

ISSN 1682-296X (Print)

ISSN 1682-2978 (Online)



Bio Technology



ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Influence of Various Ultraviolet Light Intensities on Pathogenic Determinants of *Candida albicans*

¹Mohammad K. Abu Sini, ¹Khaled H. Abu-Elteen, ¹Ali Z. Elkarmi,

¹Mawieh A. Hamad and ²Rula F. Khuzaie

¹Department of Biological Sciences and Biotechnology, Faculty of Science, Hashemite University, Zarqa, Jordan

²Department of Medical Technology, Faculty of Allied Medical Sciences, Applied Science Private University, Amman, Jordan

Abstract: The effect of pretreatment of *Candida albicans* with different UV light (360 nm) intensities (4, 6 and 10 W m⁻²) at different time intervals (6, 12, 24 and 48 h) in an attempt to investigate its influence on proteinase and phospholipase activities in correlation with *in vitro* adherence to Buccal Epithelial Cells (BECs) and lethality to mice were studied. Irradiated *C. albicans* cells were found to be less virulent than non-irradiated. Exposure of *C. albicans* to various doses of radiation led to a reduction in adherence between 6.8 and 66.7% of the control value. This reduction was dose intensity and time related. The secretion of extracellular proteinase and phospholipase was also reduced to statistically significant between irradiated and non-irradiated *C. albicans*. Experimental mouse groups were intraperitoneally injected with irradiated *C. albicans* and a statistically significant difference was observed between irradiated and non-irradiated *C. albicans* cells from kidneys, spleen, stomach and whole intestine, while no significant difference was found between both groups of *C. albicans* isolated from the liver. These results clearly demonstrated the significant role of ultraviolet light on phospholipase and proteinase activities and on adherence of *C. albicans* and their overall influence on the pathogenesis of *Candida* species.

Key words: UV light intensities, *C. albicans*, pathogenic determinants, virulence factor

INTRODUCTION

Ultraviolet (UV) light is being used as a disinfectant by the water industry because it appears to be very effective for the disinfection of a wide range of microorganisms (Rochelle, 2005; Gagnon *et al.*, 2005; Zimmer and Slawson, 2002). A number of studies examined the effects of ambient UV radiation on microorganisms, plants and marine animals (Rinnan *et al.*, 2005; Keski-Saari *et al.*, 2005; Turtola *et al.*, 2005; Ben-Yosef *et al.*, 2006). Furthermore, UV have been reported to light damage DNA and interacts with human replication protein A which is an essential component of the initial steps of nucleotide excision repair (Lysetska *et al.*, 2002). Other mechanisms for UV damage of cellular membranes (Moss and Smith, 1981) or inhibition of tRNA synthesis (Kubitschek and Doyle, 1981) have also been reported. Nevertheless, few studies have examined the effects of UV on fungal species particularly pathogenic ones.

Candida, as an organism under normal conditions, is a common human saprophyte. It becomes an

opportunistic pathogen when present in large numbers in association with immuno-depressed or immuno-compromised patients (Ellepola and Samaranayake, 2001; Cowen *et al.*, 2000; Edmond *et al.*, 1999; Levin *et al.*, 1998). Candidiasis is a term used to describe a primary or secondary infection caused by yeast that belongs to the genus *Candida*. Candidiasis may be manifested as a superficial skin infection (cutaneous and mucocutaneous) affecting mouth, vagina, throat, nails and associated frequently with fatal systemic disease that may involve the lungs, heart, gastrointestinal tract and other organs (Calderone and Fonzi, 2001; Dupont, 1995). With the increased occurrence of AIDS cases worldwide, many studies showed that among the many fatal opportunistic infections observed in HIV-positive patients, Oropharyngeal Candidiasis (OPC) is the most common, especially in the advanced stages of their disease (Al-Abeid *et al.*, 2004; Pelletier *et al.*, 2000; Boerlin *et al.*, 1995).

Most previous studies have focused on the effects of UV radiation on hyphal growth and blastospore formation; UV mutagenic and fungicidal effects and on

the expression of several virulence factors and morphogenesis (Brasch and Kay, 2004). On the other hand, there is a lack of information on tissue colonization and virulence changes due to exposure to UV radiation. Therefore, the aim of this study is to examine the influence of different doses of UV radiation on proteinase secretion, phospholipase production and adherence to epithelial cells which are taken as means to reflect changes in colonization and virulence of *Candida albicans*.

MATERIALS AND METHODS

Yeast isolates and growth conditions: *C. albicans* ATCC 36082, kindly provided by M.A. Ghannoum (Center for Medical Mycology, Mycology Reference Laboratory, University Hospital of Cleveland, Ohio, USA) and clinical *C. albicans* (O-74) isolate obtained from the oral cavity of a patient with denture stomatitis were used throughout this study. Methods for isolation, identification and antifungal susceptibility testing have been described elsewhere (Abu-Elteen and Abu-Elteen, 1998) The isolates were maintained on Sabouraud dextrose agar slant (SDA) (Difco, Detroit, Mich., USA) supplemented with 50 $\mu\text{g mL}^{-1}$ chloramphenicol (Sigma, St. Louis, Miss., USA), stored at 4°C and subcultured at 3 month intervals. For inoculation, overnight cultures of *C. albicans* were grown at 37°C in Trypton Soy Broth (TSB) (HiMedia Laboratories Limited, Mumbai-400 086, India); flasks containing 50 mL of the same medium were inoculated with 1 mL of the overnight culture and grown for 24 h in a shaking water bath at 37°C. Cells were harvested, washed twice in sterile physiological-buffered saline (SPBS) (pH 7.2).

