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Infectious Differences between the Dermatophytes-Induced Dermatophytosis in Wistar Rat

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Authors' contributions

This work was carried out in collaboration between all authors. Author SGO designed the study, wrote the protocol and wrote the first draft of the manuscript. Author AOM managed the literature searches, analyses of the study and performed the microscopy analysis. Author AMA managed the experimental process and identified the species of fungi. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study investigates the infectious differences between the two dermatophytes *Trichophyton rubrum* (*T. rubrum*) and *Epidermophyton floccosum* (*E. floccosum*), and analysed their growing effects and morphology on the skin of Wistar rats.
Study Design: The histology and histochemistry studies were investigated.
Methodology: Nine animals were used and divided into 3 equal groups. Group A was the control (not infected), group B was infected with *T. rubrum* and group C was infected with *E. floccosum*. 2 ml of washed fungal organisms were spilled on shaved, cleaned and disinfected skin of the Wistar rats with cotton swab saturated with 70% ethanol separately. The dermatophytes were allowed to incubate with noticeable changes when physically observed. Animals were sacrificed using cervical dislocation under chloroform anaesthesia, after 12 days of infection for both histological and histochemical studies.
Results: The skin of the animals were observed with the falling of furs on the skin of the Wistar rats infected with different organisms compare with the control group. The histochemical studies of melanoaldehyde (MDA), superioxide dismutase (SOD) and total protein (T.P) were carried out with

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T. rubrum infected group observed to have highest level of SOD that is 778.02±4.00 as against 741.01±4.00 of *E. floccosum*, which suggested some protecting reactions in the skin against reactive oxygen species. Also, the total protein level of the infected groups increased slightly compare to the control group. The little increase in the level of MDA was observed in *E. floccosum* infected group. The histological appearance in the cellular architecture and arrangement in group B compared with group C with obvious differences observed. Thus, *T. rubrum* infected group digested keratin more than *E. floccosum*-infected group, and may be as a result of serious invasion of the epidermis and its rete ridges by the fungi.

Conclusion: It can therefore be concluded that, *T. rubrum* is more infectious in terms on its commonest and keratin digestion when compared with *E. floccosum.*

Keywords: T. rubrum; E. floccosum; SOD; MDA; TP.

1. INTRODUCTION

Dermatophytes are a fungal group with capacity to invade keratinized tissues (skin, hair and nails) of man and other animals causing dermatophytosis [1]. Researchers from South and North America and Europe mentioned this micro-organism as one of the most commonly isolated in case of dermatophytosis in these regions and, with recognized local to therapy [2]. In Brazil, it also remains the most frequently isolated [3]. The dermatophytes belong to the category of disease organisms that almost every human alive will be affected by it at some point or the other. A huge amount of money was reported to be devoting for antifungal drugs every year targeted against dermatophytoses [4]. This has been a cause of great concern among researchers around the world.

However, Identification of individual dermatophytes species causing infections remains important for several reasons [5]. Dermatophytosis has been connected with the sudden outbreak with different factors bring about its reinfection [6], and this determines its vary treatment regimens with different organisms of dermatophyte species: for example, there is a different in time required for the treatment of *Trichophyton tonsurans* in *tinea capitis* and *Microsporum canis,*with former requires shorter time [7].

Early descriptions of dermatophytes were often ambiguous in regard to whether the object of description was a fungus per se or a mycotic disease condition. For example, P.H. Malmsten's 1845 description of *T. tonsurans* [8] was heavily based on clinical sign as well as fungal structures seen in host materials; the drawings included in the article showed only infected hairs and follicular structures. A few important dermatophyte species, such as *Epidermophyton floccosum* and *Trichophyton soudanense* (also currently considered synonymous with *Trichophyton rubrum*), resisted typing with this system and remain of unknown mating type status [9], eventually pointed out the obvious ecological factor linking the asexual, unifactorial species: they all infected animals (including *Homo sapiens*) not maintaining a soil-based burrow or den habitation suitable for the sexual processes forming *Arthrodermatelemorphs* to take place on shed hair or similar keratinous debris.

