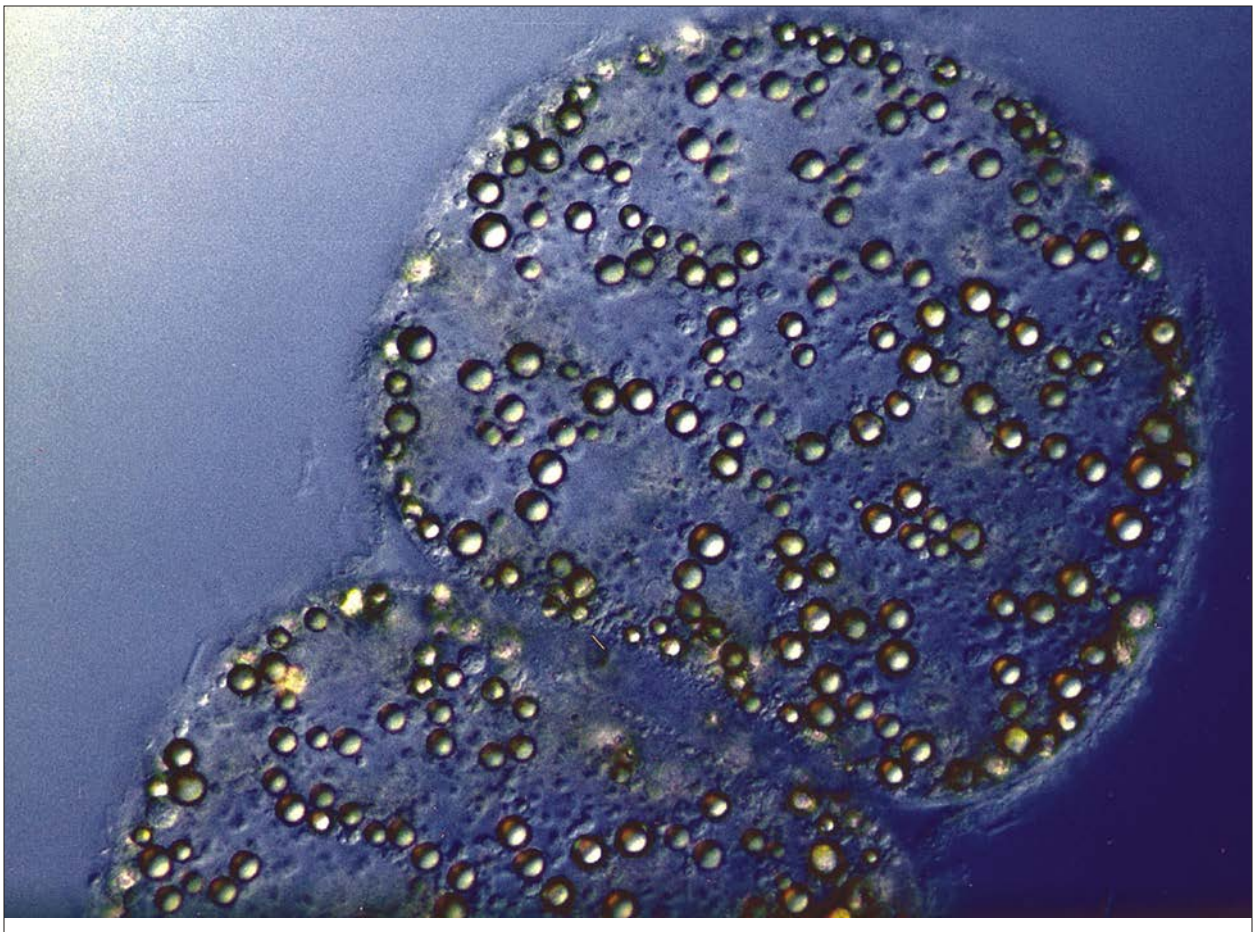

Microbiology

Journal

ISSN 2153-0696

Academic
Journals Inc.

www.academicjournals.com

Microbiology Journal 1 (1): 40-45, 2011
ISSN 2153-0696 / DOI: 10.3923/mj.2011.40.45

In vitro Evaluation of Antifungal Activity of Peroxy Acetic Acid Component (Percidine) on a Group of Fungi

