Treatment of Tinea Corporis by 0.50% Topical Ointment Prepared from Calvatia Craniformis Mushroom

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Abstract

Background: Tinea Corporis or Ringworm is a fungal disease caused by Trichophyton rubrum,T.mentagrophytes , Microsporum canis andT.tonsurans.It is transmitted by direct contact with infected animals and humans or by indirect contact through fomites.

The Aim of Study: This study aims to evaluate the efficacy of 0.50% ointment prepared from Calvatia craniformis mushroom in treatment of Tinea Corporis in human.

The Patients and Methods : The study is done in dermatology clinic in Baquba city. Three different concentration of Calvatia craniformis mushroom ointment are prepared by mixing three different weights of mushroom powder (0.3 ,0.4 and 0.5gm) with vaseline and completed up to 100 gm for each concentration to obtain (0.3%,0.4% and 0.5%) of the mushroom ointment respectively and then applied topically to lesion twice daily.

Sixty patients were involved in our study and divided into two groups, the treatment group includes thirty patients which is divided into three subgroups, each subgroup include ten patients they were treated by one of the three concentrations (Ten patients 0.3% , Ten patients 0.4%, Ten patients 0.5% concentrations). The control group included thirty patients was treated by 1% clotrimazol cream.

Results and Conclusion: 0.5% mushroom ointment significantly reduce clearance time ($P<0.05$)in comparison with the control group , while other concentration produce non significant changes in clearance time , therefore this substance is effective in the treatment of tinea corporis with less time, more patient compliance compared with the control group.

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Introduction

Tinea Corporis or Ring worm, is a rash that is indeed ring-shaped, but it has nothing to do with worms. It is a common superficial fungal infection that appears as circular red scaly patches on truck and extremites , also occurs in adults and children of both sexes and all races [1]. Tinea corporis is caused by *Trichophyton rubrum,T. mentagrophytes* , *Microsporum canis, T.tonsurans*. Transmission of the disease agent by direct contact with infected animals and humans or by indirect contact through fomites.

Diagnosis is made by clinical and microscopic examination of scrabed skin lesion cleared with 10% potassium hydroxide and by culture on sabouraud dextrose agar.

Many topical preparations were used for treatment such as miconazole, tolnaftate or clotrimazole for 2-4 weeks. Griseofulvine , itraconazole and terbinafine are the oral antifungal agents [2].

The mushroom used in this study is puffball mushroom, belongs to Basidiomycota division, Lycoperdaceae family, *Calvatia* genus, *craniformis* species (Figure-1a) [3].

Calvatia craniformis mushroom is edible when it is young, firm and white in color. Perhaps the most frequently collected puffball in Kansas, southern and eastern north America [4].

Ghosh, (2004) elicits many essential amino acids from *Calvatia craniformis* mushroom and vitamins like A,D,C,K and B-complex group and free from fatty acids and cholesterol. As a result the fungus make an ideal protein- rich food for heart diseases[5]. These objectives are essential to the repair or healing of infected epidermis and the disappearance of the lesion.

Kim et al., (1992) observed that protein-bound polysaccharides extracted from cultured *C. craniformis* mycelim suppressed the growth of sarcoma in mice by up to 74.1% . The anti-tumor activity of at least one of extract fracton referred to as calvatane was believed to be as a result of immuno-potentiation rather than cytotoxicity[6].

The (figure 1b) reveals the *Calvatia craniformis* mushroom was discovered in Jadidat Al-shat village in Hibhib city and Bani saad city- Diyala province for first time in Iraq according to the diagnosis of laboratory of fungus researches and plant disease in the college of Agriculture- Baghdad university .

Figure- 1a: represent the mushroom in the world.

Figure- 1b: represent the mushroom in Iraq.

Materials and Methods

1-Preparation of topical antifungal agent:

- *Calvatia craniformis* mushroom:

a- Fruiting body is dried and crushed in sterile Petri dish to obtain a yellow- brown powder.

b- By using a balance weigh, three weights (0.30,0.40,050 gm) each one singly alone.

c- Each weight is completed to 100gms of Vaseline to reach 0.30%, 0.40% and 0.50% concentrations.

2- The patients:

The number of patients is sixty , their ages are from 10-30 years and divided to two groups, each group consist of thirty patients. All the cases was seen sporadic during one year between June 2008 to June 2009 in dermatology clinic in Baquba city.

The diagnosis of the disease was depend upon the clinical features, which is characterized by circular, red, well - demarcated , scaly plugs accompanied by itching and confirmed by 10% KOH examination .

The first group was the tested group which include thirty patients was divided into three subgroup ; each subgroup consist of ten patients treated by different concentrations of

topical mushroom ointment twice daily for one month, while the second group was control group which include thirty patients treated by 1% clotrimazol applied topical twice daily for one month.

Statistical Analysis

The differences are compared by using (F-Test) at $p<0.05$ (7).

The study include sixty patients ,the first group(tested)shows twenty three patients responses to ointment but in different duration of treatment and this depend upon the concentration of preparation , seven patients are dropped from the study for unknown reasons, while the control group, only (twenty patients) complete the treatment course and ten patients were considered as defaulter.

