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INHIBITORY EFFECTS OF DL-TRYPTOPHAN AND L-METHIONINE ON *MICROSPORIUM CANIS* AND *TRICHOPHYTON RUBRUM* GROWTH IN SAFE PEOPLES AND SUFFERING PEOPLE TO DERMATOPHYTOSIS UNDER IN-VIVO AND IN-VITRO CONDITIONS

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ABSTRACT

In this study, the effect of DL-thryptophan and L-methionin on *Microsporium canis* and *Trichophyton rubrum* in safe peoples and suffering peoples to dermatophytosis under invivo and invitro conditions were studied. From safe and suffering to dermatophytosis peoples blood sample were taken and amount of Tryptophan and Methionine in this peoples serum by HPLC method were measured and so noted fungus were cultured in cultures media with difference concentrations of Tryptophan and Methionine. Each of samples repeated in 3 times and after 2 week the diameter of colonies was measured, the results of research statistically were analyzed by using the SAS software and comparisons of mean by using the ANOVA were done. Result has shown that the diameter of colony in difference concentration of Tryptophan and Methionin were decreased in experimental fungus than control group. This appears that the Tryptophan and Methionin were cause of decreases in the *Microsporium canis* and *Trichophyton rubrum* growth. Thus, probably the noted amino acids have inhibitory effects on experimental fungus growth.

Keywords: DL-Tryptophan, L-Methionine, *Microsporium canis*, *Trichophyton rubrum*, Dermatophytosis

INTRODUCTION

Many skin diseases such as tinea and ringworm caused by dermatophytes exist in tropical and semitropical areas. In general, these fungi live in the dead, top layer of skin cells in moist areas of the body, such as between the toes, the groin, and under the breasts. Dermatophytosis is one of the dermal mycosis that results from the group of fungus actions in the keratinized tissue (such as hair, nail, and skin keratinized tissue) that called dermatophytes. Nowadays 41 species of dermatophytes were identified that totally divided into three geniuses (with notice to the asexual phase) with names *Microsporium*, *Trichophyton* and *Epidermophyton*. However, their clinical differentiation is difficult. The clinical care is required by a physician or other healthcare professional in the treatment of these diseases **[1]**. physical and chemical agents can be effective in reveals of dermatophytosis pathogenesis in human which some people are sensitive and some other are resistance and might be dermatophytes also shown difference susceptible against of this agent. It is clear that human is continuously contact with dermatophytes, therefore, fortunately low amount show disease signs. For example, leg ringworm is one of the most prevalent types of tinea. However, appearance of empirical infection in volunteer peoples has been shown a high percentage of a natural resistance in against of sufferance from infection. Recent studies has shown that composition and rate of amino acids in perspiration of patients with ringworm disease were different with natural cases and were imaged that this is one of the effective agent in appearance of chronic infections **[2]**. In this study, we compared Inhibitory effects of DL-tryptophan and L-methionine on *Microsporium canis* and *Trichophyton ruberum* growth in safe peoples and suffering people to Dermatophytosis under in-vivo and in-vitro conditions.

MATERIALS AND METHODS

In-vivo Pathway

In this study during the November 2012 to November 2013 on 340 male that their lesions with regarding the dermatophytal infection were positive in direct experiment were done. From the people with the average age 20-40 we selected 76 individuals that have tinea acorposis disease, from these all peoples by receiving testimonial and by voluntary wanted that morning of next day as fasting refer to laboratory to blood sampling. After blood sampling, the samples to separating the serum were centrifuged (2500 round per minute for 10 minutes). Then samples were maintained in refrigerator at -60^{o}C. The cutaneous sample

of these peoples were cultured in mycobiotic agar while the fungus strain by macroscopic and microscopic studies were designated as only on peoples serum that suffers to dermatophytosis with *Microsporium canis* and *Trichophyton rubrum* agents to determination of DL-tryptophan and L-methionine amino acids, HPLC experiment were done.

***In-vitro* Pathway**

Materials

a) *Microsporium canis* and *Trichophyton rubrum* counterfoils provided from fungus collections and industrial and infectious bacterials dependent on Iran scientific and industrial researches organization.

b) Mycobiotic agar culture media produced by Germany Merck factory.

c) DL-tryptophan and L-methionine amino acids produced by Germany Merck factory.

d) Tween 80

e) Saboroud glucose broth culture media produced by Germany Merck factory.

Equipment were used in this study includes heater equipped to magnetic mixer, autoclave, disposable 8 centimeter plate, flame light connected to gas, 10 centimeter glassy tube, erlenmeyer flask. This study is tentative types of studies. First, the saboroud glucose broth culture media was provided. Thereby that 30 gram of ready powder scaled and added to 1 litter distilled water. Erlenmeyer contain culture media and distilled water was occupied on the magnetic heater and during the boiling mixed. Environment was shaded into 10 centimeter head screwed tubes and was autoclaved. 0.5cc of tween 80 were sheded into other head screwed tubes and steriled. By spike beak fieldoplatin some of dermatophyte colony were achieved and were resolved in tween 80. Contents of each saboroud glucose broth tubes were empties on one of the dermatophytes resolved in tween 80. The samples after closing the curved (the curved should not be quite sealed) for 21 days were kept in laboratory temperature and after 21 days, tubes were centrifuged and upper portion were outed and from their sediments used to culturing in solid media. 36 gram of mycobiotic agar powder were scaled and added into 2 litter erlenmeyer that 1 litter of this was distilled water. After occupation of magnet into Erlenmeyer were located on magnetic heater while during the boiling assimilated quietly (from this media were provided in more amounts). Into ten of 250cc erlenmeyer that each of them contains 200cc culture media by turn were provided 5 concentrations of each amino acids (1, 0.75, 0.5, 0.25, and 0.1 percent). As concentration

