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Formulation and *In Vitro*Evaluation of Fluconazole Loaded NanoemulgelAgainst*Candida albicans*

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Abstract

Fungal infections have grown as a threat to the health of human beings over the past few years. In healthy state the human body houses many commensal fungalspecies, *Candida albicans* being one of them. In human beings who are healthy, the fungus is present in the body without causing any disease. However, the same fungus has potential to proliferate and produce a disease under diverse conditions. As per reports, presence of Candida species accounts for the most prevalent infection in surgical patients who are critically ill. A number of therapeutics have been used for treating candidal infections. Among them, Fluconazole is reported to be effective. Fluconazole is available commercially either as tablets or for IV administration for the treatment of infections occurring by Candida infestation. However, both the dosage forms formulated suffer from certain disadvantages. In order to overcome their drawbacks, in the present study, Fluconazole loaded nanoemulsion based gel was formulated and its efficacy evaluated as an attractive alternative treatment option for Candida infections. *In vitro* efficacy of Fluconazole loaded nanoemulgel, placebo formulation, fluconazole gel and control (pure fluconazole) against *Candida albicans* (MTCC NO: 227) was compared using cup-plate method microbiological assay method. The

nanoemulgel was formulated by addition of carbopol 934P (1% w/w) in optimized Fluconazole loaded nanoemulsion.A significantly higher ($p \leq 0.05$)zone of inhibition was observed for Fluconazole nanoemulgel (35±1.1 mm) in comparison to fluconazole gel (15±1.2 mm), placebo formulation (9±1.6 mm) and control (9.8±1.2 mm) after 24 hours. Further, after 72 hours, the Fluconazole nanoemulgel exhibited zone of inhibition (34.9± 1.6 mm) while the other three formulations (fluconazole gel, placebo formulation and pure fluconazole) did not exhibit any zone of inhibition.It was concluded that Fluconazole loaded nanoemulgel formulation due to its nanometricsize was effective in inhibiting the growth of *Candida albicans* more efficiently as compared to all the other formulations evaluated.

Keywords: Fluconazole, nanoemulgel,*C. albicans*

INTRODUCTION

The past few decades have witnessed a substantial increase in the advent of fungal infections affecting human beings. Inspite of the fact that the fungal infections constitute a serious threat to human health, they have been underrated [1,2]. Fungal infestations account for approximately 15% of infections occurring in human beings [3].Candida species are the most common fungi causing disease in human, *C. albicans*and *C. glabrata*being first andsecond in frequency of isolation, respectively [4]. Candida species account for 70–90% of all fungal infestations which are invasive [3] and is also reported to be the commonest pathogen causing fungal infections in majority of the clinical setups [5]. The morphological features of *C.albicans*have an important effect on several aspects of infection as well as on recognition of the host [6].

*C. albicans*can either causesuperficial infections (examples include oral and skin candidiasis) or can result in systemic infections which can also be a serious threat to life. Since, *C. albicans* is present in 75% of the surface of mucosa even in healthy individuals; its outgrowth needs to be recognized inorder to prevent the fungal disease [7].

Fluconazole, an imidazole derivative is reported to be effective against most of the pathogenic fungi present [8]. Among the triazoles, it is one of the most frequently prescribed drugs [9]. The drug not onlyexhibits good tolerance, but is also safe for the treatment of several infections due to low toxicity associated with its use [10]. It is currently reported to be the most preferred drug for treating AIDS patients with oropharyngeal candidiasis [11,12].

Fluconazole, an oral bis-triazole synthetic compound acts as a antifungal agent by inhibition of the cytochrome P450-dependent 14 alphademethylation step in production of ergosterol, which results in alterations in many membrane-associated cell functions.

The marketed formulations of the drug include tablets and IV formulation [13]. No local drug delivery system for application to oral mucosa for the treatment candidiasis is available commercially. The oral administration of the drug, although a convenient and patient compliant method of delivery of the drug, is associated with many side

effects, which include gastric distress, abdominal pain, first pass metabolism etc. Besides this, the drug is a BCS class II drug with low solubility (< 1 mg/ml) [14]. Therefore, in order to improve solubility, enhance penetration and reduce systemic side effects, there is necessity of the development of a nanoformulation of fluconazole for local application.