Exposure of *Candida* colonies to UV light: Single colonies of *C. albicans* were produced by spreading of 10 μL of yeast suspension on SDA plates and incubated at 37°C for 24 h. *C. albicans* plates were exposed to different UV light (365 nm) intensities (4, 6 and 10 W m^{-2}) for different time intervals (6, 12, 24 and 48 h). At the end of each time intervals, irradiated colonies were collected and placed in SPBS. Cells were then harvested by centrifugation (3000 rpm for 10 min), washed twice with SPBS. This suspension was serially diluted and the cells, counted with a hemocytometer, were standardized to 1×10^7 cells mL^{-1} to examine the effect of different UV intensities on *C. albicans* colonization and virulence factors.

Mice: Twelve week-old Balb/c mice were obtained from the Hashemite University vivarium were used throughout this study. Mice were divided into two groups,

experimental group received irradiated *C. albicans* cells and control group treated with non-irradiated *C. albicans* cells. Ten microliters of standardized suspension of *C. albicans* were used for each intraperitoneal injection; a group of four mice were used for challenge by each UV light intensities at each time intervals

Tissue colonization: Mice were sacrificed by cervical dislocation after 48 h post inoculation with irradiated and non-irradiated *C. albicans*, the kidneys, spleen, stomach and whole intestine of injected animals were aseptically removed and placed in 10 mL SPBS. Superfluous tissue was trimmed away and the remaining tissue was then homogenized in 5 mL SPBS in a sterile glass homogenizer. Serial 10-fold dilutions in SPBS were prepared from these homogenates. One milliliter samples of appropriate dilutions were plated in triplicate onto SDA plates containing chloramphenicol (50 $\mu\text{g mL}^{-1}$) and the plates were left to dry then incubated at 37°C for 24-48 h, after which number of Colony-Forming Units (CFU) (one colony = 1 CFU) was determined. Values were expressed as the mean number of cells mL^{-1} per organ based on data from four animals.

Adherence of *C. albicans* to human buccal epithelial cells: The methodology for adherence assay has been described by Abu-Elteen and Hamad (2006). Human Buccal Epithelial Cells (HBEC) were collected from healthy volunteers by gentle rubbing of the mucosal surface of the cheeks with sterile tongue depressor. The cells were washed with SPBS and collected by centrifugation (1500 rpm for 10 min). Cells were counted using a hemocytometer and suspended in SPBS to concentration of 2×10^5 cells mL^{-1} . Only freshly prepared samples of HBEC were used in adherence assay. For adherence assay, 2 mL of the yeast cell suspension in SPBS at 1×10^7 cells mL^{-1} (irradiated and non-irradiated) was mixed with 2 mL of HBEC suspension in a sterile screw-capped vial. The mixture were incubated at 37°C for 2 h in a shaking water bath (180 rpm) and then filtered through a 20 μm filter (Retsch, Idar-Oberstein, Germany) to remove non-adherent yeast cells. The HBEC on the filter were washed twice with 5 mL of SPBS and finally suspended in 5 mL of the same buffer. A drop of this suspension were mounted on a microscope slide, air dried and heat fixed, then stained with crystal violet for 1 min. The adherence was determined microscopically by counting the mean number of yeast cells adhering to every 100 HBEC. Each assay was carried out in duplicate and student t-test was used to evaluate the difference in the adherence values; a p-value of <0.05 was considered significant.

Proteinase assay: Proteinase production was evaluated by the method of Staib (1965). Three flasks for *C. albicans* ATCC 36082 as well as O-47 isolate, each containing 50 mL of Sabouraud broth, were incubated at 25°C in shaking water bath for 24 h. Cells were harvested by centrifugation (3500 rpm for 15 min), washed twice with SPBS and resuspended in a concentration of 1×10^8 cells mL⁻¹. Ten microliters of the cell suspension were placed onto test medium (1% bactoagar [Difco], 1% [wt/vol] glucose, 0.1% [wt/vol] KH₂PO₄, 0.5% [wt/vol] MgSO₄, pH 5.0) containing 0.16% [wt/vol] bovine serum albumin (Sigma) as the sole nitrogen source. After 5 days of incubation at 37°C, the plates were fixed with 20% trichloroacetic acid and stained with 1.25% amido black (in acetic acid : methanol (10:90 vol/vol). Destaining was performed with 15% acetic acid and the clear zones around each colony were measured and used in the determination of the Pz value as described previously (Abu-Elteen *et al.*, 2001; Al-Abeid *et al.*, 2004). A digital caliper measured the diameter of the clear zone around each colony and the diameter of the *C. albicans* colony in order to estimate the Pz value. Each assay was carried out in triplicate on two different occasions and results were expressed as the triplicate mean.

