeine (13).

In previous study, caffeine demonstrated to have the ability to completely inhibit the growth of some species of *Trichophyton* that cause dermatophytosis after *in vitro* and *in vivo* tested (14).

The goals of this study were to detect the relationship between dermatophytosis disease and the rate of beverages consuming that contain caffeine in order to investigate the ability of beverages to prevent the development of such disease in human and to evaluate antifungal activity of caffeine when occurs in beverages forms.

Materials and Methods

Dermatophytosis and tea consumers

The influence of tea consuming on dermatophytosis development was screened. A total of 93 patients (2-55 years) with dermatophytic infection are asked about the number of their ordinary

consumed cups of tea per day. Their sexes are also included in this survey.

Isolation of dermatophytes

Scales of Dermatophytosis patients were examined microscopically with 20% KOH for fungal hyphi and spores. Positive scales were cultured on Sabouraud's chloramphenicol- cycloheximid glucose agar. Media were prepared by mixing the following composition (20 gm glucose, 10 gm peptone, 0.5 gm cycloheximid, 0.05 gm chloramphenicol and one liter of distill water) (15). Cultures had been incubated at 25-28 º C for one week.

Three species with two variants of *Trichophyton* genus were diagnosed according to the criteria established by Rippon (1988) (16) and Emmons (1970) (17). These species were: *Trichophyton mentagrophytes var. mentagrophytes, Trichophyton mentagrophytes var. interdigitale, Trichophyton rubrum and Trichophyton simii.*

Beverages of caffeine versus Dermatophytes

A- Preparation of test materials

Coffee seeds (*Coffea arabacia* L.), black tea leaves (*Camellia sinensis* L.) and cacao seeds (*Theobroma cacao* L.) were obtained from local Iraqi markets. The main purpose for choosing these plants in our study is the widely usage of them by people to prepare their common beverages.

Aqueous extracts of chosen plants were previously prepared (18) by grounding clean parts of plant in mortar and pestle. Pulverized parts were soaked in distill water (1 gm per 5 ml of distill water) for 24 hours at 37ºC. Aqueous extracts were filtered through Whatman No. 1 filter paper. Filtered extracts were dried at 60 º C for two hours. Various percentages (1, 5, 10, 15, and 20 %) of cola drink (Ugarit cola mark, Syria) were mixed with prepared Sabouraud's medium.

B-Antidermatophytic assay

Colony diameter method described by Özgönen and Biçici (2001) (19) was used to determine the antidermatophytic activities of beverages. Briefly, different concentrations of tested materials were mixed separately with melting Sabouraud's glucose agar in sterilized conical flask. Mixed media were poured in sterilized Petri dishes. A disk (9 mm) of old fungi culture, grown at 25-28 º C for one week, was formed using cork bore. In the center of Petri with mixing media, one well (9 mm) was performed.

A disk of each species of fungi was embedded in a well. Cultures had been incubated at 25-28º C for one week. Average of two perpendicular diameters (mm) of growth colony was measured.

Three controls were used in this experiment: 1 mg/ml of griseofulvin (standard antifungal agent), pure compound caffeine (Fluka, Switzerland) and free compound media.

Statistical analysis

All experiments had been repeated three times with triplicate for each one for statistical analyses using analyses of variance (ANOVA) under the property ($P<0.01$).

Results

Diagnosis of dermatophytosis in patients who consume tea as favorable beverage revealed that large number of patients have been infected by tinea corporis (46.23 %), followed by tinea capitis (22.58%), tinea manuum (13.97 %), tinea pedis (12.90 %), then tinea unguium (1.07 %). Furthermore females were infected by tinea corporis (25.80 %) and tinea manuum (11.82 %) more than males (table 1).

Drinking tea as beverage is the most common phenomena in Iraqi society to which consume by families throughout a day to reach approximately three to five or more cups per day by each adult person.

It was observed that most dermatophytosis patients drink approximately 2 cups tea per day (43.01 %) followed by 3-5 cups (22.58 %) and 6-8 cups (12.90 %) per day (table 1).

