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Apoptosis - Triggering Effects: UVB-irradiation and *Saccharomyces cerevisiae*

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ABSTRACT

Objectives: *The pathogenic disturbance of* Saccharomyces cerevisiae *is known as a rare but invasive nosocomial fungal infection. This survey is focused on the evaluation of apoptosis-triggering effects of UVB-irradiation in* Saccharomyces cerevisiae.

Materials and Methods: *The well-growth colonies of* Saccharomyces cerevisiae *on Sabouraud Dextrose Agar (SDA) were irradiated within an interval of 10 minutes by UVB-light (302 nm). Subsequently, the harvested DNA molecules of control and UV-exposed yeast colonies were run through the 1% agarose gel electrophoresis comprising the luminescent dye of ethidium bromide.*

Outcomes: *No unusual patterns including DNA laddering bands or smears were detected.*

Conclusions: *The applied procedure for UV exposure was not effective for inducing apoptosis in* Saccharomyces cerevisiae. *So, it needs another UV-radiation protocol for inducing apoptosis phenomenon in* Saccharomyces cerevisiae.

Keywords: *Saccharomyces cerevisiae*, UVB, DNA, Apoptosis

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OBJECTIVES

The eukaryotic ascomycetous yeast of *Saccharomyces cerevisiae* is generally used for food fermentation and is known as baker's or brewer's yeast. In other word, the yeast of *S. cerevisiae* has been usually considered as an ancient biologic tool for baking, brewing and making wine as far as 5000-8000 B.C. in old Persia (1-3).

However, in recent 2.5 decades, the number of invasive fungal infections caused by *S. cerevisiae* has been significantly increased (3,4).

S. cerevisiae as an apportunistic pathogenic yeast, may become the etiological agent of life-threatening mycosis in humans. The most important predisposing factors for developing mycosis caused by *S. cerevisiae* include prolonged antibiotic therapy and hospitalization, vascular catheterization, severe burns and immunodeficiency disorders which is almost similar to Candida albicans infections. The clinical syndrome caused by *S. cerevisiae* includes disseminated infections involving skin, gastrointestinal tract, respiratory tract and urogenital tract (5-7).

The limitation of antimycotic drugs is the main reason for increasing mortality rate among the patients who are infected by pathogenic fungal agents. According to different studies, the antimicrobial efficacy of UV beam including sporicidal and virucidal effects, has been proven. Therefore, the application of a successful protocol for phototherapy and particularly UV-therapy may be a suitable and cost-effective choice for treating the mycosis (8,9). The main goal of this study was to examine the effect of UVB light as a probable apoptosis-triggering factor in *S. cerevisiae*.

MATERIALS AND METHODS

The yeast samples were received from the microbial collection of mycology laboratory of Islamic Azad University, Shahr-e-Qods branch. The yeast cells of *S. cerevisiae* were controlled through macroscopic and microscopical properties, Gram staining and biochemical activities as bellow:

The white-cream colored and smooth colonies of *S. cerevisiae* were grown on Sabouraud dextrose agar through 72 hours at laboratory temperature. The orbiculate to elliptical yest cells with peripheral buddings were observed microscopically by KOH 10% and Lactophenol cotton Blue. The gram staining of *S. cerevisae* showed gram positive vegetative cells with no germ tubes. Positive fermentation reactions for glucose, galactose, maltose, sucrose and trehalose and negative for lactose were resulted.

The yeast colonies were categorized into 4 groups. A group as control one and the left three groups exposed by UVB ray (10-13) and treated for DNA extraction as described in Behzadi and Behzadi protocol (14). Shortly, the UVB ray (302 nm) was irradiated in 10 minutes to the yeast colonies from the distance of 8 cm and then the irradiated samples were kept into a dark chamber for respectively 1, 24 and 72 hours. In the last step, their harvested DNA molecules (by DNP kit, Cinnagen Inc.) were compared with control sample by running through 1% agarose gel electrophoresis (10-14).

For preparing total genomic DNA, 100μl suspension was provided for each control and UVB-exposed samples. 100μl ributinase buffer and 5μl protease was added to each one and the all samples were incubated in 55°C for about half an hour.

Each prepared sample was mixed with 400 μl Lysis Solution. The amount of 300 μl of Precipitation Solution was added and the sample microtube was shaked. In continue, the samples were kept in -20°C for a period time of 20 minutes and then were centrifuged at 12,000 g for 10 minutes. Nex after, the supernatant was decanted and 1 ml Wash Buffer was added to each pellet. Each sample was shaked and centrifuged at 12,000 g for 5 minutes and the supernatant was decanted. The obtained pellet was incubated at 65°C for 5 minutes. The dried pellet was suspended in of 50 μl Solvent Buffer and shaked. The suspension was incubated at 65°C for 5 minutes and finally, the precipitates were separated through centrifugation at 12,000 g for 30 seconds. The left purified DNA strands were isolated from the supernatant phase. In next step for obtaining clear and visible separated bands, respectively 10 μl of total genomic DNA belonging to control (lane 1) and 10-min UVB-exposed (lanes 2-4) yeast cells were loaded into the 1% agarose gel containing luminescent orange colored dye of ethidium bromide and the DNA weight marker III of CinnaGen company was used as a molecular criterion ruler (lane M). By the help of UV-transilluminator, the DNA bands of control and irradiated colonies were evaluated for

probable DNA fragmentotins in laddering bands known as apoptotic patterns (10-14) (Figure 1).

