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Anticancer drugs as enhancers of fluconazole sensitivity in *Candida albicans*

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Prescribing fluconazole for prophylaxis and treatment of *Candida albicans* infections in cancer patients is a common practice. The rational of using anti- *Candida* drugs along with cancer drugs is debating, because of contradictory results showing either increase or decrease in antifungal sensitivity. In an effort to analyse this, effect of short term exposure to thirty anticancer agents on minimum inhibitory concentration (MIC) of fluconazole was studied in a micro plate based assay. Antitumor antibiotic, 5-flurouracil was the most effective sensitizer of *C. albicans*, causing sixty-four fold increase in fluconazole susceptibility. Eight of the selected anticancer molecules had potential to lower fluconazole MIC by sixteen fold, so that it comes down to 0.062 µg/ml. Three of the cancer drugs caused eight fold increase in the antifungal sensitivity. Effective molecules belonged to six different classes, indicating that ability to sensitize *C. albicans* towards fluconazole was not confined to a specific group. Our *in vitro* study, for the first time reveals efficacy of the thirty anticancer drugs to act as sensitizers in *C. albicans*.

Key words: Antifungal, anticancer, *Candida albicans*, drug resistance, exposure, sensitization, drug susceptibility, fluconazole.

INTRODUCTION

Candida infections in cancer patients are associated with high morbidity and mortality rates as well as increased cost of treatment (Bensadoun et al., 2011; Davies et al., 2000; Lalla et al., 2010; Safdar and Armstrong, 2002). Antifungal drugs available for treatment of candidiasis are mainly confined to four classes of molecules that are polyenes, 5-fluoro-cytosine (5-FC), azoles and recently developed echinocandins (Raut et al., 2012). Fluconazole is a drug of choice for the treatment of *C. albicans* infections in cancer patients (Yu et al., 2006). In immunologically debilitated cancer patients, fluconazole need to be administered in high dosages ranging from 400 to 800 mg/day (Harnicar et al., 2009), which many times results in nephrotoxicity or infusion-related toxicity (Winston et al., 2000; Yu et al., 2006). Infections associated with drug resistant strains and biofilm forms have emerged as a major challenge to successful anti- *Candida* treatment (Campbell, 2012; Davies et al., 2000; Safdar et al., 2001; Shinde et al., 2012; Winston et al., 2000). In this context, better options of antifungal therapy in cancer patients need to be explored. Prescribing fluconazole for prophylaxis and treatment of *Candida* infections in cancer patients is a routine (Yu et al., 2006). Also, use of anti- *Candida* drugs along with anticancer agents is a common practice in clinics (Cornely et al., 2007; Winston et al., 2000). Interestingly, anticancer drugs alone were reported to

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possess antifungal properties. Cisplatin and 5-fluorouracil were shown to alter the morphology and growth of *C. albicans* (Kesavan et al., 2005a, b). Tamoxifen and its structural analog clomiphene were shown to possess good anti- *Candida* activities *in vitro* and *in vivo* in a mouse model (Dolan et al., 2009). However, combination of antifungal and antineoplastic drugs may not be always synergistic. Negative interactions among these drugs may result in emergence of antifungal drug resistance and failure of the treatment (Ghannoum et al., 1989, 1990). For example, exposure to anticancer drugs was found to increase *C. albicans* tolerance to antifungal drugs like amphotericin B (O'Keeffe et al., 2003). Therefore, rational of using anti- *Candida* drugs like fluconazole or amphotericin B along with cancer drugs is a debating issue. A report by Bulatova and Darwish (2008) showed that 16 different drugs, including an anticancer agent (tamoxifen), sensitize *C. albicans* towards activity of fluconazole. Exposure to 27.5 µg/ml of tamoxifen was found to lower down fluconazole MIC from 5.5 to 0.01 µg/ml (Bulatova and Darwish, 2008). Recently, a comprehensive study by our group elucidated the antifungal properties of thirty commonly prescribed drugs belonging to twelve different classes of anticancer agents. It showed that many of the anticancer drugs inhibit *in vitro* growth of *C. albicans*, at concentrations which may exist in cancer patients undergoing chemotherapy (Routh et al., 2011). Effects of these drugs in combination with the most widely prescribed antifungal, fluconazole, are not well studied. Information on the effect of exposure to these drugs on fluconazole susceptibility of *C. albicans* may help to design the antifungal drug regimens in cancer patients infected with *Candida*. In this study we report alteration of fluconazole sensitivity of *C. albicans* exposed to selected antineoplastic drugs.

MATERIALS AND METHODS

Culture and growth conditions

A standard strain of *Candida albicans,* ATCC 90028, was obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India, and maintained on yeast peptone dextrose (YPD) agar slants at 4°C. Activation of culture was done by inoculating a single colony from YPD agar plate (yeast extract 1%, peptone 2%, dextrose 2%, agar 2.5%) into 50 ml YPD broth in a 250 ml conical flask. The flasks were incubited at 30°C, at 100 rpm on an orbital shaking incubator for 24 h. Cells were harvested by centrifugation at 2000 × *g* and washed thrice with 10 mM Phosphate Buffered Saline (PBS), of pH 7.4. The cell density was determined by hemocytometer count and cells resuspended in PBS were used as inoculum for further experiments.

