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Synergistic Antifungal Activity of Bioactive Phytochemical in Combination with Standard Antifungal Drugs

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Abstract:

The research work was mainly designed to determine the antifungal activity of curcumin, a potent bioactive phytoconstituent, obtained from Curcuma longa and to explore the possibilities of its use as a combination with commercially available synthetic antifungal drugs for better therapeutic actions. Fluconazole and itraconazole were used as a model drug. Different combinations of curcumin with fluconazole as well as itraconazole was prepared and subjected to an antifungal screening study. The antifungal screening was carried out using Candida albicans fungal strain. The MIC of fluconazole, itraconazole and curcumin was found to be in the range of 32 µg/ml to 64 µg/ml, 8 µg/ml to 32µg/ml and 64 µg/ml to 256 µg/ml respectively. Further, the results of the in-vitro antifungal study performed based on the comparative zone of inhibition measurement of the prepared combination at a concentration of 10 µg/ml were determined. The result of the study indicates that the presence of curcumin significantly increases the antifungal capacity of both fluconazole and itraconazole. The Fractional inhibitory concentration index was measured, and the data thus obtained states that the increased antifungal activity may be observed due to synergistic or additive effects. Further, the topical sensitivity of the optimized combinations was determined using rabbit vaginal model and were found to be free from any major sign of sensitivity as per as histopathological study concern.

Keywords: FICI, Natural antifungal, Candida albican, Curcumin, Antifungal Combination.

Introduction:

The rate of superficial and invasive fungal infections has been dramatically increased over the last few decades1. The insufficient availability of side effect free antifungal molecules, Development of fungal resistance due to improper use of medication and slow discovery rate of new antifungal molecules may be considered as a major cause behind the crucial situation2. Therefore, the treatment management of fungal infections are becoming the most critical and challenging task.

The combination drug therapy are preferred over a single drug for the treatment of fungal infection, which, may also be considered as a suitable alternative for effective antifungal therapy3,4.

However, reported literature to indicate that the chances of side effect and toxicity may increase during combination therapy5-7. Since long, plant-derived bioactive or phytochemicals are successfully used either singly or in combination for effective treatment of several life treating infectious diseases8. Most of this bioactive compound are basically secondary metabolites like alkaloids, flavonoids, tannins, and terpenoids etc. and shows potent antimicrobial properties9. The literaturestates that bioactive phytochemicals obtained from plant sources may be considered as an attractive and effective prototype, which, facilitate the activity of existing antimicrobial agents9,10. The combination of bioactive phytochemicals with standard synthetic drugs may have several major advantages, including broad-spectrum antifungal action; lesser chances of development of drug resistance and required lesser concentration for effective therapeutic response11. Further, the side effect and toxicity individual drug can also be minimized12.

Material and Methods:

The drug samples such as fluconazole was received from Synergene India, Ahmadabad as a gift sample. Itraconazole was obtained as gift sample form alembic pharmaceuticals, Vadodara. Curcumin was purchased from Sigma-Aldrich Ltd. RPMI-1640 was purchased from HI-media ltd, Mumbai. The pathogenic fungal strain of Candida albicans was procured from MTCC Chandigarh.

In-vitro Antifungal Screening:

Preparation of Drug Solution:

Accurately 100 mg equivalent of standard drugs sample of Fluconazole, itraconazole and curcumin were respectively dissolved in 100 ml of hydroalcoholic solvent (alcohol: water =1:9) (Stock solution). The stock solution was then filtered and from that 10 ml of filtrate was pipetted out and diluted up to 100 ml with hydroalcoholic solvent (Standard solution). The final concentration of the standard solution was 100 µg/ml.13

Preparation of Inoculums:

At first, the Candida albicans (MTCC 227) strain procured from MTCC Chandigarh was cultured in Sabouraud dextrose agar media at 30°C for 48 hrs. After that, the standard inoculums of Candida albicans were prepared using freshly prepared culture (at list five colonies with more than 1mm diameter). The pathogenic cells were then suspended in 5 ml of sterile saline solution. The final cell suspension was vortexed for 30 sec and the turbidity was adjusted to 105–106 colony forming units (CFU))/ml as per McFarland standard14.

