ARTICLE

IN-VITRO ASSESSMENT OF ANTIFUNGAL EFFECT OF IBUPROFEN COMBINED WITH TERBINAFINE ON CANDIDA SPP

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ABSTRACT

Background: *One hundred and twenty-five throat swab samples were collected from the patient suffering from various disease conditions such as AIDS, cancer, diabetic and old age people. The healthy adult with no reported diseases were categories as control. Various species of Candida were identified using Gram staining, biochemical test and for the different species MIC was performed. The Candida species were also treated with ibuprofen as the cell wall integrity was affected because of it resulting in spheroplast. With the influence of the drug, minimum inhibitory concentration was estimated with Terbinafine that showed even in minor concentration of antifungal effect showed effective activity on the mortality of the Candida spp.* ***Methods:*** *The samples were screened for the presence of the Candida using plating. The organisms were confirmed using biochemical and Carbohydrate assimilation and fermentation test the organism were further tested with MIC with Terbinafine.* ***Results:*** *The Candida spp. cell were treated with the Terbinafine and effective MIC was shown, but when treated with ibuprofen the efficiency increased in the MIC results.* ***Conclusions:*** *When the Candida spp. cells were treated with Ibuprofen, the cell wall started to disintegrate that enabled the efficiency of the drug.*

KEY WORDS
Candida, Terbinafine, Ibuprofen, combined effect, spheroplast

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INTRODUCTION

Fungi had emerged as a cause of serious opportunistic infection among immuno-compromised and other critically ill patients, deep-seeded fungal infection were being recognized increasing frequency. *Candida* was recognized as one of the most frequent causes of opportunistic infections [1]. Ibuprofen was the first member of this class to be introduced in 1969 as a better tolerant alternative to aspirin [2,3,4]. They were analgesic, anti-steroidal, antipyretic and inflammatory efficacy is rated somewhat lower than high dose of aspirin which exhibit antimicrobial activity against *C.albicans* and non-albicans strains. Taking advantage of the drug's antifungal and inflammatory properties, the use of Ibuprofen along or in combination with Terbinafine [5] in the treatment of candidosis, particularly when applied topically,

In the present study attempt was carried out to isolate and identify the prevalence of *Candida* species among from different immune status people. Antifungal activity [6] was also studied by macro tube dilution method using Ibuprofen and Terbinafine.

MATERIALS AND METHODS

Selection of patients

Patients with AIDS [7], Cancer, diabetic, old age and adult with healthy medical record were acting as control group.

Sample collection

One hundred and twenty-five throat swab samples were collected with sterile wet cotton swabs (Hi-media Laboratories) that was moist by saline, the swabs were wiped over the thrust [8] under the supervision of medical practitioners with the written consent of the patients. The swabs were swabbed on the slant of SDA incorporated with Streptomycin. The samples were transported safely from the hospital/clinic to the laboratory where it was processed within 4 days.

Sample processing

SDA were prepared in Petri plates and the swabs were inoculated, the plates were incubated for 2 to 4 days at room temperature (28^{o}C).

Microscopic Observation
Gram staining

Kopeloff's & Beerman's modification of Gram staining was used to analyze morphological features of the *Candida* spp. cells.

Germ tube method

An isolated colony was touched and inoculated in human serum, which was incubated at 37^{o}C for 4 hrs. From that one loopful of inoculum was placed on the grease free slide and coverslip was placed over it and observed under 40 X magnification.

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Chlamydospore production

In Corn Meal Agar (CMA) plates, *Candida* spp. colony was inoculated into 3 parallel cuts made on agar. A cover slip was placed over it. Incubated for 18 to 48 hours. After incubation, the plates were examined through the coverslip under the microscope.

Carbohydrate fermentation test

An aqueous suspension of *Candida* spp. cells was prepared in saline, not to exceed the density of McFarland No.1 standard. 0.1 ml of this suspension was added to each of the fermentation broth tubes containing 1% peptone, 2 % sugar (Dextrose, Maltose, Lactose, and Sucrose) with bromo thymol blue indicator containing Durham's tube to detect gas production. The tubes were incubated for 10 – 14 days at 30^{o}C and observed for acid and gas production.