In the present study, Fluconazole loaded nanoemulgel was formulated for local delivery of the drug and its*in vitro* efficacy against *Candida albicans* was evaluated using cup-plate method microbiological assay method and the results were compared with those obtained from placebo formulation, fluconazole gel and control (pure fluconazole). The treatment of fungal infections with oral fluconazolenanoemulgel gel would have several advantages including, drug targeting to the infection site, reduced potential foroccurrence of side effects associated with the oral use of the drug, improved efficacy of treatment by improving its solubility and high patient compliance etc [15].

Materials and Methods

Nanoemulsion and Nanoemulgel components

Fluconazole was obtained as a gift sample from Ramson Remedies (Amritsar, India), Clove oil and Carbopol 934P were purchased from Sigma Aldrich Pvt. Ltd (Bangalore, India). Tween 20 was procured from Central Drug House, (New Delhi, India). All other chemicals/reagents were of analytical grade and purchased from Merck (Mumbai, India) and S.D. Fine Chem. (Mumbai, India).

Strain, growth media and culture conditions

Candida albicans(MTCC No. 227) was purchased from IMTECH (Institute of microbial technology, Chandigarh). *Candida albicans* was grown in suspension of YME (Yeast Malt Extract) and kept in aB.O.D incubator shaker.During the experiments, the viable cell concentrations present in suspension werechecked by viable counts.

Formulation of Fluconazole loaded nanoemulsion based gel and placebo formulation usingcarbopol 934 P

The formulation of Fluconazole loadednanoemulsion based gel (nanoemulgel) was prepared by using aqueous phase titration method. The formulation was prepared by adding gelling agent (carbopol 934 P) to theclove oil based fluconazole nanoemulsion formulation. The placebo formulation was also formulated by the same method but the drug was excluded.

In vitro antifungal activity

The study was performed as per the method reported by Maebashi *et al* 1995 and Vijaya*et al* 2014. 50 µl suspension was taken from the *Candida albicans*suspension ($1x10^7$cfu/ml) and spread aseptically on plates of Sabouraud dextrose agar (SDA) using sterile cotton swab. After each application, the plates were rotatedat 60° angle followed by pressing of the swab around agar surface edge. The plates werethen dried with the lid closed at room temperature. After the plates dried,using sterile core borer, four wells (3mm diameter each)were punched into the agar medium and filled with 1 gram each of Fluconazole loaded nanoemulgel, fluconazole gel (0.5%w/w of

fluconazole), placebo formulationand pure fluconazole suspension respectively. Further, in order to allow the uniform diffusion of the drug, plates refrigerated for 2 hours.The plates were then incubated at 37ºC, after which zone of inhibition around the four wells were recorded and compared [16,17].

Results

The zones of inhibition formed around the formulations on the S.D.A plate were measured. A zone of inhibition of 35±1.1 mm, 34.9 ± 1.4 and 34.9 ± 1.6 was observed for Fluconazole loaded nanoemulgel at the time interval of 24 hrs, 48 hrs and 72 hrs respectively. Fluconazole gel exhibited zone of inhibition of 15 ± 1.2; 8.2 ±1.3 ;00 ±1.2 at time interval of 24 hrs, 48 hrs and 72 hrs respectively. Placebo formulation demonstrated a zone of inhibition of 09 ± 1.6; 00 ± 1.3&00 ± 1.8 at 24 hrs, 48 hrs and 72 hrs time interval respectively; while control formulation exhibited zone of inhibition of 9.8 ± 1.2; 5.6 ± 1.5 and 00 ± 1.7 respectively at a similar time interval.

The results of the study were expressed as mean± standard deviation (S.D). The Graph Pad Instat 3 software using two tailed paired t- test was used for the statistical analysis of the data obtained. Values at $p \leq 0.05$ were considered significant.

Discussion

Preparation of Fluconazole loaded nanoemulgel and placebo formulation usingcarbopol 934 P

The fluconazole loaded nanoemulsion was formulated by aqueous titration method.

Reports in literature suggest the antimicrobial potential of extracts and oils of aromatic plants and spices, in inhibiting the growth of microorganisms which are pathogenic [18,19].

Essential oils are composed of a mixture of volatile, natural as well as aromatic compounds produced by aromatic plants and are very commonly used in traditional medicine [20]. According to the number of isoprene units present, the essential oils are classified into two major types: monoterpenes and sesquiterpenes [21]. The use of essential oils is preferred as antimicrobial agents, since they have activity against a variety of microorganisms, besides having reduced side effects as compared to other chemically synthesized antimicrobial agents [22].