Determination of Minimum Inhibitory (MIC) by Disk Diffusion Method:

The Minimum inhibitory concentration of each antifungal agent either singly or in combinations was determined by disk diffusion method. At first YEPD medium was prepared under sterile conditions. The fleshly prepared hot medium was then cooled to room temperature and standard Candida albicans cell suspension was inoculated in the medium. The fungi inoculated medium was poured into the Petri dish and allowed to cool until it gets solidified. After that, all the drugs either alone or in combination with curcumin at different concentration were dappled on 6mm diameter sterile filter paper disk. Finally, all the prepared plates with an applied concentration of antifungal drugs were incubated at 30°C for 48 hours and the respective zone of inhibition at different concentration was recorded. Determination of Minimum inhibitory concentration (MIC) by Micro dilution method:

Minimum inhibitory concentrations (MICs) of fluconazole, itraconazole, and curcumin against the Candida albicans were determined by broth micro dilution method. The study was carried out in RPMI 1640 medium using two-fold serial dilutions, following the guideline of Clinical and Laboratory Standards Institute (CLSI) method M27-A. The study was performed in 96-well microtitrater plates. A 100µl aliquot of working cell suspension was placed into microtitre plate containing RPMI 1640 medium. Further, different concentrations of fluconazole, itraconazole, and curcumin, alone as well as in combination were placed vertically and horizontally into the plates. After agitation for 60 sec, the plates were incubated at 30°C for 48 hours without shaking. After 48 hours of incubation, optical density differences were measured at 492 nm. Experiments were performed in triplicate, and the average MIC value was calculated.13,14

Determination of Fractional Inhibitory Concentration Index (IFCI):

The interaction between curcumin and different concentration of fluconazole and itraconazole was evaluated by the checkerboard method as recommended by the NCCLS. The finding of the study can be calculated in terms of the fractional inhibitory concentration index (FICI). Pharmacological screening for tissue sensitivity and irritation,Among the several infections caused due to Candida albicans, oral candidiasis and vaginal candidiasis are most common. Therefore, vaginal candidiasis model was selected to determine the effectiveness of optimized drug combinations. The optimized combination was incorporated inside 1% Carbopol P940 gel base. The study was performed on New Zealand white female rabbit of around 2 -2.5kg body weight. All the animals were kept under standard laboratory conditions. The

total numbers of animals were divided into four batches, each batch containing six animals. Approximately 0.5gm of the prepared gels were applied daily, for 10 days, through a lubricated catheter into the vagina of rabbits17,18. The external genitalia area was observed regularly for any signs of redness, genital swelling, edema, erythema and discharge including bleeding as a reaction to exposure to the prepared formulations19,20. This study was performed as per "OECD guideline 402", which states that a clear evidence of skin irritation (for example, erythema or edema) in the dermal study could be considered as a sign of irritation potential instead of performing a specific irritation study. Further, the hematological study was also conducted to measure the major changes in essential parameters like (WBC, RBC, Hb level, lymphocytes counts, Platelets counts etc.). The experimental protocol of the study was approved by the institutional animal ethics committee (Regd. No. CIP / IAEC / 2013-14/044).

Results and Discussion:

The experiment was performed to determine the therapeutic effectiveness of curcumin against fungal infections mainly caused due to Candida albicans. The aim of the research work was further extended to find out the role of curcumin to improve the therapeutic activity of existing antifungal drugs when used in combination with standard antifungal drugs. The results of minimum inhibitory concentration (MIC), measured based on the zone of inhibition as well as through microdilution method, states that the MIC of fluconazole against the pathogenic strain of Candida albicans MTCC-227 was found within the range of 32µg/ml to 64µg/ml. Similarly, MIC of Itraconazole and curcumin was found in the range of 8 µg/ml to 32µg/ml and 64 µg/ml to 256 µg/ml respectively (Table no -01). The obtained result indicates that curcumin itself has a potent antifungal activity, which complies with reported literature.