Phospholipase assay: Phospholipase secretion from *C. albicans* ATCC 36082 as well as O-47 was measured by the method of Price *et al.* (1982). The *C. albicans* isolates were cultured in Sabouraud broth containing 2% (wt/vol) glucose for 18 h at 30°C in an orbital shaker. Cells were harvested by centrifugation, washed twice by SPBS and resuspended at a density of 1×10^7 cells mL⁻¹ in SPBS. Ten microliters of the yeast suspensions were spotted onto egg yolk medium. The egg yolk medium consisted of 6.5% SDA, 5.84% NaCl, 0.55% CaCl₂ and 8% sterile egg yolk emulsion (ICN Biomedicals Inc, Ohio, USA). The plates were incubated at 37°C for 48-72 h and the diameter of the precipitation zone around the colony was measured. Each assay was carried out in triplicate and the results were expressed as the triplicate mean. Phospholipase activity (Pz) was determined as the ratio of colony diameter to the diameter of the dense white zone of precipitation using the following formula:

$$Pz = 1 - \frac{A}{B} \text{ where}$$

A = Diameter of colony + dense white zone of precipitation

B = Diameter of colony

Statistical analysis: The data were processed by a statistical package (STATISTICA 5.0) (StaSoft, USA). Statistically significant differences between means of

phospholipase and acid proteinase production, respectively and the adherence of *C. albicans* to HBEC were tested by one-way analysis of variance (ANOVA). To determine which means were significantly different from the rest Fisher's Least Significant Difference (LSD) test was used. A p-value <0.05 was considered statistically significant.

RESULTS

The effect of UV treatment on the colonization properties in the murine system: The pathogenicity of irradiated and non irradiated *C. albicans* isolates was evaluated 24 h post intraperitoneal inoculation into adult Balb/c mice. The mean number of non irradiated *C. albicans* cells (control group) colonizing the kidneys was (847±58 CFU), this number decreased in mice treated with *C. albicans* exposed to UV light with different intensities at different times (Fig. 1A).

Figure 1B shows the effect of different UV intensities on *C. albicans* colonization of spleen. It can be seen that the mean number of *C. albicans* cells colonized to spleen tissues in the control group were (467±81 CFU). The number of CFU was decreased after *C. albicans* has been exposed to UV at different intensities with different times. The mean number of non treated *C. albicans* cells colonized the stomach was ($12075 \times 10^3 \pm 7144$ CFU) in mice, this number was declined in mice with *C. albicans* treated by different UV intensities at different times (Fig. 1C). Figure 1D demonstrates the effect of different UV intensities on *C. albicans* colonization of intestine. It can be seen that the mean number of *C. albicans* cells colonized to intestine tissues in the control group were ($12 \times 10^6 \pm 7931$ CFU). While it reduced after *C. albicans* has been exposed to UV at different intensities with different times.

In vitro adherence of *C. albicans* to HBECs: There was a statistically significant difference between the mean number of adhered yeast cells of control group and experimental group at different times of exposure to UV 4 W m⁻² (p<0.05). The maximum percent reduction of *C. albicans* adherence to HBEC was measured to be 36.3% at 48 h of exposure to UV 4 W m⁻² (Table 1). As shown in Table 2, a significant difference (p<0.05) was obtained between the mean number of adhered yeast cells of control group and experimental group at different times of exposure to UV 6 W m⁻². The maximum percent reduction of *C. albicans* adherence to HBEC was measured to be 50.1% at 48 h of exposure to UV 6 W m⁻². Increasing the UV light intensity to 10 W m⁻² resulted in greater reduction in adherence. There was a statistically significant difference (p<0.05) between the mean number

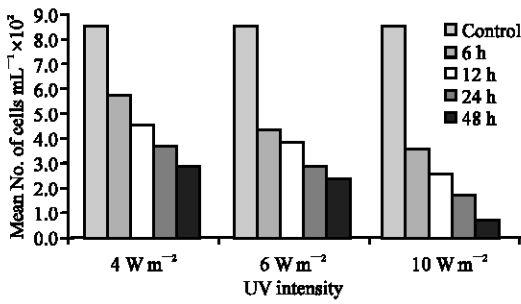


Fig. 1A: Effect of different UV intensities on *C. albicans* colonization of the kidneys

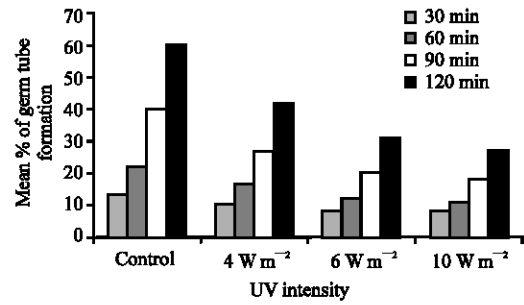


Fig. 2A: Effect of different UV intensities on germ-tube formation of *C. albicans* at 6 h

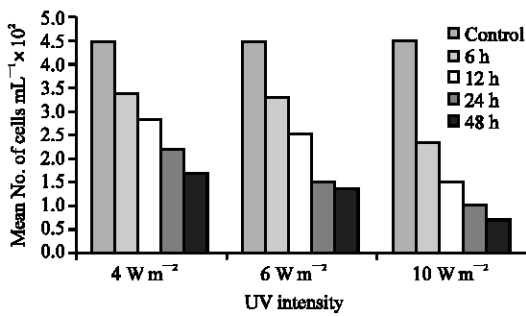


Fig. 1B: Effect of different UV intensities on *C. albicans* colonization of the spleen