Anticancer and antifungal drugs

Thirty drugs from twelve different classes of anticancer agents were obtained from the local market. Details on their classification and manufacturers are mentioned in Table 1. Twelve classes of the drugs were varying in their mode of action and their cellular targets (Table 1). Fluconazole (Forcan) the standard antifungal drug was purchased from, Cipla Pharm. Ltd., India.

Anticancer drug exposure assay

Activated cells of *C. albicans* were exposed to various anticancer drugs in an *in vitro* assay as per standard methodology with modification (Bulatova and Darwish, 2008). Briefly, a concentration half the known minimum inhibitory concentration (MICs) of each anticancer drug for *C. albicans* was selected and this sub-inhibitory concentration was used to treat the cells. In the exposure assay, cells were incubated in ½ × MIC of each anticancer drug for 1 h, at 30°C with mild shaking and then washed with PBS to remove any traces of the drugs. Washed cells were used in growth assay to check the effect of exposure on the MIC of fluconazole.

Growth assay

Growth susceptibility study was carried out by the standard micro broth dilution methodology as per Clinical Laboratory Standards Institute (CLSI) guidelines (Routh et al., 2011). Briefly, various concentrations of the antifungal drug fluconazole were prepared in RPMI-1640 medium by double dilution in the 96 well plates. Cells obtained in the exposure assay were used as inoculums for growth assay. Each well contained 1×10^3 cells/ml in the final volume of 200 µl RPMI-1640 medium. Cells without any pre- exposure of anticancer drugs were used as inoculum for the control. Micro plates were incubated at 35°C for 48 h and absorbance was read at 620 nm using microplate reader (Multiskan EX, Thermo Electron Corp. USA). The lowest concentration of a drug which caused > 50 % reduction in the absorbance compared to that of control was considered as Minimum Inhibitory Concentration (MIC). MICs after fluconazole susceptibility in treatment group (that is with prior exposure to anticancer drugs and control group (without prior exposure) were compared.

Statistical analysis

Experiments were repeated three times and the values mentioned are mean with standard deviations. Values of the percentage growth inhibited by fluconazole after exposure to a drug and that of in the control (that is without exposure to the drug), were compared by using Student's 't' test. For each group representing individual drugs, a 'P' value < 0.05 was considered significant.

RESULTS

Paclitaxel and docetaxel were the most efficient sensitizers among anti- microtubule agents

Among the four antimicrotubule anticancer agents, paclitaxel was the most efficient to sensitize *C. albicans*, so that fluconazole susceptibility increased significantly ($p < 0.05$). Exposure to sub- MIC concentration of paclitaxel was found to lower MIC of fluconazole from 1 to 0.062 µg/ml. Docetaxel followed an effect similar to that of paclitaxel and decreased the fluconazole sensitivity by eight folds to obtain MIC at 0.125 µg/ml with ($p < 0.05$).

Table 1. List of thirty anticancer drugs used in this study. Drugs belonged to twelve different classes of anticancer agents. Details on manufacturers and their brand names are mentioned.

Cancer Drug	Class of the anticancer agent	Generic name	Brand name
Docetaxel	Antimicrotubule Agents	Docet	Samarth Pharma, India
Paclitaxel		Paclistar	Lupin Oncology, India
Vinblastine		Cytoblastin	Cipla, India
Vincristine		Cytocristin	Cipla, India
Bleomycin	Antitumor Antibiotic	Bleocip	Cadila, India
Doxorubicin		Cadria	Cadila, India
Daunorubicin		Daunocin	Cytocare, India
5-flurouracil		Florac	Cadila, India
Mitoxantrone		Mitozan	Cytocare, India
Mitomycin-C		Mitocin	Cadila, India
Epirubicin		Alrubicin	Alkem, India
Dactinomycin		Dacmozen	Cytocare, India
Busulfan	Alkalyting Agent	Myran	Cytogenx , India
Carmustine		Mustine	Knoll, USA
Cyclo-phosphamide		Cycram	Cytogenx , India
Ifosfamide		Isoxan	Cytocare, India
Melphalan		Alkeran	Glaxo Smith Kline
Gemcitabine	Antimetabolite	Gemspera	Oncospera, India
Hydroxyurea		Alttrex	Cytogenx , India
Methotrexate		Cytrosar	Cytocare, India
Carboplatin	Platinum analogue	Carbokem	Alkem, India
Cisplatin		Cytoplati	Cipla, India
Oxaliplatin		Oplatin	United Biotech, India
Leucovorine	Reduced Folate	Fastivorin	Alkem, India
Tamoxifen	Antiestrogen	Tamifen	Alkem, India
Formestane	Aromatase Inhibitor	Lentaron IM Depot	Novartis, India
Etoposide	Epipodo-phyllotoxin	Eside	Cytogenx, India
Leuprolide	LHRH Agonist	Leuprofact	Zydus, India
Dacarbazine	Nonclassic Alkalyting Agent	Dacarin	Cytogenx , India
Irinotecan	Topoisomerase Inhibitor	Irinotel	Dabur, India

Treatment with vinblastine resulted in lowering of fluconazole MIC by four fold, while vincristine exposure did not alter the sensitivity significantly (Table 2; Figure 1).

