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Cancer Drugs Inhibit Germ Tube Elongation in *Candida albicans*

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Authors' contributions

This work was carried out in collaboration between all authors. Author SMK conceived the study. Majority of the work has been done by authors MMR and SBR. Author NMC is responsible for designing the manuscript. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: Hyphal forms of human fungal pathogen *Candida albicans* play a major role in infection process. During mucosal infections, they invade epithelial and endothelial cells and cause damage probably through the release of hydrolytic enzymes. Targeting yeast to hyphal form morphogenetic switching may be useful against invasive candidiasis. In this study, we report the germ tube elongation inhibitory properties of thirty cancer drugs from 12 different classes.
Methodology: *Candida albicans*, ATCC 90028 was used throughout the study and *Candida* filamentation assay was performed on polystyrene coated 96-well microtitre plates.
Results: Most of the drugs inhibited elongation at concentrations lower than their minimum Inhibitory Concentration. These concentrations were much lower than prescribed doses. The drugs were found to inhibit *Candida* growth at higher concentrations, suggesting that chemotherapy with anticancer drugs may not favor invasive candidiasis caused by *C. albicans.*
Conclusion: Our study indicates the possibility of repositioning cancer drugs as anti-morphogenetic agents in *C. albicans*.

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Keywords: Antifungal antibiotics; cancer drugs; germ tube elongation; morphogenetic switching.

1. INTRODUCTION

The ability of *C. albicans* to switch its lifestyle from a budding yeast cell to an elongating hypha is believed to confer considerable advantage to this organism to invade a wide range of tissues and evade the host defense mechanisms to cause invasive fungal infections [1]. *Candida* forms long tube like filaments with completely parallel sides and no constrictions at the site of septation. They play key role in infection process. During mucosal infections, the hyphal forms invade epithelial and endothelial cells and cause damage probably through the release of hydrolytic enzymes [2]. Access to blood stream and to establish candidemia requires crossing over of mucosal barriers, whereas infection of internal organs requires penetration of endothelia [3]. This yeast to hyphal form transition is dependent on various external signals, including elevated temperature, pH, nitrogen starvation, carbon starvation, presence of host macrophages and growth in serum as well as other chemicals such as N-acetylglucosamine [4].

Invasive fungal infections in cancer patients are primarily caused by *C. albicans* [5-7]. In critically ill cancer patients, *C. albicans* proliferates in heart valve, liver cells and intestinal tract by forming invasive and lethal filamentous forms and becomes a major cause of infection related mortality [8]. Treatment of such deep *Candida* infection frequently requires prolonged therapy and often associated with severe toxicity in patients, especially pediatric invasive infections [9].

We have reported the anti- *C. albicans* potential of different sub toxic concentrations of 30 commonly prescribed drugs belonging to 12 different classes of anticancer agents using standardized (i.e. CLSI) *in vitro* susceptibility assays. The results of our *in vitro* study have established the MIC values of 30 anticancer drugs against *C. albicans* using two standard strains ATCC90028 and ATCC10231. These drugs exhibited growth inhibitory effects on fluconazole sensitive as well as fluconazole-resistant strains [10]. There are very few studies which report about the inhibition of germ tube elongation by certain compounds. However, among few comprehensive studies Origanol oil and Carvacrol were found to affect both germination and mycelial elongation of *C. albicans in vitro* and also in a mouse model [11]. *Pongamia pinnata* root extracts, which is a plant used in Sri Lankan traditional medicinal systems to cure oral infections, inhibited germ tube elongation of five different clinical isolates of *C. albicans* [12]. However, detail role of molecules that particularly target elongation of germ tubes is yet to be revealed in *C. albicans*.

Here in this study for the first time we are reporting the effect of thirty anticancer agents on germ tube elongation of *C. albicans* ATCC90028.

2. MATERIALS AND METHODS

2.1 Cultures

Candida albicans, ATCC 90028 was obtained from Institute of Microbial Technology, Chandigarh, India and was used throughout the study. The strain was maintained on Yeast-Peptone-Dextrose [YPD] agar slants at 4ºC.