Caffeine showed much better results than its beverages as antidermatophytic agent depending upon the colony diameter measurements of grown fungi on medium containing aqueous extract of either coffee or tea.

Pure Compound caffeine completely inhibited growth of all tested fungi at concentration of 2 mg/ml. at this concentration, caffeine had been showed perfect effects than griseofulvin. Meanwhile, less than 2 mg/ml of caffeine (1 mg/ml and 0.5 mg/ml) revealed significantly effects comparable to that of free medium (control). Aqueous extracts of tested plants exhibited no activities against all *Trichophyton* species with no significant differences ($P<0.01$) when compared with free compound control (table 2). Furthermore, cola drink slightly increased the growth rates of the two variants of *T. mentagrophytes* in simulating with elevated all tested percentages of cola (table 3).

Discussion

The effects of caffeine on fungal cells are not restricted to inhibit growth, but also to prevent synthesis most of metabolic molecules in these cells. Production of aflatoxin by *Aspergillus parasiticus* was completely abolished in parallel with partially inhibition of fungal growth after culturing of this fungus in medium containing of 2 mg caffeine per ml (20). Production of Chitinase by opportunistic fungal pathogen, *Aspergillus fumigatus*, was terminated after extensive interaction with caffeine (21).

The results of table (1) illustrated that consumption of tea has been shown no significantly effects to prevent development of dermatophytosis. Although, each cup of tea contains about 50 mg of caffeine (22), small numbers of identified infectious lesions (tinea) had been observed in individuals who consume large amount of tea every day as main beverage. The variation of tea activities may be related to the influence of various factors, including the variety and growth of tea plant, environment, manufacturing conditions, grade (particle size) of the tea leaves and final infusion compositions (caffeine and polyphenolic contents). Other factors may also be important, such as the methods of preparation which are included the amount of tea and used water; time of infusion; and amount of agitation (23).

In previous study, aqueous extracts of coffee and tea revealed fungicidal effects against *Pityrosporum* species with completely inhibited of their growth. Meanwhile, pure caffeine showed no action against the same fungi (14). The present study has illustrated a reflect results than that of previous study. Pure caffeine exhibited obvious inhibition activities than those of plant extracts against *Trichophyton* species up to 5 mg /ml of aqueous extracts of coffee, tea and cacao with revealed a good results in compare with standard antifungal agent (griseofulvin) (table 2).

Although a bottle (360 ml) of cola drink contains about 40-50 mg of caffeine (22), the colony diameter of two variants of *T. mentagrophytes* was simultaneously increased compared to control (free compound media) when it is cultured in media containing high percentage of cola (table 3). A good explanation of the above results can be attributed to the high concentrations of glucose involving via the manufacturing of cola drink and these concentrations is usually elevated with the increasing of the percentage of tested cola.

The mode of caffeine action in living cells can be explained by three mechanisms: Inhibition of cAMP phosphodiesterase enzyme; Ca^{2+} mobilization; and adenosine receptor antagonism (24). Recently, stimulation of prostacyclin (vasodilator agent) production in the human body is acceptable mechanism to illustrate the action of caffeine to reduce bronchoconstraction symptom (25).

Several studies have been reported that none of the above mechanisms are used by caffeine to perform its activities in fungal cells. Buchanan *et al.*(1983) (20)results

indicated that growth inhibition of *Aspergillus parasiticus*, in part, did not depend on the inhibition of cAMP phosphodiesterase of the fungus, but due to an alteration of either purine metabolism or purine function. Caffeine could be also induced override in *Shizosaccharomyces pombe* via caffeine action on kinase enzymes that is responsible for DNA replication in fungal cells (26).

The caffeine molecule is closely resembled other metabolically important compounds in chemical structure, including purine (adenine, guanine), adenosine, xanthine and uric acid (2). Therefore, the suggested site of caffeine action may be based on the similarity in chemical structure of caffeine with purine group of nucleic acid.

In conclusion, the beverages that contain caffeine have no activities against dermatophytes. The collected data revealed weak correlation between consuming of plants and beverages containing caffeine and dermatophytosis.