Table No. 1: Determination Zone of Inhibition by Disk Diffusion Method

Curcumin		Itraconazole		Fluconazole	
Concentration (µg/ml)	Zone of inhibition (mm)	Concentration (µg/ml)	Zone of inhibition (mm)	Concentration (µg/ml)	Zone of inhibition (mm)
0.125	0	0.125	3	0.125	0
0.25	0	0.25	6	0.25	2
.5	2	.5	9	.5	3
1	4	1	11	1	6
2	6	2	15	2	10
4	9	4	18	4	13
8	12	8	29	8	18
16	14	16	42	16	23
32	19	32	45	32	34
64	23	64	46	64	42
128	39	128	47	128	43
256	49	-	-	-	-

Table No. 2: Determination Zone of Inhibition by Individual Durg/Combination of Durgs in Diferent Ratios at 10µg/ml Concentration

Combination code	Individual Drug/ Drug combination at different ratio	Zone of inhibition(mm)*
AFC1	Curcumin + Itraconzole (CUR:ITR=1:1 ratio)	39 ± 1.0
AFC2	Curcumin + Itraconzole (CUR:ITR=2:1 ratio)	42±2.0
AFC3	Curcumin + Itraconzole (CUR:ITR=3:1 ratio)	44± 1.5
AFC4	Itraconzole + Curcumin (ITR:CUR=2:1 ratio)	46± 2.0
AFC5	Itraconzole + Curcumin (ITR:CUR=3:1 ratio)	39± 2.0
AFC6	Curcumin + Fluconazole (CUR+FLU 1:1 ratio)	38 ± 1.0
AFC7	Curcumin + Fluconazole (CUR+ FLU 2:1 ratio)	36 ±1.5
AFC8	Fluconazole + Curcumin (CUR+ FLU 3:1 ratio)	39 ± 1.0
AFC9	Fluconazole + Curcumin (FLU+ CUR 2:1 ratio)	42 ± 2.5
AFC10	Fluconazole + Curcumin (FLU+ CUR 3:1 ratio)	44 ± 2.0
AFCUR	Curcumin at a concentration of 10 µg/ml	14 ± 1.0
AFFLU	Fluconazole at a concentration of 10 µg/ml	21± 2.0
AFITR	Itraconazole at a concentration of 10 µg/ml	33 ± 1.5
AFSTD	Standard drug (Ketoconazole) at a concentration of 10 µg/ml	34 ± 1.0

*Experiments were perfomed three times and the results presented in the fomat ± Standard Deviation.

Further, the effectiveness of different drug combination was evaluated by disk diffusion method. The final concentration of individual drugs, standard drug (Ketoconazole) and all the prepared drug combination was fixed up to 10 µg/ml and further subjected to in-vitro antifungal study. The results thus obtained was compared based on measurement of the zone of inhibition. The result states that a combination of curcumin with fluconazole and itraconazole shows a better zone of inhibition as compared to the individual drug at the same concentration. Which indicates the presence of curcumin significantly increases the antifungal capacity of fluconazole and itraconazole. (Table no - 2). Among all the combination, Itraconazole with curcumin 2:1 ratio (AFC4) and fluconazole with curcumin 3 :1 ratio (AFC8) at a concentration 10 µg/ ml shows a comparatively higher zone of inhibition. (Fig-02). Further, the results of fractional inhibitory concentration index (FICI) indicates that a combination of curcumin with fluconazole and itraconazole may also show synergism effect at different concentration ratios. Among the several prepared combinations of itraconazole and curcumin at the different molar ratio, CIC-C, CIC-G and CIC-I combination shows FICI of 0.346, 0.489 and 0.407 respectively.

Similarly in case of combinations prepared using fluconazole and curcumin, CFC-B, CFC-C, CFC-F combination shows FICI value of 0.364, 0.452, and 0.412 respectively, which may be considered as a clear indication of potential synergism (Table no-3). Further, the tissue sensitivity of most effective combinations from each drug combination was performed. The selection of optimized combinations was done based on probable potential

synergism at the lowest drug concentration. The hematological analysis of blood sample and tissue histopathology study indicates no sign of major abnormalities and sensitive allergic reaction. (Table no-4 and Fig no -3). Which indicates the tested combinations are free from any tissue sensitivity issue and also not produce any major abnormal changes in hematological profile of the tested animals, hence may be considered safe.