2.2 Media Chemicals and Culture Conditions

YPD medium was prepared by dissolving individual components (Yeast extract 1 %, Peptone 2 % and Dextrose 2 %) in distilled water. For culture activation, a single colony from YPD plates was inoculated in 25 ml of YPD broth, pH 6.5 in 250 ml conical flasks and incubated at 30ºC on an orbital shaker at 120 rpm for 24 hrs. Cells were harvested by centrifugation for 5 minutes at 2000 rpm speed and washed three times with PBS [10 mM Phosphate buffer, 2.7 mM potassium chloride and 137 mM Sodium chloride, pH 7.4]. Washed cells were resuspended in PBS.10 % solution of serum was made in sterile distilled water [13].

2.3 Anticancer Drugs

Thirty anticancer drugs from twelve different classes were used for this work. All the drugs were purchased from the local market.

2.4 Germ Tube Elongation Assay

Experiments were performed in 96-well microtiter plate to see the effect of various cancer drugs on germ tube elongation. Various concentrations of cancer drugs (ranging from 0.781 - 50 µg/ml) were added to preexisting germ tubes induced by 10% serum. Cells were incubated for 2 hours at 37 °C again [13] and after incubation; lengths of germ tube were measured by micrometry method. Ocular micrometer was put inside the eyepiece and was calibrated by superimposing the graduation of ocular micrometer over the graduation of stage micrometer, which is accomplished by rotating the ocular lens. By determining the number of ocular micrometer divisions coinciding with the number of divisions on stage micrometer, the calibration factor for one ocular division (O.D) is calculated for the particular combination of objective and ocular lens used by applying the formula [12,14].

$$One\ ocular\ division\ (\mu m)\ = \frac{No.of\ division\ on\ stage\ micrometer}{No.of\ division\ on\ ocular\ micrometer} X\ 100$$

After calibrating, an ocular micrometer can be used to determine the length of germ tube by using the following formula:

Length (µm) = No. of Ocular Division x Calibration Factor of the Ocular Division Occupied from Germ Tube Length.

All the experiments were done in triplicates. For each well, lengths of 10 germ tubes were measured and mean value was calculated. Percentage inhibition of germ tube elongation was calculated by comparing to that of control.

3. RESULTS AND DISCUSSION

3.1 Docetaxel Most Efficiently Inhibited Germ Tube Elongation

Docetaxel was the most efficient inhibitor of germ tube elongation among the antimicrotubule agents tested. It inhibited 100% germ tube elongation at 50 µg/ml (Tabel 1A; Fig. 1B). Vinblastine was not effective in inhibiting the length up to 50 µg/ml. Whereas, Vincristine and Paclitaxel caused inhibition of 50% length at 50 µg/ml (Table 1A).

3.2 Platinum Analogues are Good in Inhibiting Pre Formed Germ Tubes

Cisplatin and Oxaliplatin inhibited 60% of length of filamentation at 12.5 µg/ml whereas, 78% and 84% inhibition of germ tube elongation was seen at 50 µg/ml respectively. Carboplatin showed 64% germ tube length inhibition at 50 µg/ml (Table 1A and 1B).

3.3 Pre Formed Germ Tube Inhibitory Potential of Alklyting Agents

Cyclophosphamide and Busulfan were the most efficient among the alkalyting agents tested. They inhibited 89% and 76% of germ tube elongation at 25 µg/ml and 95 % at 50 µg/ml (Table 1B; Fig. 1C and E). Ifosfamide halted 88% of germ tube elongation at 50 µg/ml whereas, Melphalan caused 75% reduction in germ tube length at 50 µg/ml (Table 1B and 1C).

3.4 Activity of Antimetabolites

Among the two antimetabolites tested, Methotrexate inhibited 74% germ tube length at 50 µg/ml, followed by Gemcitabine which showed 69% inhibition in length at 50 µg/ml. (Table 1C).

3.5 Antitumor Antibiotics Inhibited Pre Formed Germ Tubes

Eight antitumor antibiotics were included in this study resulted in great degree of variations in effect on germ tube elongation. Doxorubicin and 5-flurouracil inhibited 50% length at 12.5 µg/ml and 6.25 µg/ml respectively and complete i.e. 100% germ tube length was inhibited at 50 µg/ml and 25 µg/ml respectively (Table 1C; Fig. 1D and 1F). Bleomycin, Daunorubicin, Mitoxantrone, Epirubicin and Mitomycin-C caused 50% inhibition at 25 µg/ml but fail to inhibit elongation completely event at 50 µg/ml (Table 1D).