of 1%, 2gram, for concentration of 75%, 1.5 gram, for concentration of 0.5%, 1gram, for concentration of 0.25%, 0.5 gram and for concentration of 0.1%, 0.2 gram of each DL-tryptophan and L-methionine amino acids were scaled and added. In testifier erlenmeyer no added any amino acid. Erlenmeyer after autoclaving in temperature at 121ºC and pressure of 15 atmospheres, were spreaded into 8 centimeter plates and on each plate name of each amino acid and their concentration were noted. Each of two dermatophytes counterfoil were cultured in plates contains amino acid and also in plates without amino acid. Cultured plates were located into incubator at temperature of 25◦c and after 14 days the diameter of grown colonies were measured. All fungus culturing were done near the gas flame and under sterile conditions. All this stages for each dermatophyte and amino acid concentration were repeated in 3 times and growth average of each dermatophyte in each concentration of two amino acids was determined and all results by using of SAS statistical software were analyzed **[3]**. The size of colonies has been exhibited by average. Comparison of fungus colonies size in presence of under studied amino acids, were done by using the ANOVA that amount of $p<0.05$, were exhibited the significant differences **[4]**.

RESULTS

Observing the results are indicates that colonies diameter in *Microsporium canis* and *Tricophyton rubrum* at different concentration of DL-tryptophan in compare with control group were significantly decreased ($p<0.05$) **(Table 1 and 2)**. In DL- tryptophan, all concentration rather than each other have significant different and minimum average is related to concentration of 1%. Also, minimum colony diameter of *Microsporium canis* and *Tricophyton rubrum* is related to concentration of 1%. According to table 1 and 2 comparison of colonies diameter between difference concentrations of L-methionine with control group has shown that colonies diameter of difference concentration of L-methionine has significant decreases than control group ($p<0.05$). In L-methionine maximum average is associated with concentration of 1%.

The results showed that DL-tryptophan had most inhibitory effect but L-methionine had moderate effects on the dermatophytes caused by *Microsporium canis* and *Tricophyton rubrum*.

Table 1: Comparison of Colonies Diameter in Different Concentration of DL-Tryptophan and L-Methionine With Control Group in *Microsporium canis*

Treat	Level	Mean±Sd
DL-tryptophan	**1**	**14.33±1.00**
	0.75	**22.00±1.00**
	0.5	**30.33±1.00**
	0.25	**33.00±1.00**
	0	**44.00±1.00**
L-methionine	**1**	**35.33±0.50**
	0.75	**36.33±0.50**
	0.5	**38.00±0.50**
	0.25	**39.33±0.50**
	0	**44.00±0.50**

Table 2: Comparison of Colonies Diameter in Different Concentration of DL-Tryptophan and L-Methionine With Control Group in *Tricophyton rubrum*

Treat	Level	Mean±Sd
DL-tryptophan	**1**	**16.66±0.50**
	0.75	**19.00±0.50**
	0.5	**19.66±0.50**
	0.25	**20.33±0.50**
	0	**28.33±0.50**
L-methionine	**1**	**28.00±0.50**
	0.75	**29.66±0.50**
	0.5	**30.00±0.50**
	0.25	**31.33±0.50**
	0	**33.33±0.50**

DISCUSSION

In this study *Trichophyton rubrum* and *Microsporum canis* showed greater sensitivity to DL-tryptophan and none of them were able to grow in a concentration of 0.25%. Also in Sarasgani and Firozrai study none of them were inhibited growth of dermatophytes with exception the L-lusin that were elicited to growth inhibition of *Microsporium gypseum* **[3]**. The study that were done on *Microsporium gypseum* and *Trichophyton mentagrophytes* in india has shown that cysteine hydrochloride and aspartic acid have inhibitory effect and minimal inhibitory concentration of cysteine hydrochloride for *Microsporium gypseum* is 0.5 gr/dl and for *Trichophyton mentagrophytesis* 0.4 gr/dl were reported **[5]**. Acid aspartic also with 1gr/dl concentration, were decreased the growth of *Microsporium gypseum* to 100 percent and growth of trichophyton mentagrophytes to 48 percent **[5]**. Also in one study with adding androgen hormones to dermatophyts culture media, the diameter of colonies were decreased and among hormones, and erstenedion has most inhibitory effect and

Trichophyton verrucosum and *Trichopyton rubrum* has high susceptibility **[6,7]**. In other study shown that from 24 experimented dermatophyt species, only *Trichopyton mentagrophytes* had ability to growth in presence of cysteine 4% molar concentration [8]. In one other study that was done reveled that aspargin and methionine amino acids causes decrease *Trichophyton rubrum* and *Trichophyton verrucosum* growth **[9]**. Amino acids also either was shown inhibitory effect on two dermatophytes that the acid aspartic inhibitory effects on *Microsporium gypseum* growth were determined in pandy study **[5]** In one other study by Gharachorlou, A. *et al*. reveled that histidine has inhibitory effect on *Trichophyton Mentagrophytes* Growth **[10]**. In current study the inhibitory effect of DL-tryptophan and L-methionine on *Trichophyton rubrum* and *Microsporium canis* were assessed and shown that concentration of 1% DL-tryptophan causes maximum decrease in *Trichophyton rubrum* and *Microsporium canis* growth but L-methionine had moderate effects on the dermatophytes caused by *Microsporium canis* and *Tricophyton rubrum*. The colony diameter in different concentrations of DL-tryptophan in experimental fungi than control group was decreased. Therefore DL-tryptophan and L-methinine amino acids probably have inhibitory effect on growth of *Microsporium canis* and *Trichophyton rubrum*.

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