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Table (1): Number of Dermatophytoses patients consumed a cup of tea per day.

Tea cup	Tinea corporis		Tinea pedis		Tinea capitis		Tinea cruris		Tinea manuum		Tinea unguium		Total No.
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	
Zero	6 6.45 %	3 3.22 %	2 2.15 %	1 1.07 %	1 1.07 %	2 2.15 %	1 1.07 %	1 1.07 %	1 1.07 %	2 2.15 %	zero	zero	20 21.50 %
1-2	5 5.37 %	12 12.90 %	5 5.37 %	4 4.30 %	6 6.45 %	3 3.22 %	zero	1 1.07 %	zero	3 3.22 %	zero	1 1.07 %	40 43.01 %
3-5	4 4.30 %	8 8.60 %	zero	zero	4 4.30 %	1 1.07 %	zero	zero	1 1.07 %	3 3.22 %	zero	zero	21 22.58 %
6-8	4 4.30 %	1 1.07 %	zero	zero	4 4.30 %	zero	zero	zero	zero	3 3.22 %	zero	zero	12 12.90 %
Total No.	19 20.43 %	24 25.80 %	7 7.52 %	5 5.37 %	15 16.12 %	6 6.45 %	1 1.07 %	2 2.15 %	2 2.15 %	11 11.82 %	zero	1 1.07 %	93
	43 46.23 %		12 12.90 %		21 22.58 %		3 3.22 %		13 13.97 %		1 1.07 %		

Table (2): Colony diameter (mm) of Dermatophytes grown on Sabouraud's glucose agar containing various concentrations of coffee, tea and cacao aqueous extracts at 25-28 °C for one week.

Plant type	Conc. mg/ml	*T ment. var. mentagrophytes*	*T ment. var.interdigitale*	*T. rubrum*	*T. simii*
Control		63 ± 1	64 ± 1.8	32 ± 2	54 ± 1
Coffee	0.5	62 ± 1	61 ± 2	30 ± 1.8	48 ± 2.5
	1	60 ± 2	58 ± 2	29 ± 2	47 ± 1
	5	43 ± 1	50 ± 2.5	25 ± 1.5	26 ± 2
Tea	0.5	63 ± 2.5	63 ± 3	28 ± 1.8	48 ± 1
	1	58 ± 1	60 ± 2	27 ± 2	41 ± 1
	5	39 ± 1.5	51 ± 0.9	25 ± 2.5	32 ± 1.5
Cacao	0.5	56 ± 2.5	54 ± 1.5	31 ± 3	48 ± 1
	1	51 ± 1	49 ± 2	28 ± 2.5	47 ± 2
	5	48 ± 1.8	45 ± 3	28 ± 1	41 ± 1.9
Caffeine	0.5	26 ± 0.9	29 ± 2	19 ± 2	25 ± 3
	1	12 ± 1 *	12 ± 1.5 *	13 ± 1.5 *	12 ± 1 *
	2	Zero *	Zero *	Zero *	Zero *
Griseofulvin	1	12 ± 1	13 ± 0.8	11 ± 0.5	11 ± 1

M ± SE

*** Significant differences (P<0.01) between compound and control**

Table (3): Colony diameter (mm) of Dermatophytes grown on Sabouraud's glucose agar containing various percentages of cola drink at 25-28 °C for one week.

Conc. (%)	*T ment. var. mentagrophytes*	*T ment. var.interdigitale*	*T. rubrum*	*T. simii*
Control	**63 ± 1**	**64 ± 1.8**	**32 ± 2**	**54 ± 1**
1	**53 ± 2**	**51 ± 1**	**28 ± 0.9**	**48 ± 1**
5	**54 ± 1.8**	**56 ± 2**	**27 ± 2**	**38 ± 0.9**
10	**55 ± 3**	**58 ± 1.8**	**26 ± 1**	**39 ± 2.5**
15	**56 ± 1**	**58 ± 0.9**	**24 ± 1.5**	**38 ± 1**
20	**60 ± 1.5**	**61 ± 0.5**	**25 ± 2**	**38 ± 2**

M ± SE