3.6 Other Anticancer Agents

Tamoxifen inhibited 88% of germ tube elongation at the highest concentration studied i.e 50 µg/ml (Table 1E; Fig. 1H). Etoposide exhibited 75% inhibition on length of germ tubes at 25 µg/ml and 87% at 50 µg/ml of concentration (Table 1E; Fig. 1G). Non classic alkalyting agent i.e. dacarbazine reduced 50% length at 12.5 µg/ml and 74% at 50 µg/ml of concentration. Leuprolide and Irinotecan halted elongation of serum induced morphogenesis in *C. albicans* by 61 and 64% respectively at 50 µg/ml of concentration. Formestane did not show any activity on elongation of germ tubes (Table 1E).

Penetration of host tissue is an exclusively fungal-driven process, which is mediated by hyphal extension and activities such as production of secreted aspartic proteases (SAP 4-6) [15]. Extension of hyphae is also essential for inter epithelial dissemination, penetration into adjacent epithelial cells following initial epithelial invasion and these subsequent events may be responsible for the gross damage of epithelial tissues [16-17].

In *C. albicans*, *EED1* gene plays a crucial role in the extension and maintenance of filamentous growth. *EED1* mutant cells failed to extend germ tubes into long filaments and remained in yeast form even after three hrs of incubation during growth on plastic surfaces [18]. Expression of *EED1* is regulated by transcription factor *Efg1*. Transcriptional profiling of *EED1* mutant during infection of oral tissues revealed down regulation of hyphal associated

genes including *UME6*, encoding a key transcriptional factor. These data suggest that *EED1* and *UME6* plays a major role in regulation of hyphal growth, thereby repressing hyphal-to-yeast transition permitting dissemination of *C. albicans* within epithelial tissues [19]. *UME6* which is induced in response to multiple host environmental factors is specifically important for hyphal extension. *UME6* mutants are capable of germ tube formation but exhibit clear defect in hyphal extension both *in vitro* and during infection *in vivo* [20].

Thus blocking the ability of *C. albicans* to invade tissues may prevent invasive fungal infections. With this idea, we tested thirty commonly prescribed cancer drugs targeting different cell targets in human for its efficacy on germ tube elongation.

Antimicrotubule agent Docetaxel was the most efficient inhibitor of germ tube elongation and it inhibited 100 % length at 50 µg/ml about one half of MIC_{50}. This was followed by antitumor antibiotics, 5-flurouracil and Doxorubicin. Both inhibited germ tube elongation upto 98 % at half of its MIC value i.e. 50 µg/ml. Alkalyting agents targeting cellular thiol groups and nucleic acids to form DNA-RNA and DNA-protein cross links resulting in inhibition of DNA synthesis and respective function showed good inhibition of germ tube elongation. Among various alkalyting agents tested, Busulfan and Cyclophosphamide inhibited 95 % of germ tube elongation which correspond to MIC value of 50 µg/ml. Apart from these drugs, etoposide which is an epipodophyllotoxins exhibited elongation of induced morphogenesis at 50 µg/ml which is doubled from its MIC value i.e. 25 µg/ml, suggesting that the inhibition is due to inhibition of growth. However, other drugs did not showed any promising effect on elongation, suggesting that the above drugs that has showed good results such as Docetaxel, Busulfan, Cyclophosphamide, 5-flurouracil and Doxorubicin may represent an good antifungal agent for tomorrow (Table 1A-E; Fig. 1).

Despite the general limitations of targeting distinct virulence attributes, the inhibition of germ tube elongation may be an attractive option for controlling *C. albicans* infections in future. However, such antibiotics are not in practice currently. Most of the drugs tested by us inhibited germ tube elongation at concentrations lower than their MIC and inhibited *Candida* growth at higher concentrations, suggesting that analogs of these drugs could be repositioned as good antifungal agents against invasive *Candida* infections for future.

Triplicate experiments were conducted at 37ºC in presence of 10% serum. Germ tube length was measured after 2 hours. Values are the mean of 10 germ tubes length. Values in parenthesis indicate standard deviation.