5-Flurouracil the most effective anticancer agent to modulate fluconazole susceptibility of *C. albicans*

Efficacy of anti- tumor antibiotics to potentiate susceptibility to fluconazole varied over a broad range. 5- Flurouracil showed the highest that is sixty-four fold decreases in MIC of fluconazole, followed by doxorubicin and mitoxantrone with sixteen folds ($p < 0.01$) and daunorubicin with 8 fold increase in susceptibility to fluconazole. Remaining of the antitumor molecules did not show significant effect on the MIC of fluconazole as indicated by $p > 0.05$ (Table 2; Figure 2).

Two of the alkalyting agents efficiently sensitized the *C. albicans* cells to fluconazole

Treatment of *C. albicans* cells to busulfan and cyclophosphamide resulted in 16 fold decrease in the fluconazole susceptibility with a significant p value of 0.01. Melphalan and carmustine exposure brought down the fluconazole MIC from 1 to 0.25 µg/ml ($p < 0.05$), while ifosamide treatment exerted no change (Table 2; Figure 3).

Table 2. Fluconazole MICs for growth of C. albicans ATCC 90028, after exposure to thirty drugs from twelve classes of anticancer agents. Change in fluconazole MICs of the cells exposed to various anticancer drugs was indicated in terms of fold increase in fluconazole sensitivity compared to that of unexposed cells.

Class	Cancer Drug	Fluconazole MIC(µg/ml) after exposure to cancer drugs	Fold increase in fluconazole sensitivity
Anti-microtubule agents	Docetaxel	0.125	8*
	Paclitaxel	0.062	16**
	Vinblastine	0.25	4*
	Vincristine	0.5	2
Antitumor antibiotics	Bleomycin	0.5	2
	Doxorubicin	0.062	16**
	Daunorubicin	0.125	8*
	5-flurouracil	0.0156	64**
	Mitoxantrone	0.062	16**
	Mitomycin-C	0.50	2
	Epirubicin	1	0
	Dactinomycin	0.5	2
Alkalyting agents	Busulfan	0.062	16**
	Carmustine	0.25	4*
	Cyclo-phosphamide	0.062	16**
	Ifosfamide	1	0
	Melphalan	0.25	4*
Antimetabolites	Gemcitabine	1	0
	Hydroxyurea	0.5	2
	Methotrexate	0.062	16**
Platinum analogs	Carboplatin	0.062	16**
	Cisplatin	0.125	8*
	Oxaliplatin	0.50	2
Reduced folate	Leucovorine	1	0
Antiestrogens	Tamoxifen	0.25	4*
Aromatase inhibitor	Formestane	0.25	4*
Epipodo-phyllotoxins	Etoposide	0.062	16**
LHRH Agonist	Leuprolide	0.25	4*
Non classis alkalyting agents	Dacarbazine	0.25	4*
Topoisomerase inhibitor	Irinotecan	4	-

Significant results were indicated by * $p < 0.05$, while ** stands for $p < 0.01$.

Methotrexate exhibited ability to sensitize *C. albicans*

Among the three anti-metabolites, methotrexate was the most potential one. Exposure of *C. albicans* cells to sub-inhibitory concentrations of methotrexate lowered the MIC of fluconazole by sixteen fold ($p < 0.01$). Two other anti-metabolites anticancer agents, gemcitabine and hydroxyurea were not very effective in this regard and no significant change in the fluconazole MIC could be observed (Table 2; Figure 4).

Exposure to platinum analogs showed their prominent effect

Prior exposure of *C. albicans* to three platinum analogs enhanced the susceptibility of *C. albicans* to fluconazole. Carboplatin exposure altered the fluconazole susceptibility, making it sixteen times more sensitive ($p < 0.01$). Cisplatin lowered the MIC of fluconazole to 0.125 µg/ml followed by oxaliplatin MIC at 0.5 µg/ml (Table 2; Figure 5).