2. MATERIALS AND METHODS

2.1 Animals Care

Nine (9) animals were used for this research. The animals were acclimatized in the Animal holding of the University of Ilorin, Nigeria. The rats were fed with rat pellets and given water.

2.2 Excision of the Skins

The animals were sacrificed by the cervical dislocation under chloroform anaesthesia; the skins were excised and fixed in 10% formol saline for 24 hours, and those for quantitative histochemistry were put in 0.5% sucrose solution.

2.3 Histological Procedure

The tissues were processed using Leica TP 1020 Automatic tissue processor and embedded using Leica EG 1160 Embedding system.Sections were cut at 3 microns on a Leica rotary microtome and dried for 30 minutes at 60℃. All sections were stained with haematoxylin plus eosin for cellular morphological analysis {(H&E) Pearse, 1981} and GrocottMethenamine (Hexamine) Silver for Fungi (Gomori,1946, Grocott, 1955). The sections were examined with

the light microscope and photomicrographs of the sections were taken.

2.3.1 Histochemical procedure

The following steps are followed for the histochemical analysis.

2.3.2 Assay of total protein

The levels of total protein were determined by the commercially available kits (Beacon laboratory kit). All analysis was done according to the instructions of the manufacturer.

2.3.3 Skin homogenate preparation

The skin of different groups was rinsed thoroughly with iced-cold normal saline. It was smashed in a homogenization buffer and solution was sonicated in an ice bath for 30 s, then centrifuged at 13000 rpm for another 4 min at 4℃. The supernatant was stored at 80℃, which later used to determine level of activities of both SOD and MDA.

2.3.4 Assay of SOD

SOD activity was determined by routine procedure and the activity of SOD was expressed in µml/L.

2.3.5 Assay of MDA

Lipid peroxidation was evaluated on the basis of MDA level and MDA in the skin was determined in µml/L.

3. RESULTS

The tables and figures below present the morphological changes, quantitative histochemistry and histological analyses of the experiment.

Table 1. Morphological changes in dermatophytes-infected groups (within 12 days)

S/N	Group	Changes
1	Group A	fur started growing in 4th day
2	Group B	brownish colour appeared on the skin and little fur dropped in 7th day. However, there was a heavy loss of fur on the 9th day which spread beyond the shaved portion
3	Group C	little brownish colouration on the skin & heavy fur dropped in 7th day. There was fur loss as well on the 9th day, but not as pronounce as in Group B

3.1 Quantitative Histochemistry

Table 2. Quantitative histochemical parameters using descriptive statistics

S/no	Sample identity (I.D)	MDA(µml/L)	SOD(µml/L)	Total protein/T.P(G/L)
1	Group A	16.01±0.03	772.01±2.01	12.03±2.00
2	Group B	14.01±0.42	778.02±4.00	12.02±0.02
3	Group C	17.01±0.01	741.01±4.00	13.02±0.12

3.2 Histological Observations

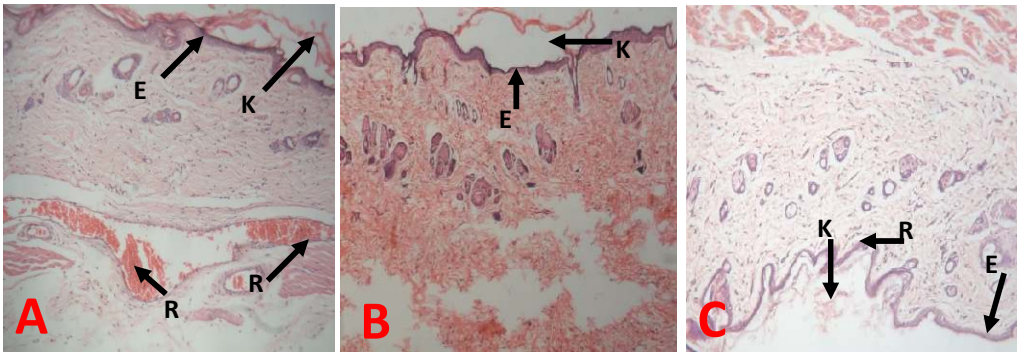

Fig. 1. Skin of the control group (A), *T. rubrum-infected* group(B) and *E. floccosum-infected* group (C) of Wistar rats and stained with (H&E) x100. K, R &E represent Keratin, Rete ridges & Epidermis of the skin respectively in the photomicrographs above