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ABSTRACT

The aim of this study was *in vitro* evaluation of antiseptic effect of different concentrations of Peroxy Acetic Acid (PAA) as percidine and percidine 513 on *Microsporum gypseum, Candida albicans* and *Aspergillus niger*. Standard strains (PTCC) were cultured on malt extract agar medium. Fungal suspensions were prepared containing 4×10^7 cfu mL^{-1} conidia and yeast cells. Afterward, they were treated with 1, 3, 5, 10 and 20% of percidine in 3, 5, 10, 20, 30 min and 0.2% of percidine 513 in 3, 5, 15, 30, 45, 60 min. Eventually, the number of conidia and yeast cells were counted. The numbers of colonies were also counted after culturing the treated suspensions on malt extract agar medium. On the basis of protocol 6986 of Institute of Standards and Industrial Research of IRAN (ISIR), a logarithmic reduction of 10^4 in vegetative conidia will reflects the effectiveness of the studied substrate. For *C. albicans* and *M.gypseum,* the satisfactory results were obtained by using 1 and 3% of percidine after 10 min. However, in case of percidine 5%, the aim was obtained after 3 min. On the other hands, in case of *A. niger,* acceptable results were obtained after 20 min for percidine 3%, 10 min for percidine 5 and 10% and finally 5 min for percidine 20%. According to ISIR, PAA can be considered as a reliable antiseptic for using in general application due to its high potency for reducing the intensity of microbial population specially the most important species of fungi.

Key words: Percidine, peroxy acetic acid, antifungal, behban shimi

INTRODUCTION

Disinfectants are a group of chemical substances which are able to inhibit growth or even kill bacteria, viruses, fungi, bacterial spores and other microorganisms. Applying such materials is essentially needed even in modern societies. Among the disinfectants, the eco-friendly new generation is more likely to be considered as suitable substrates for reducing the intensity of a wide spectrum of microbial pathogens.

Fungal infections are one of the most known nosocomial infections which have been well-studied since 200 years ago (Larone, 1995). In recent years, the prevalence of nosocomial fungal infections has been increasing around the world. Under certain conditions, they frequently are responsible for inducing life-threatening systemic infections which eventually lead to high rate

of mortality, despite using appropriate treatments. Therefore, prevention and transmission controlling of the infections is a priority in public health (Roberson *et al.*, 2006).

The rate of contributed mortality of nosocomial infections was more considerable before the advance of disinfectants by Lister and Semilvaies in 19th century. Before that time, hospitals were called as a place of slaughterhouse. Nowadays, it has been documented that the estimated prevalence of nosocomial infections is about 6-10%. Meanwhile, a large number of these infections can be prevented with much more attention to hygienic rules and providing appropriate disinfection procedures in hospitals.

In 2002, Jursch CA reported the effect of acetic acid compounds on HBV viruses (Jursch *et al.*, 2002). Moreover, Thamlikitkul V reported high appropriate effect of acetic acid on wide spectrum bacteria (Thamlikitkul *et al.*, 2001). The anti prion activity of acetic acid components on medical devices has been reported in 2002 (Antloga *et al.*, 2000). Kang compared the effect of different kinds of disinfectant on HBS antigen in 1997 and observed that compounds containing acetic acid are more effective than formalin and a so called chinese substrate disinfectant (Kang *et al.*, 1997). In 2006, Mignard published the absolute effect of acetic acid compounds on bacteria and their spores and also on viruses contaminating endoscope equipment after immersing them in acetic acid for 30 min (Mignard, 2006). In 1980, Baldry and Coworkers reported the antimicrobial effect of proxy acetic acid compounds (PAA) on sewage (Baldry and French, 1989; Baldry *et al.*, 1991). Some reports have documented the effectiveness of PAA on intestinal bacteria during refinement of water. However, there are some contradictions: some studies reported that PAA has no effect on spore of bacteria, viruses and protozoa cysts (Wagner *et al.*, 2002; Veschetti *et al.*, 2003). In 1996, Bundgaard-Nielsen and colleagues failed to report the effectiveness of 0.3% acetic acid and other disinfectant component, available in markets, on 25 saprophytic species of fungi which were isolated from bread and cheese (Bundgaard-Nielsen and Nielsen, 1996).

The aim of the present research was evaluation of antifungal activity of proxy acetic acid component on some medically important fungi.

MATERIALS AND METHODS

Isolates and chemicals: The present study was conducted at medical mycology laboratory of Golestan University of Medical Science during 2008. The study was performed according to Standard protocol No. 6986 published by Institute of Standards and Industrial Research of Iran. Standard strains of *Candida albicans* and *Aspergillus niger* were used. *Microsporum gypseum* was also added to the testing strains because of the high possibility of afflicting people with dermatophytes via cross-transmission in barbers.