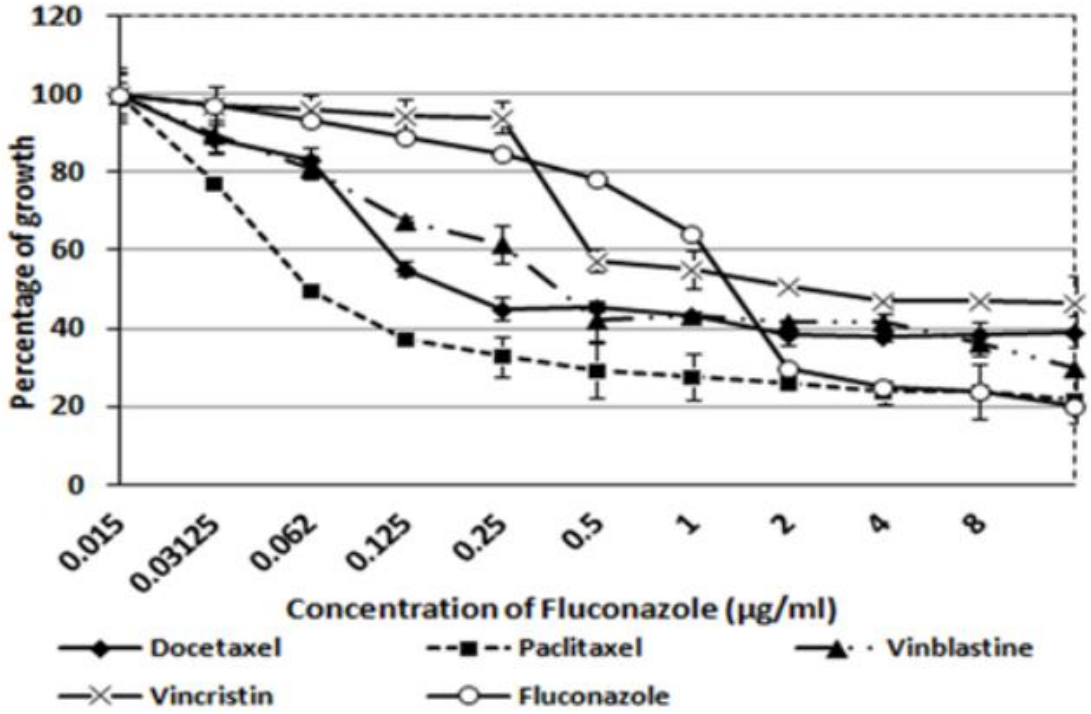

Figure 1. Growth of *C. albicans* ATCC 90028 in presence of fluconazole, after a short term exposure to anticancer anti-microtubule agents. Note down the difference in percentage of growth compared to the unexposed cells.

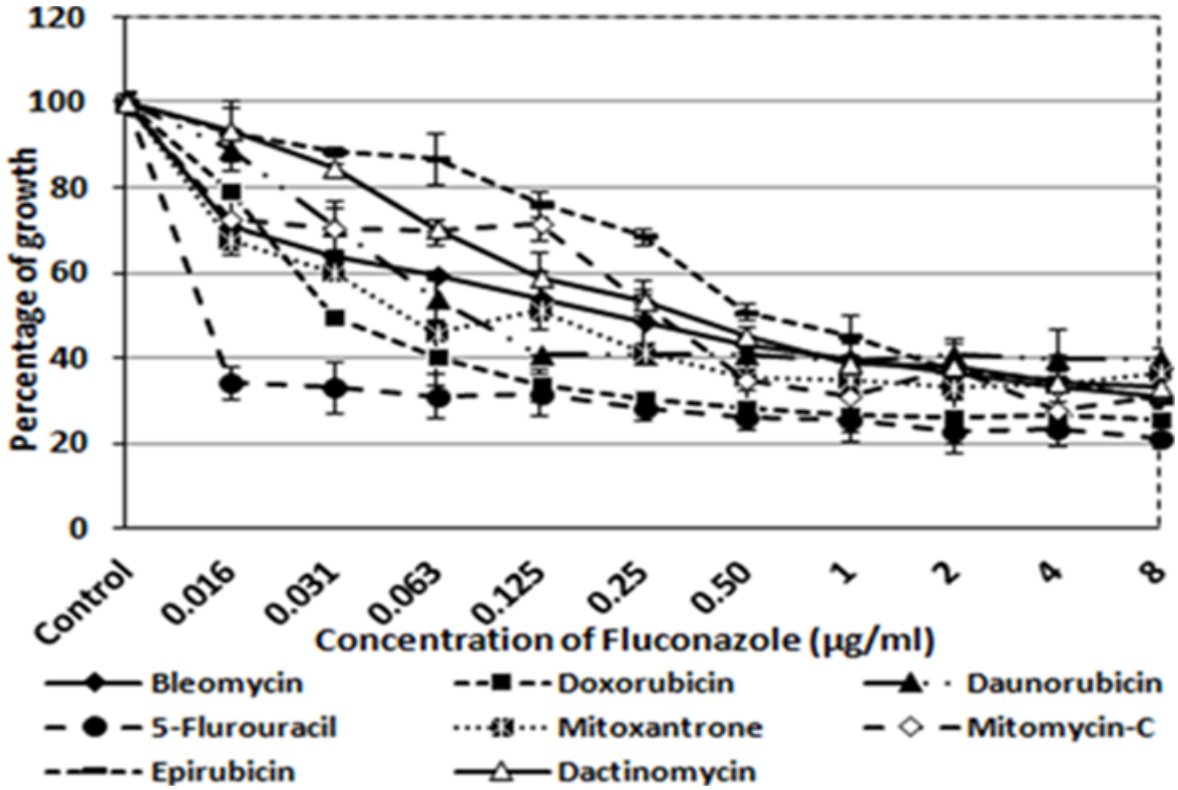

Figure 2. Fluconazole sensitivity of *C. albicans* ATCC 90028, exposed to various antitumor antibiotics.