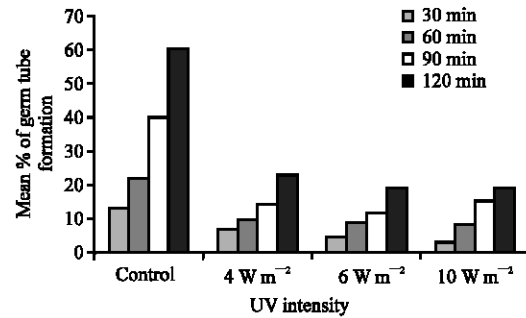


Fig. 2B: Effect of different UV intensities on germ-tube formation of *C. albicans* at 12 h

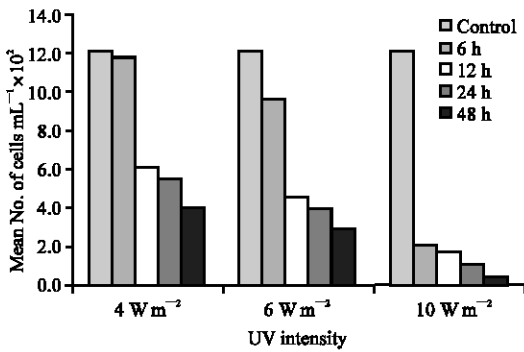


Fig. 1C: Effect of different UV intensities on *C. albicans* colonization of the stomach

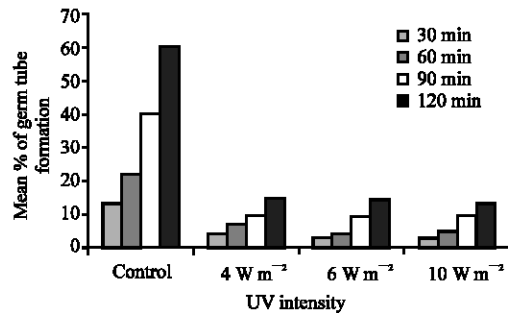


Fig. 2C: Effect of different UV intensities on germ-tube formation of *C. albicans* at 24 h

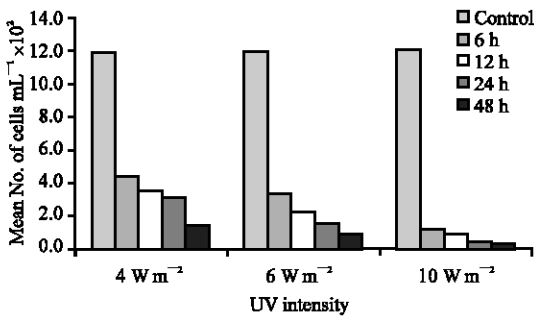


Fig. 1D: Effect of different UV intensities on *C. albicans* colonization of the intestine

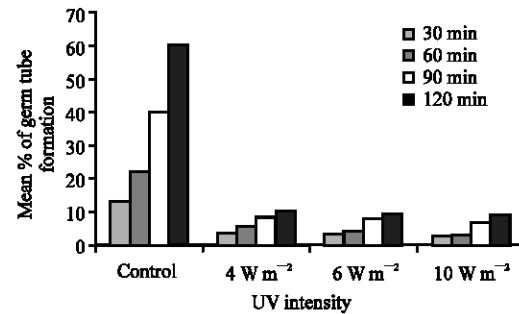


Fig. 2D: Effect of different UV intensities on germ-tube formation of *C. albicans* at 48 h

of adhered yeast cells of control group and experimental group at different times of exposure to UV. The maximum percent reduction of *C. albicans* adherence to HBEC was 66.7% at 48 h of exposure to UV 10 W m⁻² (Table 3). To try to relate the effect of radiation of *C. albicans* on adherence to cell viability, the effect of exposing the yeast to different intensities of UV light was performed. High intensity (10 W m⁻²) of UV light led to decrease in cell viability (a decrease of between 10 and 23% of the control value at different time of exposure).

Determination of germ tube formation: The mean percentages of germ tube formed by *C. albicans* ATCC 36082 were 13±1, 22±1, 42±2 and 63±3 at 30, 60, 90 and 120 min, respectively, while this mean percentage was decreased after exposure of *C. albicans* to UV light with different intensities at different times (Fig. 2A-D).

Extracellular enzyme production: The mean proteinase production (Pz) of control *C. albicans* was 0.85 mm. As shown in Table 1, the one-way ANOVA and LSD test of multiple comparison clearly indicated a statistically significant difference (p<0.05) between control group and experimental group exposed to 4 W m⁻² at different times (Table 1) were the mean proteinase production was

reduced to 0.49 mm at 6 h to 0.21 mm at 48 h of exposure. For 6 W m⁻² with different exposure times, a significant difference (p<0.05) between control group and experimental group was obtained (Table 2). Table 3 shows that the significant difference (p<0.05) between control group and experimental group exposed to 10 W m⁻² at different times.