Among the various essential oils used as antimicrobial agent, Clove oil has been extensively explored by researchers due to its reduced side effectsas well due to the presence of high content of essential oil (eugenol) [23]. Clove oil has been reported to inhibit the growth of yeasts, moulds and bacteria. The oil acts by altering the permeability of phospholipids present in the cell wall, thereby, inhibiting the growth of bacteria and different types of yeast [24]. The oil has also been reported to inhibit the growth of *Candida albicans* [25].

Based on the above reports, in the present study, for the formulation of nanoemulsion of fluconazole, clove oil was chosen as the oil phase. To the optimized nanoemulsion formulation, carbopol 934P was added as the gelling agent to produce the nanoemulgel formulation.

***In vitro* anti-fungal activity**

Evaluation of the *in vitro* anti-fungal activity was done by using cup and plate microbiological assay.The zones of inhibition formed around the formulations on the S.D.A plate were measured. A significantly higher ($p \leq 0.05$) inhibition zone was observed for Fluconazole loaded nanoemulgel (35±1.1 mm) as compared to fluconazole gel formulation (0.5%w/w of fluconazole) (15±1.2 mm), placeboformulation (9±1.6) and control(9.8±1.2 mm) after 24 hours. The larger inhibition zone for Fluconazole nanoemulgel could be attributed to the nanosize of the drug present in the gel, which caused its greater SDA diffusion, thereby resulting in its greater penetration through fungal cell walls resulting in fungicidal activity. Besides this, the clove oil which is also reported to be a fungicidal agent due to its ability to change permeability of phospholipids present in the fungal cell membrane also could have contributed to enhanced zone of inhibition of fluconazole loaded nanoemulgel formulations as compared to other formulations evaluated. Further, even after 72 h, the Fluconazole loaded nanoemulgel exhibited zone of inhibition (34.9± 1.6 mm) unlike the other three formulations fluconazolegel, placebo formulation and pure fluconazole which did not exhibit any zone of inhibition. From the results of cup-plate method microbiological assay method, it was concluded that the formulated fluconazole loaded nanoemulgel formulation exhibitedfungicidal activity as compared to fluconazole gel, placebo formulation and pure fluconazole which exhibited fungistaticactivity. The results are given in table 1, table 2 and figure 1.

Table 1: Zone of inhibition for different formulations against *Candida albicans* strain

Strain No.	**Formulation(s)**	**Zone of Inhibition (mm) mean ± S.D (n=3)**			**Inference**
		24 hrs	**48hrs**	**72 hrs**	
MTCC No. 227	FluconazoleNanoemulgel	35 ± 1.1	34.9 ± 1.4	34.9 ±1.6	Fungicidal action
	Fluconazole gel	15 ± 1.2	8.2 ±1.3	00 ±1.2	Fungistatic action
	Placebo formulation	09 ± 1.6	00 ± 1.3	00 ± 1.8	Fungistatic action
	Control (Pure Fluconazole)	9.8 ± 1.2	5.6 ± 1.5	00 ± 1.7	Fungistatic action

Figure 1: Comparison of zones of inhibition for different formulations during *in vitro* anti-fungal activity against *Candidaalbicans* (MTCC No: 227)