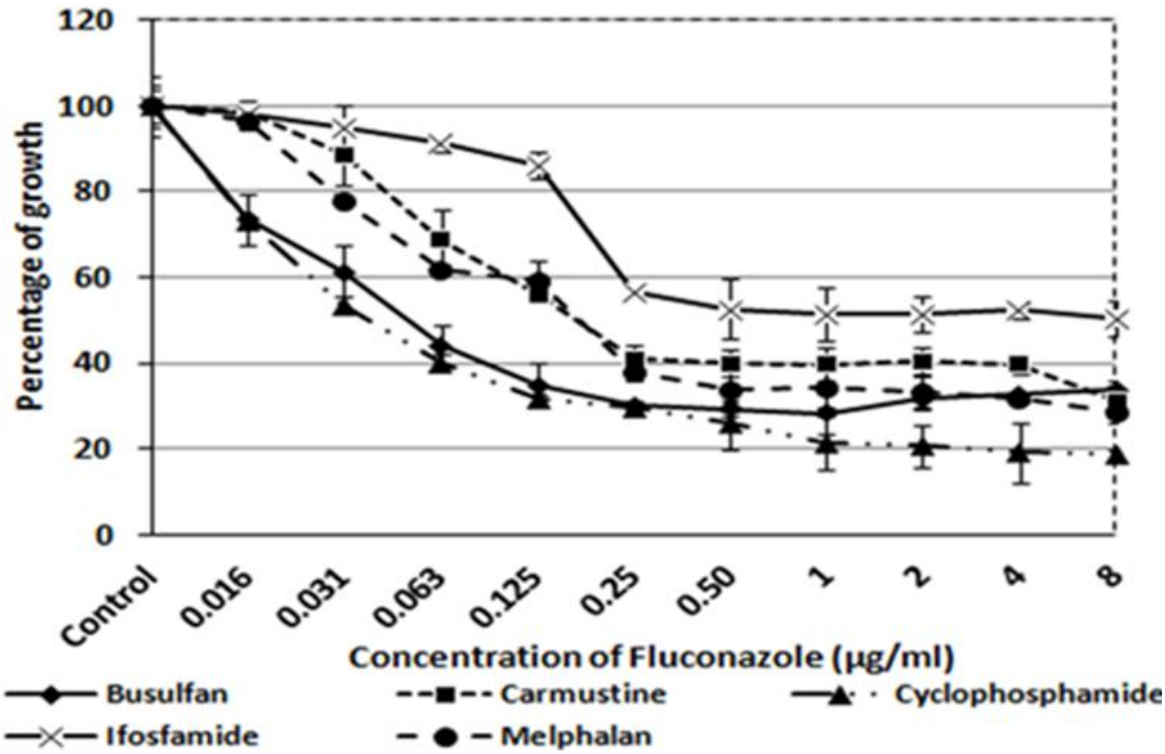

Figure 3. Growth of *C. albicans* in fluconazole containing medium after exposure to selected alkalyting anticancer drugs.

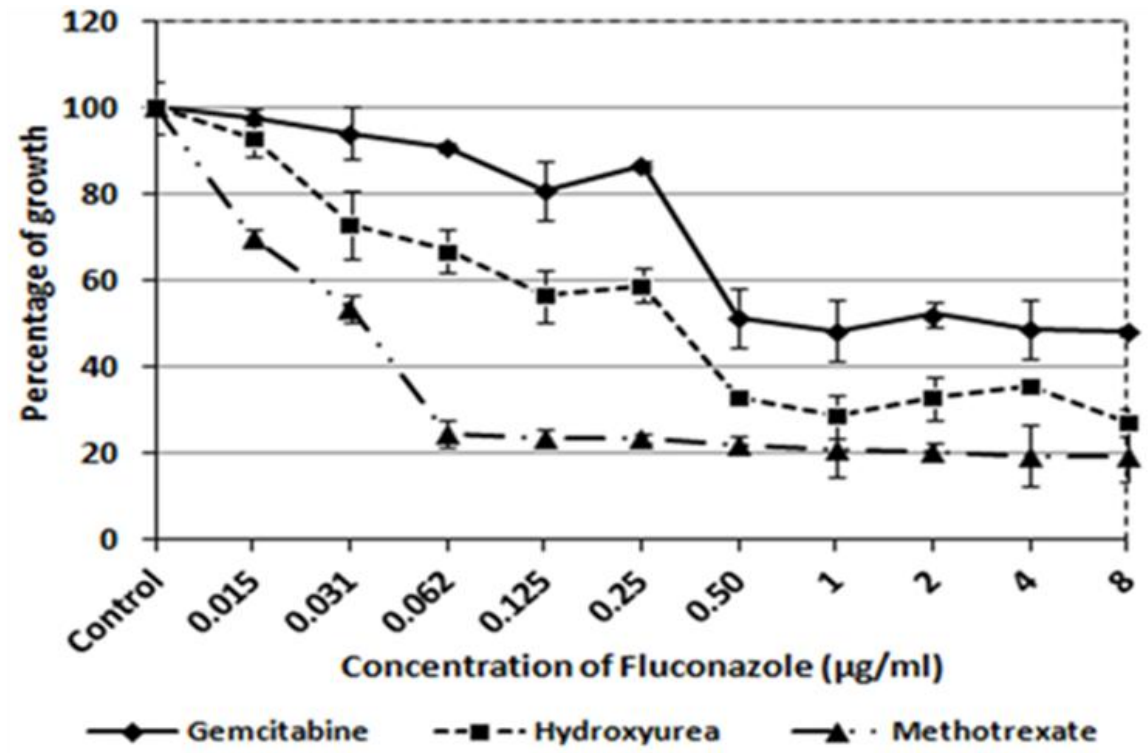

Figure 4. Fluconazole susceptibility of *C. albicans* ATCC 90028 cells, sensitized by various anticancer anti-metabolites.