Candida albicans (PTCC5027), *Aspergillus niger* (PTCC5010) and *Microsporum gypseum*(PTCC 5070) were provided by Industrial and Scientific Iranian Research Center (Persian Type Culture Collections). Proxy acetic acid component (PAA), Percidine and percidine 513 (0.2%), were provided by Behban Chimi Co.

Testing method: To maintain the strains, they were cultured on malt extract agar medium for 48-42 h and were sub-cultured for three times.

Fungal suspensions containing vegetative cells of *Candida albicans,* conidia of *Aspergillus niger* and *Microsporum gypseum* were then prepared. They were diluted with tryptone sodium chloride and then counted by using a hemocyometer in such a way that each suspension contains 4×10^7 cfu mL^{-1}.

Afterward, 1 mL of each suspension was added to a tube containing 8 mL of percidine and percidine 513 (1, 3, 5, 10 and 20%) plus 1 mL of distilled water. Shacked well and incubated on a shaker incubator at 20°C for 3, 5, 10, 20, 30 min and 3, 5, 15, 30, 45, 60 min for percidine and percidine 513, respectively.

One millileter of each testing solution was added to a tube containing 8 mL of lecithin (as neutralizer) plus 1 mL of distilled water and incubated on shaker incubator at 20°C afterward. The number of fungal yeast cells and conidia were then counted and their probable colonies were counted by using pour plate method. Consequently, 12-15 mL of hot ready to pour malt extract medium was quickly added to 1 mL of the neutralized solutions to evaluate the potency of the disinfectants. The media were incubated at room temperature for 22-48 h. The experiment was repeated on two separate occasions.

RESULTS

The acceptable effect (logarithmic reduction of 10^4) of antifungal activity of percidine 1 and 3% was observed after 10 min in case of *C. albicans* and *M. gypseum*. Minimum effective time was reported after 3 min by using percidin 5%.

Percidine 1% had no effect on *A. niger*. None of the applied concentration was effective on *A. niger* during 3 min and its effectiveness was started after 5 min by using of percidin 20%. The minimum concentration for getting suitable effect was obtained for percidin 3% after 20 min. Obviously, decreasing the concentration of percidin lead to increase the time of incubation. So that, percidin 5 and 10% gave us acceptable effect after 10 min (Table 1).

In case of percidin 513 with 0.2% of concentration, logarithmic reduction of 10^4 was observed after 5, 15 and 30 min for *C. albicans*, *M. gypseum* and *A. niger*, respectively. Moreover, no vital fungal cell was detected after 15, 15 and 30 min for *C. albicans*, *M. gypseum* and *A. niger*, respectively. Apparently, *Candida* is more sensitive to percidin 513 than others and *Aspergillus* is less sensitive to it (Table 2).

Table 1: Effect of percidine on fungi in different concentration and times

		Times (min)				
No. 4×10^7 mL^{-1}	PAA concentration (%)	3	5	10	20	30
Aspergillus niger	1	-	-	-	-	-
PTCC5010	3	-	-	-	+	+
	5	-	-	+	+	+
	10	-	-	+	+	+
	20	-	+	+	+	+
Candida albicanss	1	-	-	+	+	+
PTCC5027	3	-	-	+	+	+
	5	+	+	+	+	+
	10	+	+	+	+	+
	20	+	+	+	+	+
Microsporum gypseum	1	-	-	+	+	+
PTCC5070	3	-	-	+	+	+
	5	+	+	+	+	+
	10	+	+	+	+	+
	20	+	+	+	+	+

(+) >10^4 reduce in count, SUITABLE (In base protocol No. 6986 ISIRI). (-) < 10^4 reduce in count, UNSUITABLE (In base protocol No. 6986 ISIRI)

Table 2: Effect of percidine 513 on fungi in different times

		Times (min)					
	Percidine 513						
No. 4×10^7 mL^{-1}	concentration 0.2%	3	5	15	30	45	60
Aspergillus niger	Vc	42	40	40	0	0	0
PTCC5010	Na	6×10^5	4.5×10^4	4.2×10^3	3×10^3	2×10^2	2×10^2
	R	$< 10^4$	$<10^4$	$<10^4$	$>10^4$	$>10^4$	$>10^4$
Candida albicans	Vc	>150	41	0	0	0	0
PTCC5027	Na	3.8×10^4	3×10^3	3×10^2	2×10^2	1.5×10^2	1.5×10^2
	R	$<10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$
Microsporum gypseum	Vc	>150	64	0	0	0	0
PTCC5070	Na	4.5×10^4	4.1×10^3	3.5×10^2	2.8×10^2	1.5×10^2	1.5×10^2
	R	$< 10^4$	$<10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$