Secretion of phospholipase: Overall, the level of phospholipase activities of *C. albicans* was consistently higher than that of proteinase produced (mean control phospholipase production was 1.5 mm). There was a statistically significant difference (p<0.05) between control group and experimental group exposed to 4 W m⁻² at different times (Table 1) with range phospholipase production was 0.89 mm at 6 h and 0.63 mm at 48 h of exposure. As shown in Table 2 a statistically significant difference (p<0.05) was achieved between control group and experimental group exposed to 6 W m⁻² at different times. The ability of *C. albicans* ATCC 36082 for phospholipase secretion was reduced after exposed to 10 W m⁻² with different times. There was a significant difference (p<0.05) between control group and experimental group exposed to UV 10 W m⁻² with different times (Table 3).

Table 1: Effect of 4W m⁻² on adherence to HBEC, proteinase and phospholipase secretion of *C. albicans*

Exposure time to UV	Mean number of yeast cells adhere to 100 HBEC±SE*	Reduction (%)	p-value	Proteinase Pz* in mm±SE**	p-value	Phospholipase Pz* in mm±SE**	p-value
Control	383±23	6.8	0.014571	0.85±0.01	0.0008	1.5±0.1	0.0173
6 h	357±18			0.49±0.01		0.89±0.06	
Control	383±23	15.4	0.000392	0.85±0.01	0.0004	1.5±0.1	0.0101
12 h	324±19			0.37±0.01		0.81±0.01	
Control	383±23	23.8	0.000048	0.85±0.01	0.0003	1.5±0.1	0.0090
24 h	292±17			0.29±0.01		0.76±0.01	
Control	383±23	36.3	0.000006	0.85±0.01	0.0012	1.5±0.1	0.0065
48 h	244±21			0.21±0.03		0.63±0.01	

*[Pz = (A/B) - 1]; A, diameter of colony + zone diameter; B, diameter of colony, **SE: Standard Error

Table 2: Effect of 6 W m⁻² on adherence to HBEC, proteinase and phospholipase secretion of *C. albicans*

Exposure time to UV	Mean number of yeast cells adhere to 100 HBEC±SE*	Reduction (%)	p-value	Proteinase Pz* in mm±SE**	p-value	Phospholipase Pz* in mm±SE**	p-value
Control	383±23	11	0.003023	0.85±0.01	0.0105	1.5±0.1	0.0100
6 h	341±21			0.37±0.07		0.80±0.02	
Control	383±23	21.1	0.000141	0.85±0.01	0.0003	1.5±0.1	0.0085
12 h	302±21			0.26±0.01		0.74±0.01	
Control	383±23	34.7	0.000012	0.85±0.01	0.0006	1.5±0.1	0.0076
24 h	250±19			0.20±0.02		0.68±0.02	
Control	383±23	50.1	0.000002	0.85±0.01	0.0002	1.5±0.1	0.0060
48 h	191±18			0.16±0.01		0.58±0.02	

*[Pz = (A/B) - 1]; A, diameter of colony + zone diameter; B, diameter of colony, ** SE: Standard Error

Table 3: Effect of 10 W m⁻² on adherence to HBEC, proteinase and phospholipase secretion of *C. albicans*

Exposure time to UV	Mean number of yeast cells adhere to 100 HBEC±SE*	Reduction (%)	p-value	Proteinase Pz* in mm±SE**	p-value	Phospholipase Pz* in mm±SE**	p-value
Control	383±23	20.9	0.00052	0.85±0.01	0.0003	1.5±0.1	0.0079
6 h	303±22			0.27±0.01		0.7±0.02	
Control	383±23	36	0.000038	0.85±0.01	0.0006	1.5±0.1	0.0072
12 h	245±21			0.18±0.02		0.64±0.03	
Control	383±23	50.4	0.000007	0.85±0.01	0.0002	1.5±0.1	0.006
24 h	190±22			0.13±0.01		0.59±0.01	
Control	383±23	66.7	0.000002	0.85±0.01	0.0008	1.5±0.1	0.0051
48 h	128±23			0.07±0.03		0.44±0.04	

*[Pz = (A/B) - 1]; A, diameter of colony + zone diameter; B, diameter of colony, **SE: Standard Error

DISCUSSION

The results of this work indicate that the adherence of non-irradiated *C. albicans* was significantly higher than those exposed to UV light with different intensities at different times ($p < 0.05$). This reduction was dose intensity and exposure time related. In this investigation low intensity of UV light (4 W m^{-2}) did not affect the viability of the yeast cells, whereas higher intensity (10 W m^{-2}) did. Death undoubtedly results at a point where the probability of circumventing the damaged systems by means of nutritional supplement or alternate pathways of repair become extremely low (Ghannoum *et al.*, 1988). Since the reduction of adherence was significant at all the radiation doses used in this study, this would imply that the effect on adherence is due to reason other than viability per se. This is in agreement with Khare *et al.* (1982) who proposed that radiation-induced changes in electrophoretic mobility, membrane transport and membrane receptors which have been regarded as varied manifestations of damage to plasma membrane. Furthermore this suggest that the plasma membrane of yeast may be one of the primary targets where radiation-induced damage precedes nuclear injury. On the other hand, Lee and King (1983) studying the adherence of *C. albicans* to human vaginal epithelial cells showed that viability was not essential for adherence, but severe methods used to kill the blastospores did reduce their attachment. To what extent viability is vital has yet to be established. It may be possible to suggest that radiation sensitize the yeast cells by affecting DNA degradation in different ways and this in itself might lead to severe alterations of the outer layers of the cells, affecting the adhesions/receptor sites present in the cells and as such leading to more inhibition of adherence. Moreover, germination of *C. albicans* exposed to UV was significantly lower from those of control group ($p < 0.05$). On the other hand, the difference between *C. albicans* exposed to high doses and low doses of UV was statistically significant ($p < 0.05$). This agrees with reported results by Gilberto *et al.* (2002) who stated that increase UV-B exposure time decreased relative percent culturability for *Verticillium lecanii* and *Aphanocladium album* and a strong delay in the germination of surviving conidia was observed, also both swelling and germ tube emergence were strongly inhibited by radiation.