Results

Table(1): The response of the patients to a different concentrations of the mushroom ointment as indicated by there disease clearance time.

Patients group	Ointment concentration %	Period of clearance
6 Patients	0.30	15-20 Days
8Patients	0.40	12-15 Days
9 Patients	0.50*	10-12 Days
20 Patients	1% clotrimazol	30-60Days

$p<0.05$

Discussion

These results reflects the medical importance of the *Calvatia craniformis* mushroom. The medical analysis of this mushroom proved the presence of three components; the first is calvatic acid which has chemical formation P-carboxyphenyl-azoxycarbonitrile[8]. This calvatic acid reveals strong antimicrobial activity against the Gram- positive bacteria, and weak action against the Gram- negative bacteria and against the yeast and fungi like *Saccharomyces cerevisiae* and some *Candida* species and *Trichophyton asteroids* [9].

The second component from chemical analysis and spectroscopic means of the mushroom is hydroxyphenylazoformamide derivatives which has three chemical compounds, 4-hydroxyphenyl-1azoformamid, 4-hydroxyphenyl-ONN-azoformamid and 2-methylsulfonyl-4-hydroxy-6-methylthiophenyl-1-azoformamid, which we named it craniformin (phenolic tautomer of rubroflavin), and the third component known steroid which include ; ergosta-4,6,8 (14), 22-tetraene- 3-one, ergosta-7,22-diene-3-01 and ergosterol peroxide [10].

The hydroxyphenylazoformamide derivatives or craniformin have phenolics in its formation which are endowed with interesting biological activities as a broad spectrum bactericidal and fungicidal effect represented by *Candida albicans, Aspergillus niger* [11]. Also the craniformin has azol compound which acts as antifungal azol derivatives for example Fluconazol and Itrakanazol [12]. They inhibits the synthesis of ergosterol by blocking the action of 14-alpha-demethylase and stop proliferation of the fungus [13]. The action of azol compounds reveals

inhibition fungal mRNA transcription and treating fungal infections in human and animal subjects and fungal infestations in plants [14].

The third component which resulted from chemical analysis is three steroid compounds, and these are lipophilic and this character facilitates entry into the cells. Also the specific binding proteins which are present in any animal cells may facilitate steroids entry into target tissues [15].

Foiani et al., (1994) proved that the B subunit of the DNA polymerase alpha- primase complex in *Saccharomyces cerevisiae* has essential function at initial stage of DNA replication and this should be inhibited by ergosterol peroxidase which results in inhibition of the proliferation of the yeasts and fungus[16].

The synergistic action of all components of the mushroom are shared in treatment of the lesion and display in (Figure-2 a,b,c and Figure-3 a,b,c) in less time and without side effect.

Statistical analysis reveals significant difference at $p<0.05$ when we are used our preparation in treating the disease with less time when compared with topical antifungal such as 1% clotrimazol cream which needs more time for clearance between 30-60 days, while our preparation needs less time between 10-20 days.

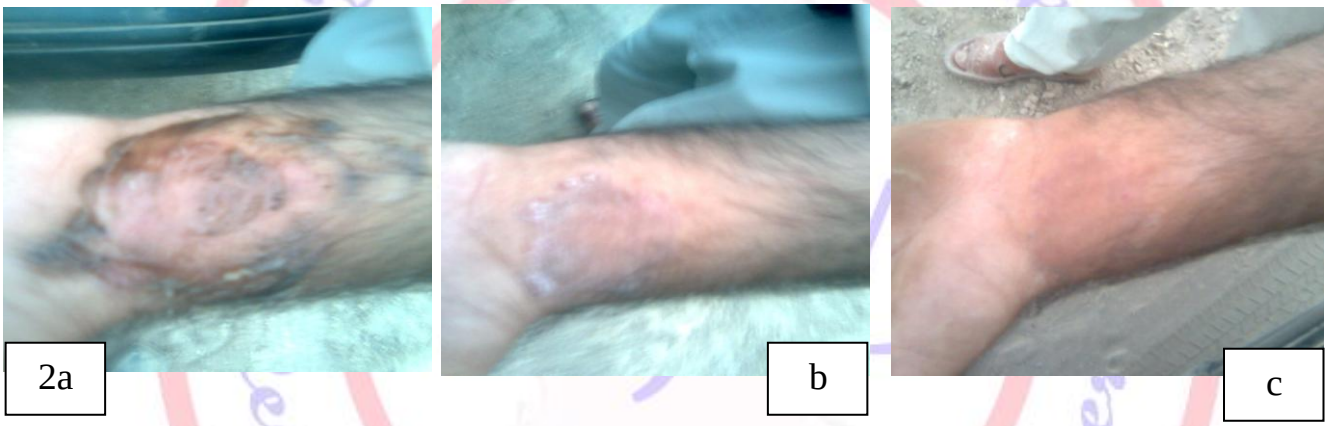

Figure- 2 a: represent the lesion and the application of the ointment.
Figure- 2 b: represent the gradual cure of the lesion .
Figure- 2 c: represent the complete clearance of the lesion .