Table 1A. Effect of cancer drugs on elongation of germ tube in *C. albicans*

Drug name	Conc. of drug(µg/ml)	Length of germ tube after 4 hrs (µm) (A)	Length of germ tube after 2 hrs (µm) (B)	Elongation of germ tubes in 2 hrs (µm) (A-B)	% Inhibition of elongation compared to control
Docetaxel	0	16.3	7.9	8.4(±0.0)	0
	0.781	14.5		7.5(±0.2)	11
	1.562	14.2		6.3(±0.4)	25
	3.152	13.1		5.2(±0.2)	38
	6.25	11.3		3.4(±0.0)	60
	12.5	9.7		1.8(±0.4)	79
	25	8.9		1(±0.3)	88
	50	7.93		0.03(±0.3)	**100**
Vinblastin	0	16.3	7.9	8.4(±0.1)	0
	0.781	15.7		7.8(±0.4)	7
	1.562	14.1		6.2(±0.3)	26
	3.152	13.9		6(±0.2)	29
	6.25	13.7		5.8(±0.3)	31
	12.5	13.2		5.3(±0.2)	37
	25	13		5.1(±0.2)	39
	50	13		5.1(±0.1)	**39**
Vincristine	0	16.3	7.9	8.4(±0.0)	0
	0.781	15.4		7.5(±0.4)	11
	1.562	14.1		6.2(±0.3)	26
	3.152	13.5		5.6(±0.4)	33
	6.25	13.7		5.8(±0.1)	31
	12.5	12.9		5(±0.0)	40
	25	12.6		4.7(±0.1)	44
	50	12.1		4.2(±0.2)	**50**
Paclitaxel	0	16.3	7.9	8.4(±0)	0
	0.781	14.6		6.7(±0.4)	20
	1.562	14.4		6.5(±0.3)	23
	3.152	13.3		5.4(±0.4)	36
	6.25	13.2		5.3(±0.1)	37
	12.5	12.9		5(±0.0)	40
	25	12.8		4.9(±0.1)	42
	50	12.7		4.8(±0.2)	**43**
Cisplatin	0	16.3	7.9	8.4(±0.3)	0
	0.781	15.3		7.4(±0.4)	12
	1.562	14.5		6.6(±0.2)	21
	3.152	13.6		5.7(±0.3)	32
	6.25	12.1		4.2(±0.1)	50
	12.5	11.4		3.5(±0.0)	58
	25	10		2.1(±0.3)	75
	50	9.7		1.8(±0.1)	**79**

Table 1B. Effect of Cancer drugs on elongation of germ tube in *C. albicans*

Drug name	Conc of drug(µg/ml)	Length of germ tube after 4 hrs (µm) (A)	length of germ tube after 2 hrs (B)	Elongation of germ tubes in 2hrs (A-B)	% inhibition of elongation compared to control
Oxaliplatin	0	16.3	7.9	8.4(±0.3)	0
	0.781	15.3		7.4(±0.4)	12
	1.562	14.5		6.6(±0.2)	21
	3.152	13.6		5.7(±0.3)	32
	6.25	12.1		4.2(±0.1)	50
	12.5	11.4		3.5(±0.0)	58
	25	10		2.1(±0.3)	75
	50	9.7		1.8(±0.1)	**79**
Carboplatin	0	16.3	7.9	8.4(±0.0)	0
	0.781	15.7		7.8(±0.4)	7
	1.562	14.8		6.9(±0.3)	18
	3.152	13.9		6(±0.4)	29
	6.25	13.2		5.3(±0.1)	37
	12.5	12.7		4.8(±0.0)	43
	25	12.2		4.3(±0.1)	49
	50	10.9		3(±0.2)	**64**
Cyclophos-	0	16.3	7.9	8.4(±0.1)	0
phamide	0.781	15.1		7.2(±0.4)	14
	1.562	14		6.1(±0.3)	27
	3.152	13.1		5.2(±0.2)	38
	6.25	11		3.1(±0.4)	63
	12.5	9.9		2(±0.2)	76
	25	8.8		0.9(±0.2)	89
	50	8.3		0.4(±0.1)	**95**
Busulfan	0	16.3	7.9	8.4(±0.0)	0
	0.781	15.6		7.7(±0.4)	8
	1.562	14.1		6.2(±0.3)	26
	3.152	13.7		5.8(±0.4)	31
	6.25	12.1		4.2(±0.0)	50
	12.5	11.4		3.5(±0.1)	58
	25	9.9		2(±0.1)	76
	50	8.3		0.4(±0.2)	**95**
Ifosfamide	0	16.3	7.9	8.4(±0.0)	0
	0.781	14.6		6.7(±0.4)	20
	1.562	13.8		5.9(±0.3)	30
	3.152	13		5.1(±0.1)	39
	6.25	12.2		4.3(±0.0)	49
	12.5	11		3.1(±0.1)	63
	25	10.7		2.8(±0.2)	67
	50	8.9		1(±0.1)	**88**

