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Effects of hydroxyl alkaline compounds on dermatophytic cells

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ABSTRACT

Objective: To determine the effect of hydroxide compounds on the viability of dermatophytic cells.

Methods: Two strains (*Trichophyton mentagrophytes* and *Epidermophyton floccosum*) of dermatophytes were clinical isolated from an old male patient with tinea corporis at AL-Hussein General Hospital of Karbala Province. Skin scales of fungal lesion were cultured on Sabouraud dextrose agar containing 0.05 g chloramphenicol. The hydroxide compounds were dissolved in melted fungal media to obtain different percentages (0.625%, 1.25%, 2.5%, 5%). Then the fungal growth on solid media containing tested compounds was determined by using colony diameter method and different concentrations of KOH, NaOH, $Mg(OH)_2$ and $Ca(OH)_2$ were tested against two species of dermatophytes. Experiments were repeated triplicate for statistical analysis and the data were analyzed for SE of each experiment.

Results: KOH and NaOH (down to 1.25%) showed the ability to completely inhibit the growth of both strains of dermatophytes (*Trichophyton mentagrophytes* and *Epidermophyton floccosum*). Other two hydroxide compounds revealed variable effects on dermatophytes cells.

Conclusions: The strong hydroxide compounds showed a harmful effect on fungal structures and functions. In addition to diagnosis, pathogenic fungi like dermatophytes were killed after treating with these hydroxide agents.

1. Introduction

Dermatophytes are a specific group of pathogenic fungi that cause a very common skin disease which is called tinea or dermatophytosis in human and animals with a high prevalence all over the world. Dermatophytes infect the superficial layer of skin, resulting in a various form of clinical changes in skin layer, but without dissemination to other organs[1,2]. There are three main genera of dermatophytes including *Trichophyton*, *Microsporum* and *Epidermophyton*[3]. All of dermatophytes species like to consume a keratin protein which is found in skin, hair and nail[4].

Alkaline chemicals containing the hydroxide ions within their structure include a wide range of compounds. Most of these compounds have an important value in various fields of science, medicine, agriculture and different branches of industry. KOH at a concentration of 10%–20% is considered as the suitable agent for diagnosis of dermatophytosis[5]. It plays an valuable role in diagnosis of such type of fungal infection due to its abilities to digest proteinaceous debris, bleach many pigments, and separate infected skin cells for the clarified observation of fungal elements[6]. For diagnosis of thick skin infection such as plantar dermatophytosis and chronic hyperkeratotic palmer eczema, a few drops of 10% of KOH can be used directly on the skin over the peripheral portion of the lesion[7].

This study was designed to evaluate the effect of KOH on dermatophytic cells but not the infected skin cells. Other types of hydroxide compounds had also been chosen to determine which one was suitable to diagnose dermatophytosis or could be used as a alternative of KOH.

2. Materials and methods

2.1. Organisms

Two strains of dermatophytes were clinical isolated from an old male patient (26 years) infected with tinea corporis at AL-Hussein General Hospital of Karbala Province. Skin scales of fungal lesion were cultured on Sabouraud dextrose agar containing 0.05 g chloramphenicol. Cultures were incubated at 28 ℃ for two weeks. Grown fungi were diagnosed according to criteria recorded by Rippon and Emmons *et al*.[8,9]. The isolated strains were *Trichophyton mentagrophytes* (*T. mentagrophytes*) and *Epidermophyton floccosum* (*E. floccosum*).

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2.2. Chemicals

Compounds containing hydroxide ions like KOH, NaOH, $Mg(OH)_2$ and $Ca(OH)_2$ were purchased from HiMedia (Mumbai, India). Sabouraud dextrose agar was also purchased from HiMedia (Mumbai, India).

2.3. Antifungal assay

Hydroxide compounds were dissolved in melted fungal media to obtain different percentages (0.625%, 1.25%, 2.5%, 5%). Based on the colony diameter method that described by Küçüc and Kivanç[10], fungal growth on solid media containing tested compounds was determined. Briefly, prepared media with tested compounds were poured into sterilized Petri dishes. A disk (9 mm) of grown fungi (at 28 ℃ for one week) was inoculated in the center of culture media. Plates were incubated at 28 ℃ for one week. Media without compounds were included as control. Perpendicular colony diameters (mm) of grown strains were measured and the percentage inhibitions were calculated according to the formula:

$$\text{Percentage inhibition} = \frac{(C - T) \times 100}{C}$$

where, C was colony diameter (mm) of control fungi, and T was colony diameter (mm) of treated fungi.