OUTCOMES

The DNA bands belonging to control and 10-minute-UVB exposed colonies showed no deformities including DNA laddering patterns (apoptosis) or smear (necrosis) on 1% agarose gel electrophoresis (Figure 1).

DISCUSSION

Nowadays, Phototherapy and chemotherapy are known as the routine and nonsurgical treatment methods in medicine. Phototherapy, particularly UV therapy may induce apoptosis pathways in irradiated cells (15). The apoptosis phenomenon is a form of regulated cell death which is important for medical research and treatments (16). There are numerous stimuli including physical stress such as UVB-beam, which triggers apoptosis in a eukaryotic cell like *S. cerevisiae*. The UV spectrum comprising UVA, UVB and UVC may induce apoptosis in irradiated cells; but the mechanism of apoptosis caused by UVA, UVB and UVC differs from each other (17). However, the UVB radiation as the most mutagenic and apoptotic UV spectrum may generate DNA damage (10-14,18,19).

According to our result, it may different alternatives which act as protective systems against the inducing effects of UVB light in the UV-exposed yeast cells of *S. cerevisiae*. The first alternative is the presence of heat shock proteins which are accepted as an important protective system (20-22).

The second one is the presence of UV radiation resistance-associated genes (UVRAGs) in eukaryotic cells. UVRAGs have key role to prevent the induction of apoptosis reaction in UV-exposed cells (15).

The third one, UV light may trigger the acetylation reaction of histone H3 via the global genome repair which modulates chromatin structure promoting efficient DNA repair in the yeast cell's genome (23).

CONCLUSION

In this survey, another problem was solved. The protective systems and cell regulators belonging to *S. cerevisiae*, prevent to induce apoptosis reaction through the applied UVB radiation protocol.

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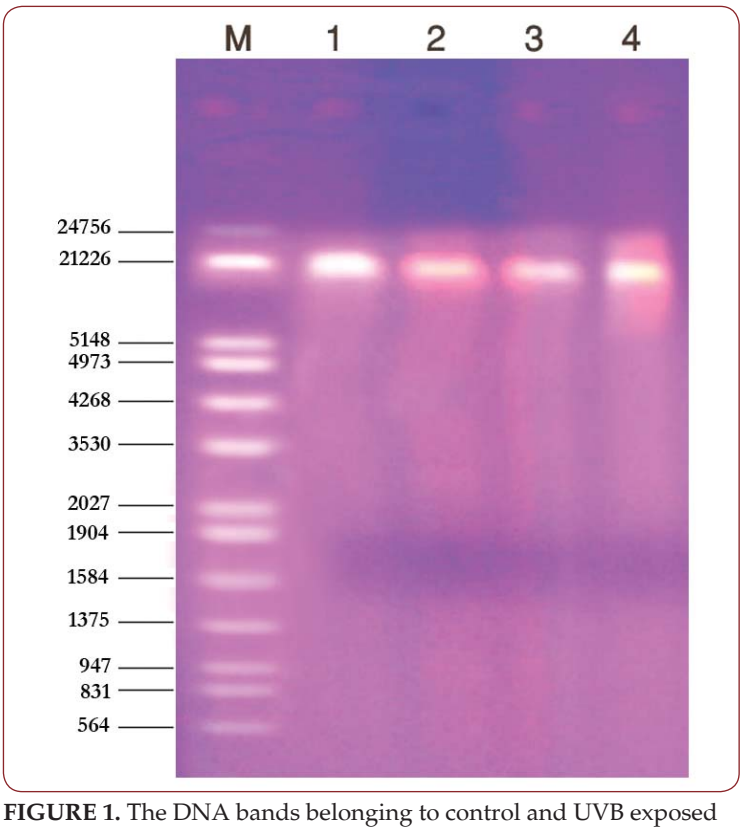

FIGURE 1. The DNA bands belonging to control and UVB exposed colonies of Saccharomyces cerevisiae.
Lane M: indicating DNA weight marker III of CinnaGen Company. The size marker shows the weight of 22000 bp.
lane 1: DNA band belonging to control colonies of Saccharomyces cerevisiae.
lane 2: DNA band belonging to 10 min-irradiated Saccharomyces cerevisiae colonies which were kept for 1 hour in a dark chamber after UVB exposure.
lane 3: DNA band belonging to 10 min-irradiated Saccharomyces cerevisiae colonies which were kept for 24 hours in a dark chamber after UVB exposure.
lane 4: DNA belonging to 10 min-irradiated Saccharomyces cerevisiae colonies which were kept for 72 hours in a dark chamber after UVB exposure.

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