Triplicate experiments were conducted at 37ºC in presence of 10 % serum. Germ tube length was measured after 2 hours. Values are the mean of 10 germ tubes length. Values in parenthesis indicate standard deviation.

Table 1C. Effect of Cancer drugs on elongation of germ tube in *C. albicans*

Drug name	Conc. of drug(µg/ml)	Length of germ tube after 4 hrs (µm) (A)	Length of germ tube after 2 hrs (µm) (B)	Elongation of germ tubes in 2hrs (µm) (A-B)	% inhibition of elongation compared to control
Melphalan	0	16.3	7.9	8.4((±0.0)	0
	0.781	15.7		7.8((±0.4)	7
	1.562	14.3		6.4((±0.3)	24
	3.152	13.6		5.7(±0.4)	32
	6.25	12.7		4.8(±0.1)	43
	12.5	11.4		3.5(±0.0)	58
	25	10.5		2.6(±0.1)	69
	50	10		2.1(±0.2)	**75**
Methotrexate	0	16.3	7.9	8.4(±0.1)	0
	0.781	15.5		7.6(±0.6)	10
	1.562	14.3		6.4(±0.5)	24
	3.152	13.8		5.9(±0.3)	30
	6.25	12.6		4.7(±0.4)	44
	12.5	11.5		3.6(±0.1)	57
	25	10.6		2.7(±0.3)	68
	50	10.1		2.2(±0.2)	**74**
Gemcitabine	0	16.3	7.9	8.4(±0.3)	0
	0.781	15.4		7.5(±0.3)	11
	1.562	14.6		6.7(±0.2)	20
	3.152	13.6		5.8(±0.5)	31
	6.25	13.5		5.7(±0.0)	32
	12.5	12.8		4.9(±0.4)	42
	25	11.5		3.6(±0.1)	57
	50	10.5		2.6(±0.1)	**69**
Doxorubicin	0	16.3	7.9	8.4(±0.0)	0
	0.781	15.8		7.9(±0.2)	6
	1.562	14.7		6.8(±0.4)	19
	3.152	13.6		5.7(±0.2)	32
	6.25	13		5.1(±0.0)	39
	12.5	12.1		4.2(±0.4)	50
	25	11		3.1(±0.3)	63
	50	8.5		0.6(±0.3)	**93**
5-Flurouracil	0	16.3	7.9	8.4(±0.0)	0
	0.781	15.3		7.4(±0.6)	12
	1.562	14.6		6.7(±0.0)	20
	3.152	13.4		5.5(±0.1)	35
	6.25	12.1		4.2(±0.2)	50
	12.5	10.9		3(±0.3)	64
	25	8.3		0.4(±0.2)	95
	50	8.1		0.2(±0.1)	**98**

Triplicate experiments were conducted at 37ºC in presence of 10% serum. Germ tube length was measured after 2 hours. Values are the mean of 10 germ tubes length. Values in parenthesis indicate standard deviation.