Conclusion:

The experiments were performed to determine the antifungal effectiveness of curcumin and to explore its possible therapeutic action in combination with standard antifungal drugs. Form the result of the performed studies it may be concluded that curcumin has a potent antifungal action against the fungi Candia albicans, Moreover, the presence of curcumin seems to significantly increase the antifungal action of fluconazole and itraconazole, hence can be an effective alternative in antifungal combination therapy. Further, considering the results of the histopathological study and hematological test, it may be concluded that the developed combinations are safe as per topical application concern. The study may be further extended to the determination of molecular mechanism involved behind the positive therapeutic effect of drug combinations and their possible side effects.

Reference:

1. Umme H, Hosakote G S, Vaghela R, Osmani RA and Shrivastava A, Candidiasis: A fungal infection- current challenges and progress in prevention and treatment, Infect. Disord.: Drug Targets, 2015, 15, 42-52
2. Choudhury A, Roy A, Bahadur S, and Saha S, Phytoconstituent based mucoadhesive antifungal vaginal formulation: An effective and innovative approach, Biosci. Biotechnol. Res. Commun., 2016, 9(4), 694-701
3. Denning D W and Hope WW, Therapy for fungal diseases: Opportunities and priorities, trends in microbiology.(IN PRESS)
4. Wagner H, and Merzenich G U, Synergy research: Approaching a new generation of phyto pharmaceuticals. J. Nat. Rem.,2009, 9(2), 121 – 141
5. Matlock K, Berlow N, Keller C, and Pal R, Combination therapy design for maximizing sensitivity and minimizing toxicity, BMC Bioinf., 2017, 18(4),17-29.
6. Badiee P and Hashemizadeh Z, Opportunistic invasive fungal infections: Diagnosis and clinical management, Indian J Med Res, 2014, 139,195-204.
7. Mohapatro SK, Dandapat MC and Padhi NC, Toxicity and side-effects of combination

chemohormonal therapy of advanced breast cancer, J Indian Med Assoc., 1992, 90(2):39-42.

8. Chanda S and Rakholiya K, Combination therapy: Synergism between natural plant extracts and antibiotics against infectious diseases. Science against microbial pathogens: communicating current research and technological advances. Formatex, 2011, 520-529
9. Doble M, Hemaiswarya S and Kruthiventi A K, Synergism between natural products and antibiotics against infectious diseases, Phytomedicine, 2008, 15, 639–652
10. Barbier R, Coppo E, Marchese A, Daglia M, Sánchez Eet al., Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity, Microbiol. Res., 2017, 196, 44-68
11. Cowan M M, Plant Products as Antimicrobial Agents, Clin. Microbiol. Rev., 1999,12(4), 564–582
12. Choudhury A, Verma A, Sinha D, Shau S and Roy A, Development and characterization of topical phyto-formulation for antifungal activity, J. Appl. Pharm. Res.2014, 1(2), 18-21.
13. Choudhury A, Roy A, Bahadur S, and Saha S, Preparation and evaluation of plant bioactive phytochemical based bioadhesive antifungal vaginal formulation. Indo Am. J. Pharm. Sci., 2016, 3 (12), 1673-1681
14. Septama AW and Pharkphoom P, Synergistic effect of artocarpin on antibacterial activity of some antibiotics against methicillin resistant Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli, Pharm Biol, 2016, 54(4), 686–691
15. Pereira A F, Gomes A S G, Curvelo J A R R and Soares MA, Curcumin acts synergistically with fluconazole to sensitize a clinical isolate of Candida albicans showing a MDR phenotype. Med. Mycol., 2012, 50, 26–32.
16. Fratinia F, Mancinia S, Turchia B, Frisciaa E, Pistellib Let al., A novel interpretation of the Fractional Inhibitory Concentration Index: The case Origanum vulgare L. and Leptospermum scoparium J. R. Forst essential oils against Staphylococcus aureus strains, Microbiol. Res.2017,195, 11–17.
17. Mehta S, Verstraelen H, Peremens K, Villeirs G, and Vermeire S, Vaginal distribution and retention of a multiparticulate drug delivery system assessed by gamma scintigraphy and magnetic resonance imaging. Int. J. Pharm.,2012, 426, 44-53