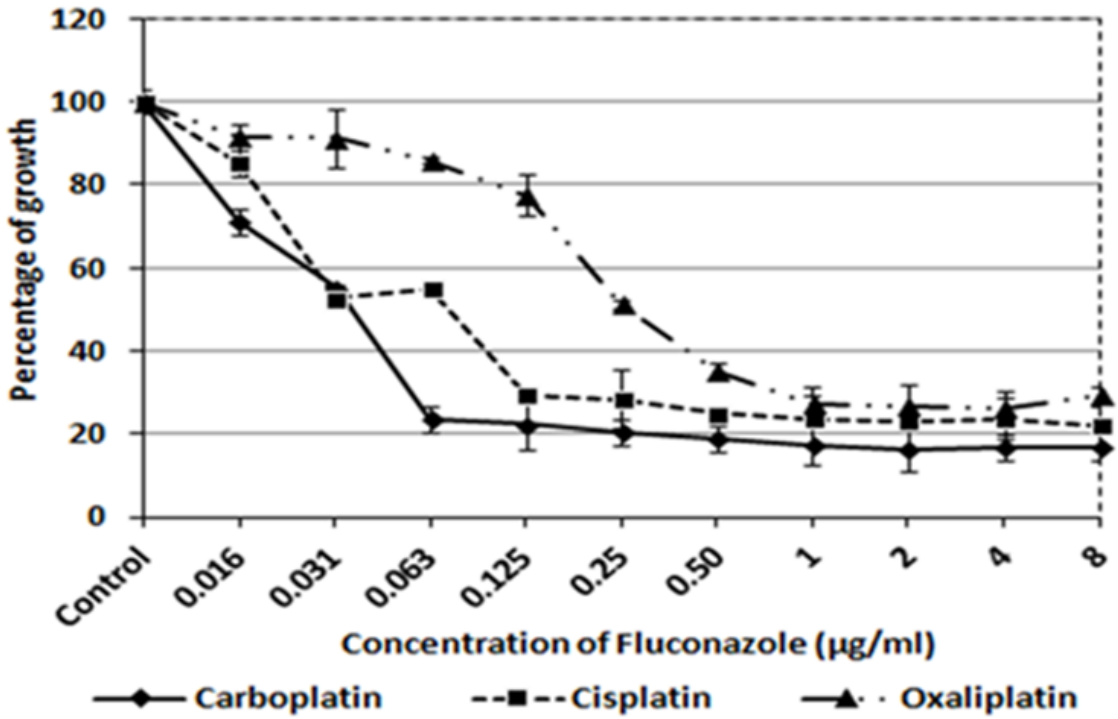

Figure 5. Sensitization of *C. albicans* to fluconazole after prior exposure to different antineoplastic platinum analogs.

Efficiency of other anticancer agents

Etoposide (an epipodophylotoxin anticancer drug), significantly ($p < 0.01$) increased the *C. albicans* susceptibility to fluconazole. MIC of fluconazole decreased from 1 to 0.62 µg/ml. Four of the drugs tamoxifen, aromatase inhibitor formestane, non-classic alkalyting anticancer agent, dacarbazine and leuprolide (LHRH agonist) altered the fluconazole sensitivity to make it four times efficient with a significant $p < 0.05$ value, while leucovorine had no effect. Interestingly, irinotecan which is an active topoisomarase I inhibitor exhibited an effect totally different from rest of the twenty nine drugs used in this study. Exposure to irinotecan increased fluconazole concentration required to inhibit *C. albicans* growth by four times compared to that of MIC for unexposed cells (Table 2; Figure 6).

DISCUSSION

In this study, effect of anticancer drug exposure on the fluconazole sensitivity of *C. albicans* was analysed. Pre-exposure for short time to many of the selected drugs sensitized *C. albicans* cells to antifungal activity of fluconazole. Out of the thirty anticancer drugs belonging to twelve different classes, antitumor antibiotic 5- flurouracil was the most effective sensitizer ($p < 0.01$) of fluconazole. A known antifungal agent flucytosine, when crosses the fungal cell wall and membrane, gets converted by cytosine deaminase to 5-flurouracil. It is then incorporated into fungal RNA in place of uracil to block the protein synthesis (Polak and Scholer, 1975). The exact reason behind strong sensitization after exposure to this drug is not very clear. Eight anticancer molecules - paclitaxel, doxorubicin, mitoxantrone, cyclo-phosphamide, methotrexate, and carboplatin had potential to lower fluconazole MIC by sixteen fold; so that it comes down to 0.062 µg/ml. Whereas, three cancer drugs- docetaxel, daunorubicin and cisplatin caused eight fold increase in the antifungal susceptibility. This was found to be a significant ($p < 0.05$) decrease in fluconazole MIC. Effective molecules belonged to six different classes, indicating that activity was not specific to a single class. Fourteen of the selected drugs enhanced the fluconazole sensitivity of *C. albicans* by two to four folds, with significant p value of 0.05. Remaining of the anticancer agents exerted no activity, while irinotecan induced the negative effect to increase the MIC of fluconazole by four fold that is at 4 µg/ml. Being eukaryotes, yeast and mammalian cells exhibit similarities in basic cellular machinery and mechanisms. In many instances, *Saccharomyces cerevisiae* have provided invaluable insights into the actions of a diverse array of anticancer agents like topoisomerase inhibitors, microtubule aggregation and disaggregation inhibitors, immunosuppressants which block T-lymphocyte function, phosphatidylinositol kinase inhibitors, and steroid receptor antagonists (Cardenas et al., 1999; Hartwell et al., 1997; Shen et al., 1992). Challenges in the development of antifungal drugs and anticancer agents are more