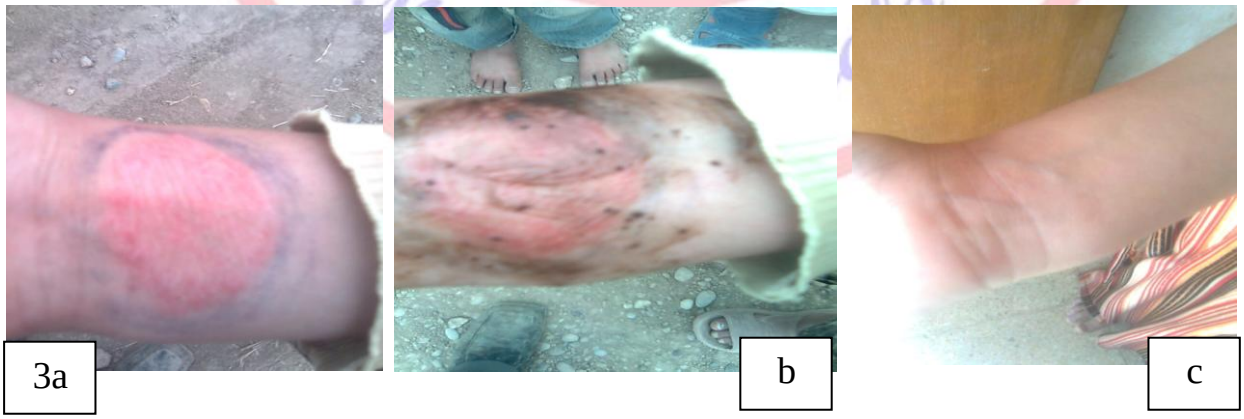

Figure- 3a: represent the lesion and the application in of the ointment.
Figure- 3b: represent the gradual cure of the lesion.
Figure- 3c: represent the complete clearance of the lesion .

References

[1]Singal A.; Pandhi D; Agrawl S. and Das S. 2005. comparative efficacy of topical 1% butenafine and 1% clotrimazole in tinea cruris and tinea corporis: a randomized, double-blind trial. J. Derm. Treatment.; 16:331-5.; Hunter J.;. Savin J and Dahi M.2008. Clincal .

[2] Weller R. Dermatology (4th)ed. Blackwell science . For Ruth . patricia and Arlene.

[3] Smith, A.H. 1951. Puffballs and their allies in Michigan Am. Arbor. University of Michigan Press. 131p.

[4] Baseia, I.G. 2003. Contribution to the study of the genus Clavatia (Lycoperdaceae) in Brazil-Mycotaxon.88:107-112.

[5] Ghosh D. 2004. Algae and fungi as food. Resonance, Vol.9, No.4.

[6] Kim BK; Kwun JY; Park YI;Bok JW and Choi,EC.1992. Amti tumor components of the cultured mycelia of Calvatia craniformis (sic). J.Korean Cancer Res. Assoc.24:1-18.

[7] Zar,J.H. 1984. Biostatistical analysis, 2nd ed., prentice Hall Inc.,Englewood,N.J.s

[8] Okuda T. and Fujiwara A.1982.Calvatic acid and product by the Lycoperdeceae 2.Distribution among the Gasteromycetes. Trans.Mycol.Soc.Jpn-23.235-239.

[9] Hamao, U.; Tomio, T. Hironobu, L. and Osamu, T. 1976. Production of a new antibiotic, calvatic acid . Assignee: Zaidan, Hojin Biseibutsu Kagaku Kenkya Kai (Tokyo, JA).

[10] Takaishi Y.; Murakami Y.; Uda M.; Ohashi T.; Hamamura N.;Kidota M. and Kadota S.1997.Phytochemistry .Vol.45,Issue-5,PP.997-1001.

[11] Bouaziz, M.; Lassoued, S.; Bouallagui, Z.; Smaoui, S.; Gargoubi, A.; Dhouib, A. and Sayadi, S.2008. Synthesis recovery of high bioactive phenolics from table- olive brine process wastewater. Bioorg . Med .Chem.; 1,(20):9238-46.

[12] Fugmann, B.; Arnold, S.; Steglich, W.; Fleischhauer, J.; Repges, C.; Koslowski, A. and Raabe, G. 2001. Pigments from the Puffball Calvatia rubro-flava- Isolation, structural Elucidation and synthesis Euro. J.of organic chemistry. Vol.2001, Issue 16, P:3097-3104.

[13] Lewis, R.E. 2006. Medical Mycology. Managing drug interactions in the patients with aspergillosis. Vol.4, No.1; PP: 349-356.

[14] Wobbe, R.C.; Bradley, J.D. and Li, Z. 2000. Antifungal agents- United States Patent 6165998. Scriptgen Pharmaceuticals, Inc.(Waltham,M.A.).

[15] Jhonson, M. and Eviritt, B. 1980. Essential Reproduction. Blackwell Scientific Publications, Oxford.

[16] Foiani, M.; Marini, F.; Gamba, D.; Luchini, G. and Plevani, P. 1994.Mol.Cell Biol.14(2):923-933.