Secreted Aspartyl Proteinases (SAPs) are believed to be virulence determinants of *C. albicans* (Abu-Elteen *et al.*, 2001; Wu *et al.*, 2000; Naglik *et al.*, 1999). The role of Saps as virulence factors was confirmed by the finding that the virulence of null mutants, *SAPs*1-3 and a triple mutant of *SAPs* 4-6 were all attenuated in a systemic mouse infection model and a guinea pig

infection model and in terms of adherence to HBECs (Hube *et al.*, 1997; Sanglard *et al.*, 1997; Watts *et al.*, 1998). In this regard, adherence of *C. albicans* to human mucosa (Watts *et al.*, 1998; Borg and Ruchel, 1988), epidermal corneocytes (Ray and Payne, 1988) and epidermal keratinocytes (El-Magharabi *et al.*, 1990) has been shown to be inhibited by pepstatin A, a hexa peptide from *Streptomyces* with strong anti-Saps potential. The results of this study indicate that a significant difference ($p < 0.05$) was seen between proteinase secreted from irradiated and non-irradiated *C. albicans*, also the difference was statistically significant between proteinase secreted from *C. albicans* exposed to different UV intensities ($p < 0.05$). Moreover, many evidence implicate the contribution of phospholipase to *Candida* pathogenicity (Abu-Elteen *et al.*, 2001; Ghannoum, 2000; Ibrahim *et al.*, 1995). These authors showed that isolates of *C. albicans* produced higher extracellular phospholipase levels were invasive in the infant mouse candidiasis model, whereas isolates with low extracellular phospholipase activity were not. The results of this study indicate that a significant difference ($p < 0.05$) between phospholipase production from irradiated and non-irradiated *C. albicans*, also the difference was statistically significant between phospholipase production from *C. albicans* exposed to different UV intensities ($p < 0.05$). In this context, it was concluded that the common virulence factors (phospholipase and proteinase production, adherence, germination, growth rate and ability to damage endothelial cells), only extracellular phospholipase activity was found to be predictive of mortality (Ibrahim *et al.*, 1995).

The results of this study provide evidence that UV light can affect *C. albicans* colonization of various organs. The results of this work indicate that a significant difference ($p < 0.05$) between irradiated *C. albicans* and non-irradiated that colonized the kidney, spleen, stomach and intestine tissues at different intensities with different times. It is possible that the correlation between low proteinase and phospholipase activities in one hand and reduce germ tube production on the other hand may inhibit the penetration of *C. albicans* through the mucosa resulted in lower tissue colonization in the kidneys, spleen, stomach and intestine, since the phospholipase activity is particularly concentrated at the tips of the hyphae (Abu-Elteen and Hamad, 2006). It was postulated that UV irradiation can affect yeast cell membrane (Khare *et al.*, 1982). It appears that there may be a relationship between cell survival and accumulation of four amino acids. Exposure of cells to increasing doses of γ radiation resulted in a significant decrease in cell survival and in the level of accumulation of lysine, glycine, glutamic acid and proline. The inhibition in amino acid accumulation following exposure to γ radiation could

be due to a change in conformation of various membrane components or permeases (Doyle and Kubitschek, 1976). Brasch and Kay (2004) found that *C. albicans* responds to low-dose UVB-irradiation with a reduction of hyphal growth and suggest that the invasive capacity of *C. albicans*, which is related to mycelial penetration, can be reduced by low-dose UVB-irradiation. A study by Sullivan and Conner-Kerr (2000) provides further evidence that short exposure times to UVC were detrimental to prokaryotic and simple unicellular eukaryotic organisms.

In conclusion, our investigation provides further evidence of a correlation between quantitative proteinase, phospholipase, germination, adherence and tissue colonization. An association of these parameters may be an important contributory factor for pathogenicity. In addition, it is reasonable to say that the findings of this research indicate that there were differences in these parameters between *C. albicans* exposed to different intensities of UV light and control group and may have interesting clinical connotations.

ACKNOWLEDGMENTS

This study was supported by a research grant (K.H. Abu-Elteen 2004\2005) from the Hashemite University, College of Graduate Studies and Scientific Research.