Table 1D. Effect of cancer drugs on elongation of germ tube in *C. albicans*

Drug name	Conc. of drug(µg/ml)	Length of germ tube after 4 hrs (µm) (A)	Length of germ tube after 2 hrs (µm) (B)	Elongation of germ tubes in 2hrs (µm) (A-B)	% Inhibition of elongation compared to control
Bleomycin	0	16.3	7.9	8.4(±0.0)	0
	0.781	15.9		8(±0.6)	5
	1.562	15.6		7.7(±0.5)	8
	3.152	15.2		7.3(±0.3)	13
	6.25	14.6		6.7(±0.4)	20
	12.5	13.5		5.6(±0.1)	33
	25	12.1		4.2(±0.3)	50
	50	12		4.1(±0.2)	**51**
Daunorubicin	0	16.3	7.9	8.4(±0.1)	0
	0.781	15.1		7.2(±0.1)	14
	1.562	13.9		6(±0.1)	29
	3.152	13.8		5.9(±0.1)	30
	6.25	12.6		4.7(±0.1)	44
	12.5	12.5		4.6(±0.1)	45
	25	12.2		4.3(±0.1)	49
	50	11.9		4(±0.1)	**52**
Mitoxantrone	0	16.3	7.9	8.4(±0.2)	0
	0.781	15.8		7.9(±0.4)	6
	1.562	15.2		7.3(±0.0)	13
	3.152	14.8		6.9(±0.1)	18
	6.25	14		6.1(±0.2)	27
	12.5	12.4		4.5(±0.1)	46
	25	12.1		4.2(±0.3)	50
	50	10		2.1 (±0.3)	**75**
Epirubicin	0	16.3	7.9	8.4(±0.2)	0
	0.781	15.9		8(±0.3)	5
	1.562	15.6		7.7(±0.3)	8
	3.152	15		7.1(±0.1)	15
	6.25	14		6.1(±0.4)	27
	12.5	12.5		4.6(±0.2)	45
	25	12.1		4.2(±0.1)	50
	50	11.8		3.9(±0.1)	**54**
Mitomycin-C	0	16.3	7.9	8.4(±0.0)	0
	0.781	15.7		7.8(±0.1)	7
	1.562	15		7.1(±0.5)	15
	3.152	14.7		6.8(±0.3)	19
	6.25	13.6		5.7(±0.4)	32
	12.5	12.4		4.5(±0.1)	46
	25	12.1		4.2(±0.3)	50
	50	11		3.1(±0.2)	**63**

Triplicate experiments were conducted at 37ºC in presence of 10% serum. Germ tube length was measured after 2 hours. Values are the mean of 10 germ tubes length. Values in parenthesis indicate standard deviation.

Table 1E. Effect of cancer drugs on elongation of germ tube in *C. albicans*

Drug name	Conc. of drug (µg/ml)	Length of germ tube after 4 hrs (µm) (A)	Length of germ tube after 2 hrs (µm) (B)	Elongation of germ tubes in 2 hrs (µm) (A-B)	% Inhibition of elongation compared to control
Etoposide	0	16.3	7.9	8.4(±0.1)	0
	0.781	15.6		7.7(±0.3)	8
	1.562	14.2		6.3(±0.2)	25
	3.152	13.8		5.9(±0.2)	30
	6.25	12.5		4.6(±0.1)	45
	12.5	11.9		4(±0.1)	52
	25	10		2.1(±0.0)	75
	50	9		1.1(±0.2)	**87**
Dacarbazine	0	16.3	7.9	8.4(±0.2)	0
	0.781	15.3		7.4(±0.3)	12
	1.562	15		7.1(±0.3)	15
	3.152	14.7		6.8(±0.0)	19
	6.25	13.3		5.4(±0.4)	36
	12.5	12.1		4.2(±0.2)	50
	25	11.8		3.9(±0.1)	54
	50	10.1		2.2(±0.1)	**74**
Leuprolide	0	16.3	7.9	8.4(±0.0)	0
	0.781	15.4		7.5(±0.6)	11
	1.562	14.9		7(±0.0)	17
	3.152	14		6.1(±0.1)	27
	6.25	13.7		5.8(±0.2)	31
	12.5	13.1		5.2(±0.3)	38
	25	12		4.1(±0.2)	51
	50	11.2		3.3(±0.0)	**61**
Tamoxifen	0	16.3	7.9	8.4(±0.0)	0
	0.781	15.6		7.7(±0.6)	8
	1.562	14.4		6.5(±0.5)	23
	3.152	14		6.1(±0.3)	27
	6.25	12		4.1(±0.4)	51
	12.5	11.5		3.6(±0.1)	57
	25	11		3.1(±0.3)	63
	50	8.9		1(±0.2)	**88**
Irinotecan	0	16.3	7.9	8.4(±0.2)	0
	0.781	15.8		7.9(±0.4)	6
	1.562	14.3		6.4(±0.3)	24
	3.152	13.6		5.7 (±0.4)	32
	6.25	12.7		4.8(±0.1)	51
	12.5	12		4.1(±0.0)	43
	25	11.5		3.6(±0.1)	57
	50	10.9		3(±0.2)	**64**