2.4. Effective time

Inoculation of a disk (9 mm) from grown fungi was shaken by hand through using sterilized erlenmeyer flasks (250 mL) containing 100 mL of media with different concentrations of hydroxide compounds. Flasks were then left for various times (up to 4 days) at room temperature. Then, 0.1 mL of inoculation was sub-cultured on plates with media free compounds. All of plates were incubated at 28 ℃ with periodically examined for visible growth.

2.5. Statistical analysis

Experiments were repeated triplicate for statistical analysis. The data were analyzed for SE of each experiment.

3. Results

The activity of four alkaline compounds containing hydroxide ions on the viability of dermatophytic cells was investigated on two species of dermatophytes. The results revealed that KOH and NaOH completely inhibited the growth of dermatophytes at concentrations of 1.25%, 2.5% and 5% respectively, after one week of incubation. While at a low concentration (0.625%), NaOH exhibited a higher percentage of inhibition against two dermatophytic species than KOH (Tables 1 and 2). The difference in sensitivity of fungal species toward the hydroxide compounds was significant when fungi had been grown on media containing $Mg(OH)_2$ and $(CaOH)_2$. Both isolated species of dermatophytes showed complete growth inhibition at 2.5% and 5% of $Mg(OH)_2$ and $Ca(OH)_2$, while *E. floccosum* exhibited more sensitivity toward $Mg(OH)_2$ with complete inhibition of growth by comparing with *T. mentagrophytes* (Tables 1 and 2).

Based on the results of effective time measurement, high percentage of KOH and NaOH (2.5% and 5%) revealed the inhibitory action on two species of dermatophytes after 30 min of culturing, meanwhile, $Ca(OH)_2$ did not effect on fungal growth up to 4 days (Table 3).

Table 1

Percentage inhibition of *T. mentagrophytes* on media containing hydroxide compounds.

Hydroxide compounds	Percentage inhibition (%)			
	5%	2.5%	1.25%	0.625%
KOH	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	35.8 ± 0.8
NaOH	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	48.7 ± 1.8
$Ca(OH)_2$	100.0 ± 0.0	100.0 ± 0.0	58.9 ± 1.2	35.8 ± 2.0
$Mg(OH)_2$	56.4 ± 0.8	56.4 ± 1.0	56.4 ± 0.7	46.1 ± 1.3

Values were expressed as mean ± SE. Colony diameter of control fungus was 39 mm.

Table 2

Percentage inhibition of *E. floccosum* on media containing hydroxide compounds.

Hydroxide compounds	Percentage inhibition (%)			
	5%	2.5%	1.25%	0.625%
KOH	100	100	100	30.4 ± 2.1
NaOH	100	100	100	30.4 ± 1.5
$Ca(OH)_2$	100	100	43.4 ± 1.3	17.3 ± 0.9
$Mg(OH)_2$	100	100	100	46.1 ± 1.2

Values were expressed as mean ± SE. Colony diameter of control fungus was 23 mm.

Table 3

Time of positive cultures of dermatophytes strains on media containing hydroxide compounds.

Hydroxide compounds	Strains	Maximum time of positive cultures			
		5%	2.5%	1.25%	0.625%
KOH	*T. mentagroph*	30 (min) ± 1.3	30 (min) ± 1.4	4 (days) ± 1.0	4 (days) ± 1.3
	E. floccosum	30 (min) ± 2.0	30 (min) ± 1.8	4 (days) ± 1.2	4 (days) ± 2.0
NaOH	*T. mentagroph*	30 (min) ± 1.4	30 (min) ± 1.6	4 (days) ± 1.0	4 (days) ± 1.3
	E. floccosum	30 (min) ± 1.4	30 (min) ± 0.8	4 (days) ± 2.0	4 (days) ± 0.9
$Ca(OH)_2$	*T. mentagroph*	4 (days) ± 0.9	4 (days) ± 0.8	4 (days) ± 0.8	4 (days) ± 1.5
	E. floccosum	4 (days) ± 0.7	4 (days) ± 0.4	4 (days) ± 0.9	4 (days) ± 2.1
$Mg(OH)_2$	*T. mentagroph*	1 (day) ± 2.0	2 (days) ± 1.2	4 (days) ± 1.0	4 (days) ± 1.2
	E. floccosum	1 (day) ± 1.5	2 (days) ± 1.7	4 (days) ± 1.8	4 (days) ± 0.7

Values were expressed as mean ± SE.