Vc: Vital fungi count Na: CFU mL^{-1} R: Reduce Log 10^4. SUITABLE : $>10^4$ reduce in count (In base protocol No. 6986 ISIRI). UNSUITABLE :$< 10^4$ reduce in count (In base protocol No. 6986 ISIRI)

DISCUSSION

According to the standard 6986 Institute of Standards and Industrial Research of Iran, a component is regarded as an effective antiseptic if it has an ability to cause a logarithmic reduction of 10^4 in the number of a certain microorganism or their conidia and spores (ISIRI, Protocol No. 6986).

Using antiseptic components are regarded as an essential strategy for fighting with microorganisms. Application of them is more stands out when they are considered as agents for a preventing the infectious diseases. Among these materials, those which are eco-friendly have been recently drawing the ecologist's attention. Alcohols and peroxy components are two materials which have little adverse effects on environment. However, we will face with some limitations using alcoholic substrates: they are not effective on all microorganism, they are incendiary and expensive. On the other hand, peroxy components are more desirable because of their minimal side effects on environment.

Antiseptics fight with microbes though several mechanisms like inhibiting of their cell wall constructions, inhibiting their protein synthesis and adverse effects on nucleic acids and also proteins (Roberson *et al*., 2006).

The main efficient mechanisms of PAA are due to its ability to release active radicals of oxygen which lead to break (SH-) and S-S bands within the cell wall construction of microorganisms (Lefevre *et al*., 1992). Another mechanism is its ability to release hydroxyl radicals which act like hydrogen peroxide (Lubello *et al*., 2002).

Percidine consists of peroxy acid, hydrogen peroxide and acetic acid and is easily prepared on the basis of the following reaction:

$$CH_3COOH+H_2O_2 \Leftrightarrow CH_3CO_3H+H_2O$$

Chemical disinfection methods are important due to sensitivity of some medical devices to high temperatures for sterilization.

Our obtained results shows the effectiveness of percidine 1% on *C. albicans* and *M. gypseum* after 10 min ($R>10^4$), but demonstrated that it has no effect on *A. niger* in thas concentrate. Treating *A. niger* conidia with percidine 5% shows desirable effects after 10 min while the same

results were obtained for *C. albicans* and *M. gypseum* in 3 min. However, the minimum concentration for getting suitable effect was obtained for percidin 3% after 20 min in case of percidin 513, desirable results were got after 5, 15 and 30 min of incubation for *C. albicans*, *M. gypseum* and *A. niger*, respectively.

Present results obviously confirm the importance of testing species on reducing the number of vital microorganisms. In other words, the more resistant of conidia, the more concentrated substrate is needed to be effective. Therefore, it is expectable that the conidia of *A. niger* need much more time or more concentrated solutions ($R>10^4$).

Nielsen reported the antiseptic effect of PAA 0.3% on some species of *Penicillium* isolating from bread and cheese. The different outcomes may be the result of using different species, methods or concentrations (Bundgaard-Nielsen and Nielsen, 1996).

On the other hand, Kitis has introduced PAA as a powerful wide-spectrum antimicrobial agent which is not toxic or mutagenic with grate adaption for pH variations and requirement of minimum time to be effective. Recently, PAA has been applied for refinement of water (Kitis, 2004; Conti, 2005). Moreover, its resistance to microbial enzymes such as catalase is a notable advantage for PAA components (Bundgaard-Nielsen and Nielsen, 1996).

CONCLUSIONS

Considering the risk of nosocomial infections caused by saprophytic fungi, especially in patients with immune deficiency or patients undergone any kind of transplantations and also the high possibility of cross-transmission of some dermatophytes, effective appropriate disinfectants and disinfection methods are necessary to be evaluated.

Besides, by application the appropriate disinfectants with suitable effective concentrations, emergence of resistant strains will be hindered. Moreover, low cost of such materials is an advantage which leads to save healthcare and patients charge. Due to the lack of similar studies on the fungus, it is recommend expanding the study and confirming the obtained results by studying more isolates including hospital and environmental samples. The PAA components are promising substrate for using in domestic, agricultural and medical industrials.

ACKNOWLEDGMENTS

This work was supported by funding from the Golestan University of Medical Sciences and Behban Shimi Company.

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