Carbohydrate assimilation test

Candida spp. suspension was prepared in saline equivalent to density of McFarland No.4 Standard. Using sterile swab the surface of medium was swabbed with *Candida* spp. suspension. Filter paper disc – prepared from Whatmann No.1 was impregnated with 20% solution of sugar (Dextrose, Maltose, Lactose, Xylose, Sucrose, Cellobiose) was placed at certain distance on the medium. Plates were incubated for 2 to 4 days. Growth around each sugar indicated assimilation

Antibiogram [Broth Macro Dilution Antifungal susceptibility testing of *Candida* spp. (CLSI, USA –M 27A)]

Macro broth dilution Antifungal test (CLSI – M27A) was intended for testing *Candida* spp. that causes invasive infection. Antifungal agents (Terbinafine) which was purchased from Sigma and stored as manufacturer's recommendation [9].

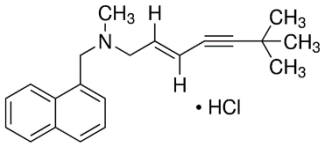

Fig.1: Molecular diagram of Terbinafine (trans-N-(6,6-Dimethyl-2-hepten-4-ylyl)-N-methyl-1-naphthylmethylamine hydrochloride).

The amount of powder or dilution need for the standard solution may be calculated by the following formula.

Volume (ml)=(Weight (mg) x Assay Potency (µg/µg))/(Concentration)

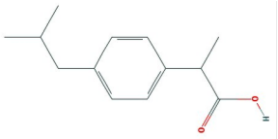

Fig. 2: Molecular diagram of Ibuprofen (2-(4-Isobutylphenyl)propanoic acid).

Ibuprofen was purchased from Cipla Pharmaceuticals Ltd. Mumbai. Ibuprofen (iso butry phyenl propionic acid) was diluted and the stock was prepared by ethanol. The diluent used was RPMI broth. Medium used was RPMI – 1640 (with glutamine, without bicarbonate and with pH indicator)

Inoculum preparation

Five colonies picked up from 72-hour culture, were suspended in 5 ml of sterile saline. The suspension was adjusted with McFarland No.0.5 used as stock. The MIC is the lowest concentration of an antifungal that substantially inhibit the growth of the organisms as detected visually [10].
Terbinafine - Sensitivity < 8 µg/ml; Intermediate 16 – 32 µg/ml; Resistance > 64 µg/ml

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Statistical analysis

The analysis was performed using SPSS version 17 software. P values < 0.05 were considered statistically significant [11].

RESULTS

The isolated organisms were categorized as [Fig. 3], that elucidate the distribution of different species of the *Candida*.
The identified organisms were used for performing MIC using Terbinafine, Ibuprofen and with combination.

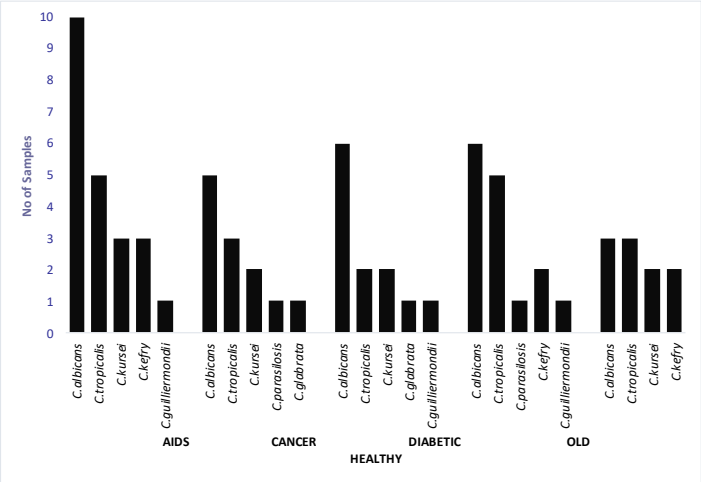

Fig. 3: Distribution of *Candida* spp. among different immune status patients.

ANTIFUNGAL SUSCEPTIABILTIY TEST

Table 1: MIC of terbinafine

Candida species	No. isolated	MIC of Terbinafine in µg/ml								
		128	64	32	16	8	4	2	1	0.5
C.albicans	30	4	5	7	5	4	2	2	1	-
C.tropicalis	19	-	2	2	6	8	-	1	-	-
C.kefyr	9	-	-	-	-	6	-	2	1	-
C.krusei	7	-	3	3	-	-	1	-	-	-
C.parapsilosis	2	-	-	2	-	-	-	-	-	-
C.guilliermondii	2	-	-	-	-	-	-	1	1	-
C.glabarata	2	-	2	-	-	-	-	-	-	-

The antifungal activity was performed on all the *Candida* species isolated from different groups of people showed the organisms were having effect on the Terbinafine at different concentration [Table 1].