4. Discussion

For diagnosis of superficial dermatophytosis, infected skin is the perfect sample to examine because of heavily distributed fungal elements (spore or hyphae) within skin cells. Thus, adding KOH (10%–20%) is very important to separate skin cells from each other, digest debris materials and to easy diagnosis of fungal cells[7]. This process becomes routinely applied in all medical laboratories. However, application of KOH is a simple, rapid, inexpensive test to perform, and requires minimum infrastructure with some amount of experience to interpret the smears[5].

In addition to KOH, other alkaline compounds containing hydroxide ions within their structures were considered as having a very large group that obtain its alkalinity from the presence of hydroxide ions. Thus, other members closely related to KOH were selected for this study to be alternative agent or might have the same properties as KOH. However, the detailed study on direct effect of KOH on the viability of dermatophyte cells found *in vitro* or outside skin cells is deficient. Based on the alkaline property, NaOH could be the best choice for replacing KOH. It showed activity against many fungi such as *Candida albicans* (*C. albicans*) which was inhibited after culturing on media containing NaOH with a high pH

value[11,12].

The activity of $Ca(OH)_2$ in fungal growth is variable. In some cases, $Ca(OH)_2$ revealed no effects on the growth of fungi, while in others, it could be considered as an inhibitory or inducer factor. In the case of no activity of $Ca(OH)_2$ in fungal cells, it had been shown that it exhibited none or a very weak inhibitory effect on *C. albicans*[13,14]. Otherwise, Ca^{2+} induced increasing weight of dry mass and growth rate of *Phytophthora cinnamomi*[15].

As inhibitor, ash of *Combretum imberbe* aqueous extracts that contains $Ca(OH)_2$ was found to inhibit the growth and enzyme production of phytopathogenic and mycotoxigenic fungi[16]. The incidence of fungal disease in apple also decreased after treating with $Ca(OH)_2$[17]. Furthermore, $Ca(OH)_2$ had reduced 65% of *Monilinia fructicola* growth and production of its polygalacturonase enzyme by comparing with the control[18]. Additionally, Ca^{2+} and OH^- that result from dissolving $Ca(OH)_2$ in media may also have inhibitory action in fungal cells. Xu found that OH^- inhibited the activity of laccases enzyme of fungi[19]. While, Ca^{2+} inhibited the germ tube and mycelia growth of *Colletotrichium* spp[19]. The main effect of Ca^{2+} on fungi may be related to its ability to inhibit cell wall-degrading enzymes produced by the pathogenic fungi[17].

Dermatophytes are usually preferring to grow on media with a wide range of pH value (7–9)[20]. In alkaline condition, tolerance time allowing dermatophytes to live in such medium is not detected. Thus, growth of dermatophytes in media containing different hydroxide compounds with different concentrations had been tested at various periods of incubation. Two strains of dermatophytes treated with KOH or NaOH showed a higher percentage inhibition after a period of incubation (30 min) than those treated with $Ca(OH)_2$. In other studies, *C. albicans* had been inhibited after treatment with 1% of NaOH for 30 min[11]. These results indicated that direct exposure of dermatophytes to KOH or NaOH for a long period of time could destroy the internal structures of fungi or even their normal shape after blocking the metabolic functions of fungal cells. Ramasamy also found that KOH in form of phosphite reduced colony growth of *Phytophthora* fungus[21]. This fact can explain the activity of KOH and NaOH on the growth rate of dermatophytes.

In conclusions, the strong hydroxide compounds showed a harmful effect on fungal structures and functions. In addition to diagnosis, pathogenic fungi like dermatophytes were killed after treating with these hydroxide agents. This treatment was also considered as a good step to restrict the infection among laboratory workers. However, the treatment of dermatophytes with strong hydroxides such as KOH and NaOH did not need long periods of time.

Conflict of interest statement

I declare that I have no conflict of interest.

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