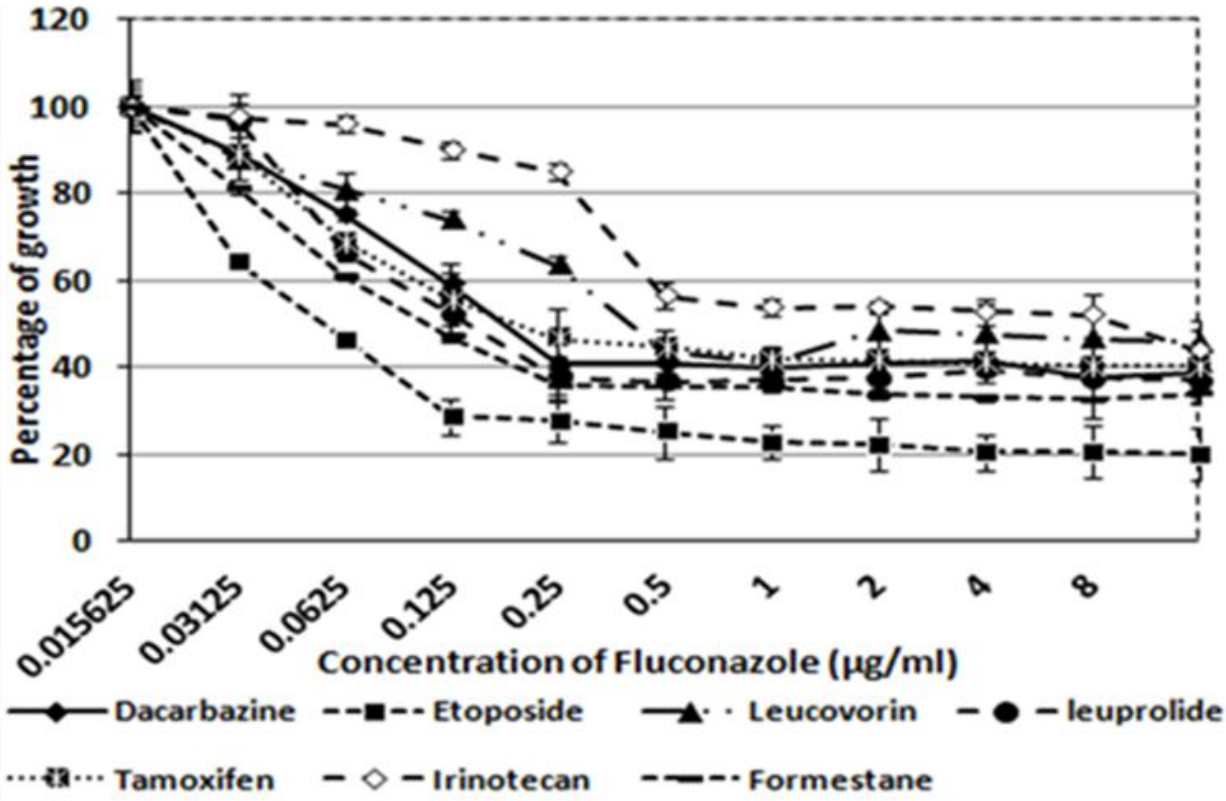

Figure 6. Exposure to selected anticancer drugs results in variation in fluconazole sensitivity of *C. albicans* ATCC 90028.

or less similar. For example, problem of drug resistance due to over expression of efflux pumps is identical in antifungal therapy and cancer chemotherapy. Several membrane pumps have been elucidated including Mdr1p and Mdr3p (Multidrug Resistance Pumps) in humans and Cdr1p (Candida Drug Resistance) in pathogenic yeast *C. albicans*. Genes *CDR1* and *CDR2* are homologous to the transmembrane human P-glycoprotein encoded by *MDR1* (Akins et al., 2005). There is a possibility that cancer drugs may inhibit *Candida* drug efflux pumps causing more accumulation of fluconazole inside the *Candida* cells and thus sensitizing them. Anticancer agents with various cellular targets may have unexplored bioactivities that results in sensitization of *C. albicans* towards fluconazole (Cardenas et al., 1999). Our study, for the first time reveals efficacy of the thirty anticancer drugs to act as sensitizers in *C. albicans*. As the anticancer drugs have strong sensitizing ability in *C. albicans*, the dose of fluconazole given for treatment of candidiasis in cancer patients could be reduced. Information obtained in this *in vitro* study may help to design the antifungal drug regimens in cancer patients experiencing *Candida* infections. However, various predisposing factors like drug interactions, immunological interactions and physiological state of the patients may modulate these effects. Hence, further studies with clinical isolates of *C. albicans* from cancer patients undergoing cancer chemotherapy as well as *in vivo* experiments may be rewarding.

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REFERENCES

Akins RA (2005). An update on antifungal targets and mechanisms of resistance in *Candida albicans*. Med. Mycol. 43:285-318.

Bensadoun RJ, Patton LL, Lalla RV, Epstein JB (2011). Oropharyngeal candidiasis in head and neck cancer patients treated with radiation: update 2011. Support. Care Cancer. 19:737–744.

Bulatova NR, Darwish RM (2008). Effect of chemosensitizers on minimum inhibitory concentrations of fluconazole in *Candida albicans*. Med. Principles Pract. 17:117–121.