REFERENCES

- Abu-Elteen, K.H. and R.M. Abu-Elteen, 1998. The prevalence of *Candida albicans* populations in the mouths of complete denture wearers. *Microbiologica*, 21: 41-48.
- Abu-Elteen, K.H., A.Z. Elkarmi and M.A. Hamad, 2001. Characterization of phenotype-based pathogenic determinants of various *Candida albicans* strains in Jordan. *Jpn. J. Infect. Dis.*, 54: 229-236.
- Abu-Elteen, K.H. and M.A. Hamad, 2006. Determination of the Various Virulence Factors of *C. albicans* and Related Species. In: *Techniques in Medical Mycology: A Molecular Approach*, Kevin Kavanough (Ed.), Wiley and Sons, London, UK.
- Al-Abeid, H., K.H. Abu-Elteen, A.Z. Elkarmi and M. Hamad, 2004. Isolation and characterization of *Candida* sp. in Jordanian cancer patients: Prevalence, pathogenic determinants and antifungal sensitivity. *Jpn. J. Infect. Dis.*, 57: 279-284.
- Ben-Yosef, D.Z., Y. Kashman and Y. Benayahu, 2006. Response of the soft coral *Heteroxenia fuscescens* to ultraviolet radiation regimes as reflected by mycosporine-like amino acid biosynthesis. *Marine Ecol.*, 27: 219-228.
- Boerlin, P., F. Boerlin-Petzold, C. Durussel, M. Addo, J. Pagani, J. Chave and J. Bille, 1995. Cluster of oral atypical *Candida albicans* isolated from human immunodeficiency virus-positive drug users. *J. Clin. Microbiol.*, 33: 1129-1135.
- Borg, M. and R. Ruchel, 1988. Expression of extracellular acid proteinase by proteolytic *Candida* sp. during experimental infection of oral mucosa. *Infect. Immun.*, 56: 626-631.
- Brasch, J. and C. Kay, 2004. Low-dose UVB reduce hyphal growth and stimulates blastospore formation of *Candida albicans*. *Mikol. Lek.*, 11: Suppl. 1.
- Calderone, R. and W. Fonzi, 2001. Virulence factors of *Candida albicans*. *Trends in Microbiol.*, 9: 327-335.
- Cowen, L.E., D. Sanglard, D. Calabrese, C. Sirjusingh, J.B. Anderson and L.M. Kohn, 2000. Evolution of drug resistance in experimental populations of *Candida albicans*. *J. Bacteriol.*, 182: 1515-1522.
- Doyle, R.J. and H.E. Kubitschek, 1976. Near ultraviolet light inactivation of an energy-independent membrane transport system in *S. cerevisiae*. *Photochem. Photobiol.*, 24: 291-293.
- Dupont, P.F., 1995. *Candida albicans*, the opportunist. A cellular and molecular perspective. *J. Am. Pediatr. Med. Assoc.*, 85: 104-115.
- Edmond, M.B., S.E. Wallace, D.K. McClish, M.A. Pfaller, R.N. Jones and R.P. Wenzel, 1999. Nosocomial blood stream infections in United States hospitals: A three-years analysis. *Clin. Infect. Dis.*, 29: 239-244.
- Ellepola, A.N. and L.P. Samaranyake, 2001. Inhalation and topical and oral candidosis: A Mini Review. *Oral. Dis.*, 7: 211-216.
- El-Magharabi, E.A., D.M. Dixon and J.W. Burnett, 1990. Characterization of *Candida albicans* epidermolytic proteinases and their role in yeast-cell adherence to keratinocytes. *Clin. Exp. Dermatol.*, 15: 183-191.
- Gagnon, G., T. Dykstra, K. Leary, R. Andrews, C. Chauret and C. Volk, 2005. Impact of UV Disinfection on Biological Stability. *Water Intelligence Online*. UNIQUE ID: 200502AF90999F.
- Ghannoum, M.A., K.H. Abu-Elteen and M.S. Motawy, 1988. Effect of antineoplastic agents and X-irradiation on the adherence of *Candida* sp. to human buccal epithelial cells *in vitro*. *Mycopathologia*, 104: 171-180.
- Ghannoum, M.A., 2000. Potential role of phospholipases in virulence and fungal pathogenesis. *Clin. Microbiol. Rev.*, 13: 122-143.
- Gilberto, U.L., E.N. Drauzio, D. Stephan, D. Charles, J. Anne and W. Donald, 2002. Damage and recovery from UV-B exposure in conidia of the entomopathogens *Verticillium lecanii* and *Aphanocladium album*. *Mycologia*, 94: 912-920.