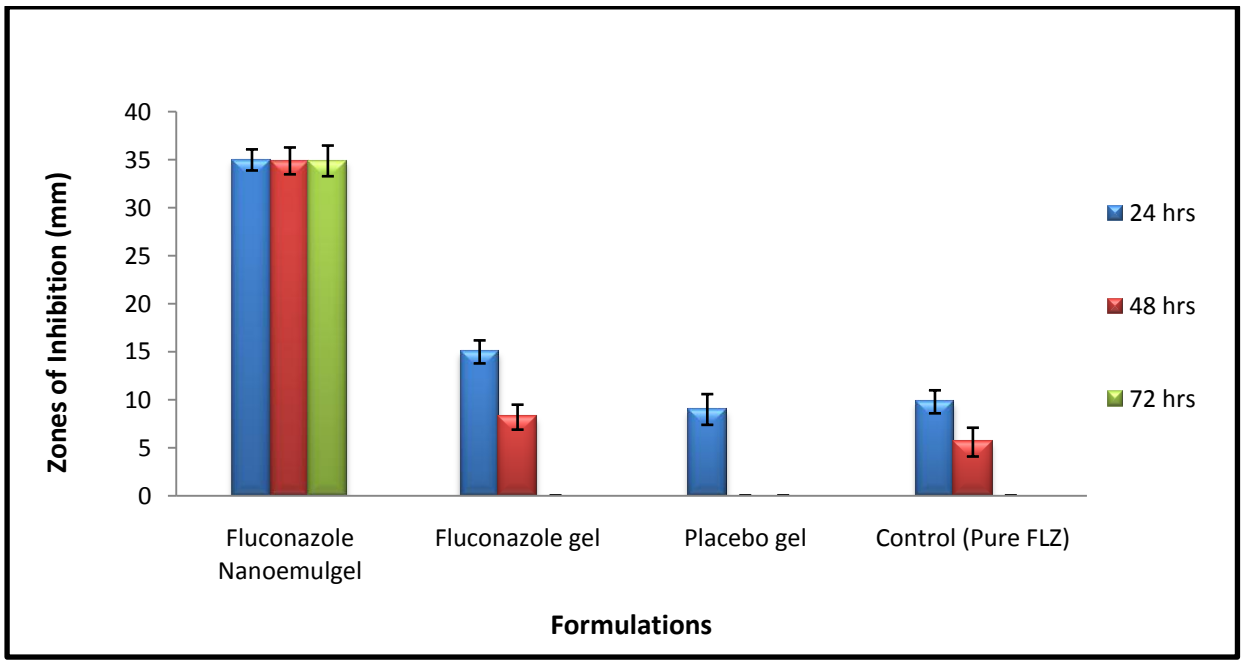
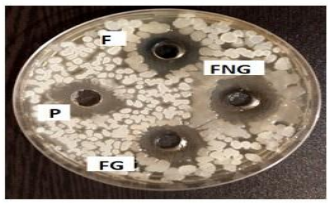
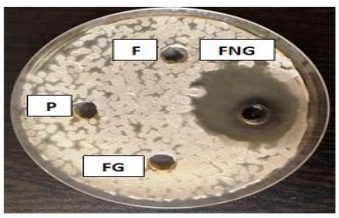


Table 2: Observations of Zones of Inhibition for different formulations evaluated against strain of *Candida albicans* at different time intervals

Incubation Time (hrs)	Observations of Zone of Inhibition for different Formulations
	Candida albicans (MTCC No. 227)
24	
48	
72	

Where, FNG = Fluconazole nanoemulgel, FG = Fluconazole Gel, F = Pure Fluconazole Suspension , P= Placebo formulation

Conclusion: The zone of inhibition for Fluconazole loaded nanoemulgel (35 ± 1.1 mm) was found to be significantly higher ($p \leq 0.05$) as compared to fluconazole gel (15 ± 1.2 mm), placebo formulation(9±1.6 mm) and control (9.8± 1.2 mm) after 24 hours. Further, even after 72 h, the Fluconazole loaded nanoemulgel exhibited zone of inhibition (34.9± 1.6 mm) unlike the other three formulations (fluconazolegel, placebo formulation and pure fluconazole) which didnot exhibit any zone of inhibition. From the results of cup-plate method microbiological assay method, it was concluded that the formulated fluconazole loaded nanoemulgel formulation exhibitedfungicidal activity as compared to the fluconazole gel, placebo and pure fluconazole which exhibitedfungistatic activity. The results prove that the formulated fluconazole loaded nanoemulsion has significantly higher antifungal activity against Candida albicans and therefore could serve as an attractive and potential option for the treatment of oral candidiasis.

