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**ORIGINAL ARTICLE**

# Survey of Tryptophan Effects on Trichophyton Verrucosum Growth in Normal and Dermatophytic Patients under in Vivo and in Vitro Conditions

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Aliasghar Gharachorlou, S.J. Hashemi, Shahram Gharachorlou, Jamshid Ghiasi Ghalekandi, Ahmad Asghari; Survey of Tryptophan Effects on Trichophyton Verrucosum Growth in Normal and Dermatophytic Patients under in Vivo and in Vitro Conditions

## ABSTRACT

In this study the effect of tryptophan amino acid on trichophyton verrucosum in normal and dermatophytic patients under in vivo and in vitro conditions were studied. From safe and suffering from dermatophytic people's blood sample was obtained and amounts of tryptophan amino acid in this people serums by HPLC method was measured and so mentioned fungi was cultured in cultures media with different concentrations of tryptophan. Each of samples repeated in 3 times and after 2 weeks the diameter of colonies was measured, the results of research statistically were analyzed by using the SAS software and comparison of mean by using the ANOVA was done. Result has shown that the diameter of colony in different concentration of tryptophan decreased in experimental fungi than control group. This appears that the tryptophan amino acid cause decrease in the trichophyton verrucosum growth. Thus, probably the mentioned amino acid has inhibitory effects on experimental fungi growth.

***Key words:*** dermatophytosis, tryptophan, trichophyton verrucosum, inhibitory effect.

## Introduction

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. Dermatophytes is a group of keratinophilic fungus that known from many years ago. Nowadays 41 species of dermatophytes were identified that totally divided into three geniuses (with notice to the asexual phase) with names microsporium, trichophyton, epidermophyton. Dermatophytosis is not contiguous disease and probably specific agents in sufferance to this disease are effective. 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. Of physical effective agents can be refer to temperature, moisture and PH that have difference effects on several dermatophytes. Several chemical factors such as hormones, fatty acids and amino acids in skin can be effective in dermatophytes growth. 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

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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 [1]. The study that were done on microsporium gypseum and trichophyton verrucosum in India has shown that cysteine hydrochloride amino acids and aspartic acid have inhibitory effect and minimal inhibitory concentration of cysteine hydrochloride for microsporium gypseum is 0.5 gr/dl and for trichophyton verrucosum is 0.4 gr/dl were reported. Also, Acid aspartic with 1gr/dl concentration decreased the growth of microsporium gypseum to 100 percent and growth of trichophyton verrucosum to 48 percent [2]. 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 [3,4]. In other study shown that from 24 experimented dermatophyt species, only trichopyton mentagrophytes had ability to growth in presence of cysteine 4% molar concentration [5].

In one study with measurement of androgen hormones in patients serum with dermatophytosis result from trichophyton verrucosum and trichophyton rubrum, were considered significant decrease in patients serum testosterone levels with trichophyton verrucosum agent in compared with healthy individual [6]. In the other study observed that fatty acids cause decreases the dermatophytes growth and unsaturated fatty acids with low amounts of carbon, acted more efficiently [7]. In this study the effect of tryptophan with different concentrations on trichophyton mentagrophytes.

## Materials and methods

*In-vivo Pathway:*

In this study during the years of 88-89 on 340 male which their lesions with regarding the dermatophyte infection were positive in direct experiment were done. Of 20-40 years old peoples we selected 76 individuals that have tinea corposis disease, from these all peoples by catching testimonial 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$^{\circ C}$. 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 from dermatophytosis due to trichophyton verrucosum agent to determination of tryptophan amino acid, HPLC experiment were exert.

*In-vitro pathway:*

Materials used in this study includes
A: trichophyton verrucosum counterfoils.
Trichophyton verrucosum provided from fungus collections and industrial and infectious bacterial dependent on Iran scientific and industrial researches organization.
B: mycobiotic agar culture media produced by germany merck factory.
C: tryptophan amino acid 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 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 screwd tubes and was autoclaved.0.5$^{CC}$ of tween 80 was shaded into other sterile and head screwed tubes. 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 discarded 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 different concentrations of tryptophan (1, 0.75, 0.5, 0.25, and 0.1 percent). As concentration of 1%, 2gram, for concentration of 75%, 1.5 kilogram, for concentration of 0.5%, 1gram, for concentration of 0.25%, 0.5 gram and for concentration of 0.1%, 2 gram of tryptophan was scaled and added. In control erlenmeyer no added any amino acid. Erlenmeyer

after autoclaving in temperature at 121 $^{c}$ and pressure of 15 atmospheres, were divided into 8 centimeter plates and on plates the name of amino acid and their concentration were wrote. Each of trichophyton verrucosum counterfoils 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. Fungus culturing were done near the gas flame and under sterile conditions. All this stages were repeated in 3 times and growth average of dermatophyt in each concentration of tryptophan was determined and all results by using of SAS statistical software were analyzed [8]. 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 [9].

*Results:*

According to results, the colonies diameter in different concentrations of tryptophan amino acid has significant decreased than control group ($p\leq0.05$). Observing the results are indicates that colonies diameter in different concentration of tryptophan amino acids in compared with control group were significantly decreased ($p\leq0.05$). In tryptophan, all concentrations rather than each other have significant differences and minimum average is related to concentration of 1%. Also, minimum colony diameter of trichophyton verrucosum is related to concentration of 1% than to maximum average (concentration of 0.1%) is lower than about 2.09 fold (tables 1 and 2).

**Table 1:** Comparison of colonies diameter in different concentration of tryptophan in normal and treated groups.

| Treat | level | Mean±Sd |
|---|---|---|
| normal | 0 | 28/33 a± 0/57 |
| tryptophan | 1 | 16/66 ± 1/15 |
| | 0/75 | 19/0 ± 1/00 |
| | 0/5 | 19/66 ± 0/57 |
| | 0/25 | 20/33 ± 0/57 |
| | 0/1 | 21/00 ± 0/00 |

**Table 2:** Tryptophan amounts in normal and patients to dermatophytosis sera.

| | tryptophan |
|---|---|
| Normal | 22/3 ±12/14 |
| Patient | 8/12 ±12/14 |
| *P value* | 0/005 |

*Discussion:*

Achieved results have shown that people distribution in associated with tryptophan amounts in serum was shown that about 50% of diseased peoples. With considering to current study this appears that increase in tryptophan level in serum probably causes hypersensitivity in people against dermatophytosis and were stimulated the dermatophytes growth. Also in Sarasgani and Firozrai study revealed that none of them were inhibited growth of dermatophytes with exception the L-lusin that were elicited to growth inhibition of microsporium gypseum. Argenine also in concentration of 1 and 0.1 have inhibitory effects but were not causes complete growth inhibition even in concentration of 1 gr/dl. Methionine also has no effect on trichophyton verrucosum and was shown mildly effect on microsporium gypseum [8]. In one other study that was done by Garachorlou *et al*., [11] reveled that aspargin and methionine amino acids causes decrease in the trichophyton rubrum and trichophyton verrucosum growth [10]. Acidic 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 [2]. In one other study by Garachorlou *et al*., [11] reveled that histidine has inhibitory effect on Trichophyton Mentagrophytes Growth [11]. In current study the inhibitory effect of tryptophan on trichophyton verrucosum were assessed and shown that concentration of 1% tryptophan causes maximum decrease in trichophyton verrucosum growth. The colony diameter in different concentrations of tryptophan in experimental fungi than control group was decreased. This appears that tryptophan causes growth decreasing in epidermophyton floccosum. Therefore tryptophan amino acid probably has inhibitory effect on growth of epidermophyton floccosum.

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