Table 2: MIC of Ibuprofen

Candida species	No. isolated	MIC of Terbinafine in mg/ml								
		10	5	2.5	1.25	0.625	0.313	0.17	0.078	0.039
C.albicans	30	-	2	5	6	9	5	3	-	-
C.tropicalis	19	-	-	5	6	6	3	-	-	-
C.kefyr	9	-	1	2	2	3	1	-	-	-
C.krusei	7	-	1	2	2	2	-	-	-	-
C.parapsilosis	2	-	-	2	-	-	-	-	-	-
C.guilliermondii	2	-	-	2	-	-	-	-	-	-
C.glabarata	2	-	1	1	-	-	-	-	-	-

The effect of Ibuprofen showed that the organism became insubstantial cell wall that enhances the penetrability of the other antifungal drug leading to the destruction of the organism that was proved in the combination of the ibuprofen and terbinafine [Table 2].

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Table 3: MIC of terbinafine combination of ibuprofen

Candida species	No. isolated	MIC of Terbinafine in µg/ml								
		64	32	16	8	4	2	1	0.5	0.25
C.albicans	30	4	2	4	5	7	4	4	-	-
C.tropicalis	19	3	2	4	2	2	2	2	2	-
C.kefyr	9	-	1	2	1	1	3	1	-	-
C.krusei	7	1	2	2	-	1	1	-	-	-
C.parapsilosis	2	-	1	1	-	-	1	-	-	-
C.guilliermondii	2	-	1	1	-	-	-	-	-	-
C.glabarata	2	2	-	-	-	-	-	-	-	-

Table 4: MIC of Ibuprofen combination of Terbinafine

Candida species	No. isolated	MIC of Terbinafine in mg/ml								
		5	2.5	1.25	0.625	0.313	0.15	0.078	0.03	0.01
C.albicans	30	-	-	4	11	9	5	1	-	-
C.tropicalis	19	-	-	2	8	3	4	2	-	-
C.kefyr	9	-	-	4	4	3	1	-	-	-
C.krusei	7	-	1	1	3	-	2	-	-	-
C.parapsilosis	2	-	-	-	2	-	-	-	-	-
C.guilliermondii	2	-	-	-	1	1	-	-	-	-
C.glabarata	2	-	-	-	1	1	-	-	-	-

Tugut et al., [12], Al-Athel et al., [13] and Alves Izabel Almeida et al.,[14] reported the effect of different drug that has an effect on *Candida* growth and enhance the effect of antifungal activity. Comparing the pervious result the significance of the combination of two different drugs had more influence on the control of *Candida*.

CONCLUSION

One hundred and twenty-five samples were collected from different categories of patients and healthy individuals to analysis the presence of *Candida* species. In AIDS patients, total number of positive samples was 88% in which *C. albicans* was about 45% followed by *C. tropicalis* – 23%. In Cancer patients, total number of positive samples was 48% in which *C. albicans* was about 41% followed by *C. tropicalis* – 25%. In Diabetic patients, total number of positive samples was 52% in which *C. albicans* was about 47% followed by *C. tropicalis* – 23%. In Old aged, total number of positive samples was 60% in which *C.albicans* was about 45% followed by *C. tropicalis* – 33%. In healthy persons, total positive sample was about 40%. Other strains isolated from the above categories were *C. krusei, C. kefyr, C. parapsilosis, C. guilliermondii* and *C. glabrata*. Minimum Inhibitor concentration (Macro dilution) done for Terbinafine, Ibuprofen alone and in combination with different strains of *Candida* reveal that they were highly susceptible when ibuprofen was diluted in the constant amount of Terbinafine when compared with that of Ibuprofen alone. From the above experiments, it proves that Ibuprofen does not inhibit the activity of Terbinafine, in turn induces the effect over *Candida* species.

CONFLICT OF INTEREST
Authors declare no conflict of interest

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None

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