Campbell BC, Chan KL, Kim JH (2012). Chemosensitization as a means to augment commercial antifungal agents. Front. in Microbiol. 3:doi:10,3389/fmicb.2012.00079.

Cardenas ME, Cruz MC, Del Poeta M, Chung N, Perfect JR, Heitman J (1999). Antifungal activities of antineoplastic agents: *Saccharomyces cerevisiae* as a model system to study drug action. Clin. Microbiol. Rev. 12:583–611.

Cornely OA, Maertens J, Winston DJ, Perfect J, Ulmann AJ, Walsh TJ, et al. (2007). Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. New Eng. J. Med. 356:348–359.

Davies A, Brailsford S, Broadley K, Beighton D (2000). Resistance amongst yeasts isolated from the oral cavities of patients with advanced cancer. Palliat. Med. 16:527–531.

Dolan K, Montgomery S, Buchheit B, DiDone L, Wellington M, Krysan D (2009). Antifungal activity of tamoxifen: *In vitro* and *in vivo* activities and mechanistic characterization. Antimicrob. Agents Chemother. 53:3337–3346.

Ghannoum MA, Abu-Elteen KH, Motaway MS, Abu-Hatab MA, Ibrahim AS, Criddle RS (1990). Combinations of antifungal and antineoplastic drugs with interactive effects on inhibition of yeast growth. Chemother. 36:308–320.

Ghannoum MA, Motaway MS, Abu-Hatab MA, Abu-Elteen KH, Criddle RS (1989). Interactive effect of antifungal and antineoplastic agents on yeasts commonly prevalent in cancer patients. Antimicrob. Agents Chemother. 33:726–730.

Harnicar S, Adel N, Jurcic J (2009). Modification of vincristine dosing during concomitant azole therapy in adult acute lymphoblastic leukemia patients. J. Oncol. Pharm. Pract. 15:175–182.

Hartwell LH, Szankasi P, Roberts CJ, Murray AW, Friend SH (1997). Integrating genetic approaches into the discovery of anticancer drugs. Science. 251:1064–1068.

Johansen HK, Gotzsche PC (2004). Amphotericin B versus fluconazole for controlling fungal infections in neutropenic cancer patients (Cochrane Review). In: The Cochrane Library, Issue 2, Chichester, UK: John Wiley & Sons, Ltd.

Kesavan C, Joyee AG (2005). 5-Fluorouracil altered morphology and inhibited growth of *C. albicans*. J. Clin. Microbiol. 43:6215–6216.

Kesavan C, Raghunathan M, Ganesan N (2005). Morphological and growth altering effects of cisplatin in *C. albicans* using fluorescence microscopy. Ann. Clin. Microbiol. Antimicrob. 4:1–4.

Lalla RV, Latortue MC, Hong CH, Ariyawardana A, D'Amato-Palumbo S, Fischer DJ, Martof A, Nicolatou-Galitis O, Patton LL, Eltinng SS, Spijerkvet FKL, Bernnan MT (2010). A systematic review of oral fungal infections in patients receiving cancer therapy. Support. Care Cancer. 18:985–992.

O'Keeffe J, Doyle S, Kavanagh K (2003).Exposure of the yeast *Candida albicans* to the anti-neoplastic agent adriamycin increases the tolerance to amphotericin B. J. Pharm. Pharmacol. 55:1-5.

Polak A, Scholer (1975). Mode of action of 5-fluorocytosine and mechanisms of resistance. Chemother. 21:113–130.

Raut JS, Shinde RB, Karuppayil SM (2012). Indole, a bacterial signaling molecule, exhibits inhibitory activity against growth, dimorphism and biofilm formation in *Candida albicans*. Afr. J. Microbiol. Res. 6:6005–6012.

Routh MM, Raut JS, Karuppayil SM (2011). Dual properties of anti-cancer agents: An exploratory study on the *in vitro* anti-*Candida* properties of thirty drugs. Chemother. 57:372–380.

Safdar A, Armstrong D (2002). Prospective evaluation of *Candida* species colonization in hospitalized cancer patients: impact on short term survival in recipients of marrow transplantation and patients with hematological malignancies. Bone Marrow Transplant. 30:931–935.

Safdar A, Chaturvedi V, Cross EW, Park S, Bernard EM, Armstrong D, et al (2001). Prospective study of *Candida* species in patients at a comprehensive cancer center. Antimicrob. Agents Chemother. 45:2129–2133.

Shen LL, Baranowski J, Fostel J, Montgomery DA, Lartey PA (1992). DNA topoisomerases from pathogenic fungi: targets for the discovery of antifungal drugs. Antimicrob. Agents Chemother. 36:2778–2784.

Shinde RB, Raut JS, Karuppayil SM (2012). Biofilm formation by *Candida albicans* on various prosthetic materials and its fluconazole sensitivity: A kinetic study. Mycoscience. 53:220–226.

Winston DJ, Hathorn JW, Schuster MG, Schiller GJ, Territo MC (2000). A multicenter, randomized trial of fluconazole versus amphotericin B for empiric antifungal therapy of febrile neutropenic patients with cancer. Am. J. Med. 108:282–289.

Yu DT, Seger DL, Peterson JF, Kumar RN, Bates DW (2006). Fluconazole for empiric antifungal therapy in cancer patients with fever neutropenia. BMC Inf. Dis. 6:1–10.