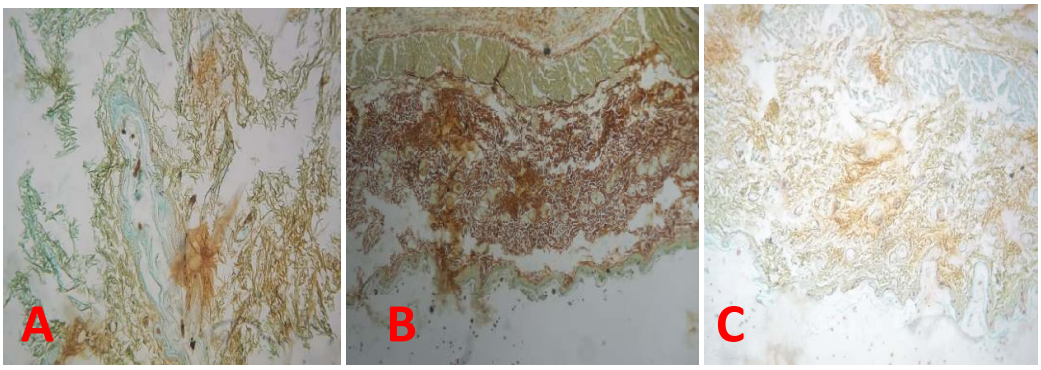

Fig. 2. Skin of the control group (A), *T. rubrum-infected* group (B) and *E. floccosum-infected* group (C) of Wistar rats and stained with (Grocott Methenamine) x100

4. DISCUSSION

From the Table 1 above, the changes in the *E. floccosum* skins colouration and falling of furs indicated the invasion of the dermatophytes, though the *E. floccosum* was first observed to be highly invasive in the 7th day of infection. But with time, *T. rubrum* was observed to be more invasive, but *T. rubrum* was with dark brown compared with light brown in *E. floccosum* at the early days of the infection. Histochemically, the antioxidant agents such as superoxide dismutase (SOD), malondialdehyde (MDA) and total protein (TP) are all supportive team of defences against Reactive Oxygen Species (ROS), the reactivity of ROS determines the biological disorderliness of systems to neutralise the free radical induced damages of tissues [10]. However, hosts trigger a defence mechanisms resulting in the generation of reactive oxygen species (ROS), superoxide anion (O_2-) radicals, hydroxyl (OH) radicals and hydrogen peroxide (H_2O_2), [11,12]. *T. rubrum* infected group was observed to have highest level of SOD, that is 778.02±4.00 as against 741.01±4.00 of, which suggested some protecting reactions in the skin against reactive oxygen species. While *E. floccosum* infected group has a bit higher level of MDA, this suggested that with time the *E. floccosum* group could be anti-oxidized. Thus, the result could support the reason while *T. rubrum* is known to be the commonest pathogen causing various superficial infections, such as *tinea-capitis*, *tinea-corporis*, *tinea-pedis* and others [13].

The histological evidence shows that, the *E. floccosum* infected group has digested the keratin from the superficial layer of the epidermis, though the epidermis and its rete ridges were fairly distorted. But, it was a total distortion in the case of *T. rubrum* infected group as the keratin was observed to be seriously digested, may be it could as a result of serious invasion of the epidermis and its rete ridges [1].

The fungi staining on the skin using Grocott methanamine (hexamine) silver (Gomori 1946, Groccot, 1955) as a special fungi demonstration technique, it is always use to demonstrate fungi infection with black/brown stain to indicate fungi infection and pale green for normal condition. However, from the photomicrographs above, *T. rubrum*-infected group was shown to retain heavy stain compared with *E. floccosum*-infected group.

5. CONCLUSION

It can therefore be concluded that, *T. rubrum* is more infectious in terms on its commonest and keratin digestion when compared with *E. floccosum.*

ETHICAL APPROVAL

All authors hereby declare that “Principle of laboratory animal care” (NH publication No. 85-23, revised 1985) was followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethic committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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