- Hube, B., D. Sanglard, F.C. Odds, D. Hess, M. Monod, W. Schafer, A.J.P. Brown and N.A.R. Gow, 1997. Disruption of each of the secreted aspartyl proteinase genes *SAP1*, *SAP2* and *SAP3* of *Candida albicans* attenuates virulence. *Infect. Immun.*, 65: 3529-3538.
- Ibrahim, A.S., F. Mirbod, S.G. Filler, Y. Banno, G.T. Gole, Y. Kitajima, J.E. Edwards, Jr., Y. Nozawa and M.A. Ghannoum, 1995. Evidence implicating phospholipase as a virulence factor of *C. albicans*. *Infect. Immun.*, 63: 1993-1998.
- Keski-Saari, S., J. Pusenius and R. Julkunen-Tiitto, 2005. Pjenolic compounds in seedlings of *Betula pubescens* and *B. pendula* are affected by enhanced UVB radiation and different nitrogen regimens during early ontogeny. *Global Change Biol.*, 11: 1180-1194.
- Khare, S., A. Trivedi, P.C. Kesavan and R. Parasad, 1982. Effect of gamma-radiation on the structure and function of yeast membrane. *Int. J. Rad. Biol.*, 42: 369-383.
- Kubitschek, H.E. and R.J. Doyle, 1981. Growth delay induced in *Escherichia coli* by near ultraviolet radiation: Relationship to membrane transport functions. *Photochem. Photobiol.*, 33: 695-702.
- Lee, J.C. and R.D. King, 1983. Characterization of *C. albicans* adherence to human vaginal epithelial cells *in vitro*. *Infect. Immun.*, 41: 1024-1030.
- Levin, A.S., S.F. Costa and N.S. Mussi, 1998. *Candida parapsilosis* fungemia associated with implantable and semi-implantable central venous catheters and the hands of health care workers. *Diagn. Microbiol. Infect. Dis.*, 30: 243-249.
- Lysytska, M., A. Knoll, D. Boehringer, T. Hey, G. Krauss and G. Krausch, 2002. UV light-damaged DNA and its interaction with human replication protein A: An atomic force microscopy study. *Nucleic Acid Res.*, 30: 2686-2691.
- Moss, S.H. and K.C. Smith, 1981. Membrane damage can be a significant factor in the inactivation of *Escherichia coli* by near-ultraviolet radiation, *Photochem. Photobiol.*, 33: 203-210.
- Naglik, J.R., G. Newport, T.C. White, L.L. Fernandes-Naglik, J.S. Greenspan, D. Greenspan, S.P. Sweet, S.J. Challacombe and N. Agabian, 1999. *In vivo* analysis of secreted aspartyl proteinase expression in human oral candidiasis. *Infect. Immun.*, 67: 2482-2490.
- Pelletier, R., J. Peter, C. Antin, C. Gonzalez, L. Wood and T.J. Walsh, 2000. Emergence of resistance of *Candida albicans* to clotrimazole in human immunodeficiency virus-infected children: *In vitro* and clinical correlations. *J. Clin. Microbiol.*, 38: 1563-1568.
- Price, M.F., I.D. Wilkinson and L.O. Gentry, 1982. Plate detection method for detection of phospholipase activity in *Candida albicans*. *Sabouraudia*, 20: 7-14.
- Ray, T.L. and C.D. Payne, 1988. Scanning electron microscopy of epidermal adherence and cavitation in murine candidiasis: A role for *Candida* acid proteinase. *Infect. Immun.*, 56: 1942-1949.
- Rinnan, R., M.M. Keinnanen, A. Kasurinen, J. Asikainen, T.K. Kekki, T. Holopainen, H. Ropoulsen, T.N. Mikkelsen and A. Michelsen, 2005. Ambient ultraviolet radiation in the Arctic reduces root biomass and alters microbial community composition but has no effects on microbial biomass. *Global Change Biol.*, 11: 564-574.
- Rochelle, P.A., 2005. An investigation of UV disinfection and repair in *Cryptosporidium parvum*. *Water Intelligence Online*. UNIQUE ID: 200503AF91015F.
- Sanglard, D., B. Hube, M. Monod, F.C. Odds and N.A.R. Gow, 1997. A triple deletion of the secreted aspartyl proteinase genes *SAP4*, *SAP5* and *SAP6* of *Candida albicans* causes attenuated virulence. *Infect. Immun.*, 65: 3539-3546.
- Staib, F., 1965. Serum-proteins as nitrogen source for yeast-like fungi. *Sabouraudia*, 4: 187-193.
- Sullivan, P.K. and T.A. Conner-Kerr, 2000. A comparative study of the effects of UV-C irradiation on selected prokaryotic and eukaryotic wound pathogens. *Ostomy Wound Manage.*, 46: 28-34.
- Turtola, S., M. Rousi, J. Pusenius, K. Yamaji, S. Heiska, V. Trikkonen, B. Meier and R. Julkunen-Tiitto, 2005. Clone-specific responses in leaf phenolics of willows exposed to enhanced UVB radiation and drought stress. *Global Change Biol.*, 11: 1655-1663.
- Watts, H.J., F.S.H. Cheah, B. Hube, D. Sanglard and N.A.R. Gow, 1998. Altered adherence in strains of *Candida albicans* harbouring null mutations in secreted aspartic proteinase genes. *FEMS Microbiol. Lett.*, 159: 129-135.
- Wu, T., K. Wright, S.F. Hurst and C.J. Morrison, 2000. Enhanced extracellular production of aspartyl proteinase, a virulence factor, by *Candida albicans* isolates following growth in subinhibitory concentrations of fluconazole. *Antimicrob. Agents Chemother.*, 44: 1200-1208.
- Zimmer, J.L. and R.M. Slawson, 2002. Potential repair of *Escherichia coli* DNA following exposure to UV radiation from both medium and low pressure UV sources used in drinking water treatment. *Applied Environ. Microbiol.*, 68: 3293-3299.