Triplicate experiments were conducted at 37 °C in presence of 10% serum. Germ tube length was measured after 2 hours. Values are the mean of 10 germ tubes length. Values in parenthesis indicate standard deviation.

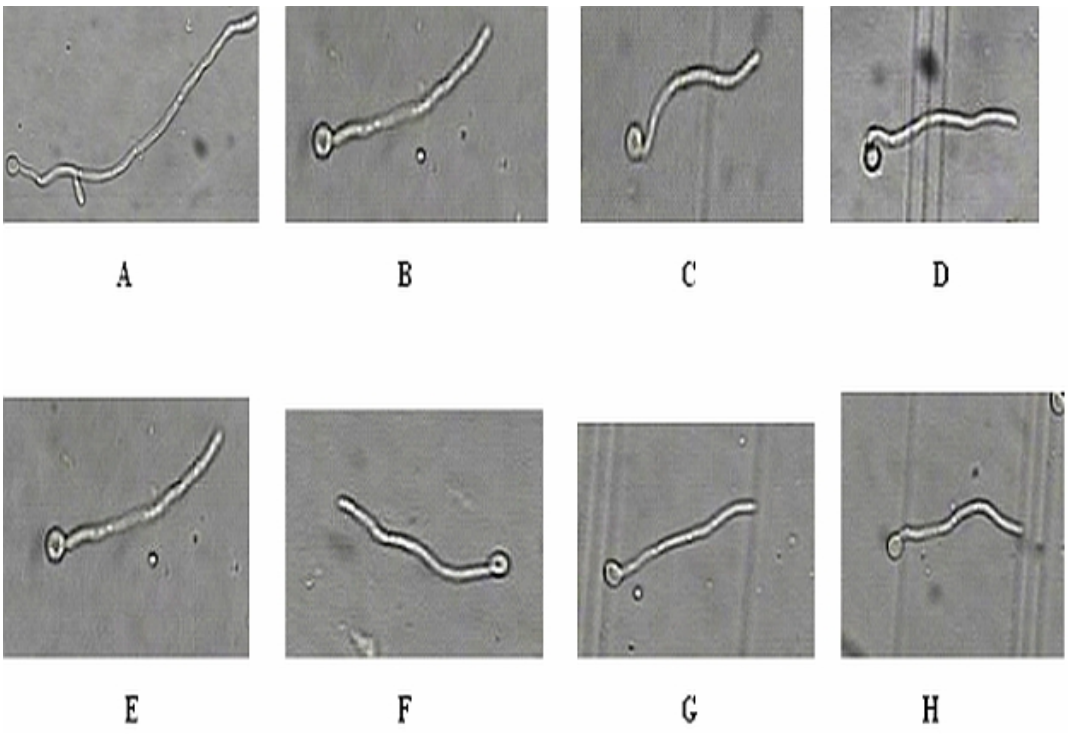

Fig. 1. Effect of various anticancer drugs on germ tube elongation. (A) Control after 4 hours of incubation in serum, (B) Docetaxel (25 µg/ml), (C) Cyclophosphamide (25 µg/ml), (D) Doxorubicin (25 µg/ml), (E) Busulfan (50 µg/ml), (F) 5-Flurouracil (25 µg/ml), (G) Etoposide (50 µg/ml), (H) Tamoxifen (50 µg/ml). Briefly, various concentrations of anticancer drugs were added to pre-existing germ tubes and germ tube length was observed under microscope after 4 hours of incubation. The difference in germ tube elongation was compared with that of control (without drugs) after 4 hours of incubation

4. CONCLUSION

Based on our study it was concluded the rethinking of possibility of repositioning cancer drugs as anti-morphogenetic agents in *C. albicans*.

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COMPETING INTEREST

The authors declare that they have no competing interest.

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