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RESEARCH ARTICLE

TREATMENT OF RINGWORM DISEASE IN SHEEP BY OINTMENT PREPARED FROM *CALVATIA CRANIIFORMIS* MUSHROOM IN DIYALA, IRAQ

***Ghassan H. Jameel**

College of Veterinary Medicine, Diyala University, Iraq

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ABSTRACT

Ringworm or (dermatophytosis) is a fungal disease caused by *Trichophyton verrucosum* and *T.mentagrophytes.* It is transmitted by direct contact with infected animals or by indirect contact through fomites. Three different concentration of *Calvatia craniformis* mushroom ointment are prepared from mushroom powder were (0.25%,0.50% and 1%), then applied topically to lesion once daily. Sixty infected ewe, lamb in different age and sex were involved in our study and divided into two groups; the first is treatment group and, the second is the control group. 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. The control group included thirty patients was treated by 1% iodine ointment. The 1% 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 ringworm with less time.

INTRODUCTION

Dermatophytosis (ringworm) in sheep is a zoonotic skin infection of keratinized tissues caused by several types of fungus. Ringworm can also be spread by contact with animals, clipping, brush, cards, lamb tubes and blankets, fence posts, wire, and the hands of handlers (Scott, 2007). *Trichophyton verrucosum* and *Trichophyton mentagrophytes* are the usual zoophilic dermatophyte involved in sheep ringworm throughout the temperate regions of the world (Quinn *et al*., 2011). Sheep ringworm mainly occurs in young animals (lambs) and is rapidly spread in the herd via infected propagates, example hyphae, and specialized fungal spores named arthrospores. The disease occurs worldwide and *T. verrucosum* is the almost exclusive etiologic agent (Kane *et al*., 1997; Weber, 2000). Aside from animal involvement, several human outbreaks of *T. mentagrophytes* infection have been reported the face, head and the body (Ming *et al*., 2006). Animals cases of ringworm infection have been successfully treated by different antifungal agents such as azole compounds , iodine preparation as local application, and griseofulvine orally (Radostits *et al*., 2000). Diagnosis is made by clinical and microscopic examination of scraped skin lesion cleared with 10% potassium hydroxide and by culture on sabouraud dextrose agar. The aims of the present study are to determine the prevalence of sheep ringworm and, to evaluate the efficiency of the *Calvatia craniformis* mushroom in treatment of the infection to determinate reverse transmission to the owners and other animals. The mushroom used in this study is puffball mushroom, belongs to Basidiomycota division, Lycoperdaceae family, *Calvatia* genus, *craniformis* species (Smith, 1951). The *Calvatia craniformis* mushroom was dignosed 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.

Corresponding author: Ghassan H. Jameel
College of Veterinary Medicine, Diyala University, Iraq

MATERIALS AND METHODS

Preparation of topical antifungal agent

a- Fruiting body of *Calvatia craniformis* mushroom is dried and crushed in sterile Petri dish to obtain a yellow- brown powder.

b- By using a balance weigh, three weights (0.25,0.50,1 gm) each one singly alone. Each weight is completed to 100gms of Vaseline to reach 0.25%, 0.50% and 1% concentrations. The ointment were used to treat the first group(treatment group). while the second group (control group) which include thirty patients treated by 1% iodine ointment applied topical once daily for two months.

Statistical analysis

The differences are compared by using (F-Test) at $p<0.05$ (Zar, 1984).

RESULTS

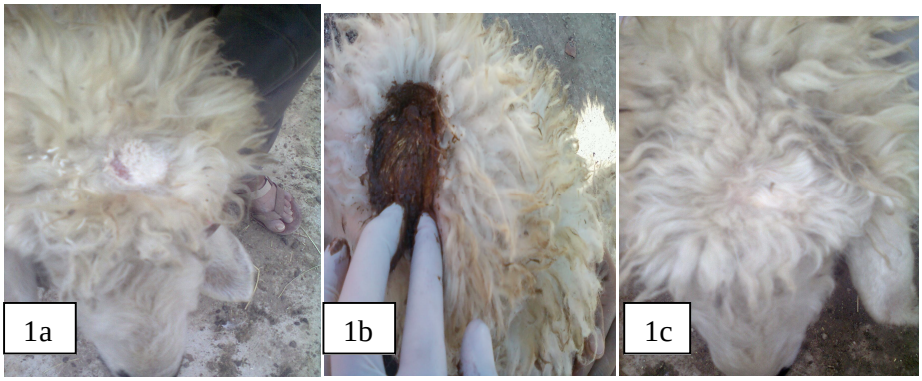
The Table (1) is revealed the response of the patients to a different concentrations of the mushroom ointment as indicated by their disease clearance time and wool regrowing.

Table 1. is revealed the patients and the concentrations of the mushroom ointment with other drug as comparison and the clearance time.

Patients group	Ointment concentration %	Period of clearance
10 Patients	0.25	35-38 Days
10Patients	0.50	23-27 Days
10 Patients	1*	10-13 Days
30 Patients	1% iodine	30-60Days

($p<0.05$) *

The Figures (1a,b.c) were represents the untreated lesion in the scalp of the sheep and the application of the mushroom ointment and, the wool regrowing after healing respectively.



The Figure (3) a: represent the untreated lesion in the scalp. b: represent the application of the mushroom ointment. c: represent the wool regrowing after healing

DISCUSSION

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% iodine ointment which needs more time for clearance between 30-60 days, while our preparation needs less time between 10-38 days according to the dose. These results reflects the medical importance of the *Calvatia craniformis* mushroom in treatment of the disease. The medical analysis of this mushroom proved the presence of three components; the first is calvatic acid which has chemical formation P-carboxyphenyl-azoxycarbonitrile(Okuda and Fujiwara,1982). This calvatic acid reveals weak action against the yeast and fungi like *Saccharomyces cerevisiae* and some *Candida* species and *Trichophyton asteroids* (Hamao *et al.*, 1976). 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-methylthio phenyl-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 (Takaishi *et al.*, 1997). 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* (Bouaziz *et al.*, 2008). Also the craniformin has azol compound which acts as antifungal azol derivatives for example Fluconazol and Itrakanazol (Fugmann *et al.*, 2001). They inhibits the synthesis of ergosterol by blocking the action of 14-alpha-demethylase and stop proliferation of the fungus (Lewis, 2006). The action of azol compounds reveals inhibition fungal mRNA transcription and treating fungal infections in human and animal subjects and fungal infestations in plants (Wobbe *et al.*, 2000). 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 (Jhonson and Eviritt, 1980). Gupta and Cooper, (2008) illustrates the action of topical antifungal infection by, the method allows the drug to penetrate the hair shaft where the fungus lives. 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. Also the chemical analysis of mushroom powder which is done in White Fields Company for Chemical and Engineering Studies and Consultations in Baghdad – Iraq proved the presence of different materials as ergothioneine and gallic acid. The ergothioneine (ET) which is an unusual sulfur-containing derivative of the amino acid, histidine. It may be represent a new vitamin whose physiologic roles include antioxidant cytoprotectant (Paul and Snyder, 2010). The last material termed gallic acid ; it is a trihydroxybenzoic acid, a type of phenolic acid. Gallic acid is found both free and as part of tannins . Gallic acid seems to have anti-fungal and anti-viral properties. (Jeremy and Nuansri, 2007). The synergistic action of all components of the mushroom are shared in healing of infected epidermis and the disappearance of the lesion and display in Figure (1 a,b,c) in less time and without side effect because the *Calvatia craniformis* is edible mushroom (Baseia, 2003). This study was coming in agreement with Rasheed *et al.* (2011) which were proved the ability of same preparation in different concentration to treat tinea corporis in human. Also it was coming in agreement with other study done by me which proved the ability of same preparation of the mushroom in different concentration in treatment of tinea capitis in human and the research was published in Al- Mustansiriyah Journal of Science (Vol. 23, No.7;2012).

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