Conflict of Interest: Nil

Funding: Nil

References

1. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC: Hidden killers: Human fungal infections. Sci.Transl. Med. 2012, 4:165rv13.10.1126/scitranslmed.3004404.
2. Nature Microbiology: Stop neglecting fungi. Nat. Microbiol.Macmillan Publishers Limited (ed.).2017.2: 17120.10.1038/nmicrobiol.2017.120 s
3. Delaloye J, Calandra T: Invasive candidiasis as a cause of sepsis in the critically ill patient. Virulence 2014, 5:161–169.10.4161/viru.26187.
4. Brunke S, Hube B: Two unlike cousins: *Candida albicans* and *C. glabrata* infection strategies. Cell. Microbiol. 2013, 15:701–708.10.1111/cmi.12091.
5. Pappas PG, Lionakis MS, Arendrup MC, Zeichner LO, Kullberg BJ: Invasive candidiasis. Nat. Rev. Dis. Prim. 2018, 4:26.10.1038/nrdp.2018.26.
6. Noble SM, Gianetti BA, Witchley JN:*Candida albicans* cell-type switching and functional plasticity in the mammalian host. Nat. Rev. Microbiol. 2017, 15:96–108.10.1038/nrmicro.2016.157.
7. Mayer FL, Wilson D, Hube B: *Candida albicans* pathogenicity mechanisms. Virulence 2013, 4:119–128.10.4161/viru.22913.
8. Holt RJ:Topical Pharmacology of Imidazole Antifungals. Journal of Cutaneous Pathology. 1976, 3:45-59.10.1111/j.1600-0560.1976.tb00846.x.
9. Pappas PG, Rex JH, Sobel JD.Filler SG, Dismukes WE, Walsh TJ, Edwards JE:Guidelines for treatment of candidiasis. Clin. Infect. Dis. 2004, 346:161-189.10.1086/380796.
10. Chandran SC, Shirwaikar A, Kuriakose MR, Sabna NS: Development and evaluation of ethosomes for transdermal delivery of Fluconazole. Journal of Chemical, Biological and Physical Sciences. 2011, 2: 254-260.
11. Ameen M: Epidemiology of Superficial Fungal Infections. Clinics in Dermatology. 2010, 28: 197-201.10.1016/j.clindermatol.2009.12.005.

12. Havlickova B, Czaika VA, Friedrich M: Epidemiological Trends in Skin Mycoses Worldwide. Mycoses. 2008, 5: 2-15.10.1111/j.1439-0507.2008.01606.x.
13. Salerno C, Carlucci AM, Bregni C: Study of *in vitro* Drug Release and Percutaneous Absorption of Fluconazole from Topical Dosage Forms. AAPS Pharm. Sci.Tech. 2010,11: 986- 993.10.1208/s12249-010-9457-1.
14. Vincent-Ballereau FN, Patey ON, Lafaix C:Fluconazole. Review and situation among antifungal drugs in the treatment of opportunistic mycoses of human immuno-deficiency virus infections. Pharm. Week bl. Sci.1991,13:45-57.10.1007/BF01974981.
15. Lee CM, Maibach HI: Deep percutaneous penetration into muscles and joints. Journal of Pharmaceutical Science. 2006,95: 1405-1413.10.1002/jps.20666.
16. Maebashi K,Itoyama T, Uchida K, Suegara N, Yamaguchi H: A novel model of cutaneous candidiasis produced in prednisolone treated guinea pigs. Journal of Veternary and Mycology 1995,19: 390-392.10.1080/02681219480000471.
17. Vijaya R, Kumar SS, Kamalakannan S: Preparation and *in vitro* evaluation of miconazole nitrate nanoemulsion using tween 20 as surfactant for effective topical / transdermal delivery. Journal of Chemical and Pharmaceutical Sciences 2014, 8: 92-98.
18. Dorman HJD, Deans SG: Antimicrobial agents from plants antibacterial activity of plant volatile oils. J Appl.Microbiol. 2000, 88: 308-316.10.1046/j.1365-2672.2000.00969.x.
19. Rauha JP, Remes S, Hemonen M, Hopia A, Kähkönen M, Kujala T, Pihlaja K, Vuorela H, Vuorela P:Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. Int J Food Microbiol. 2000, 56: 3-12.10.1016/s0168-1605(00)00218-x.
20. Nerio LS, VerbelOJ, Stashenko E: Repellent activity of essential oils: a review. Bioresour Technol. 2010, 101: 372-378.10.1016/j.biortech.2009.07.048.
21. Oulkheir S, Boumariem H, Dandi H:Antimicrobial activity of four essential oils extracted from plants commonly used in traditional medicine against some clinical strains. Herba Pol. 2019, 65: 22-29.10.2478/hepo-2019-0010.
22. Rana IS, Rana AS, Rajak RC: Evaluation of antifungal activity in essential oil of the Syzygiumaromaticum (L.) by extraction, purification and analysis of its main component eugenol. Braz J Microbiol. 2011, 42: 1269–1277.10.1590/S1517-83822011000400004.
23. Park MJ, Gwak KS, Yang I,Choi WS, Jo HJ, Chang JW, Jeung EB, Choi IG:Antifungal activities of the essential oils in Syzygiumaromaticum (L.) Merr.Et Perry and Leptospermum petersonii Bailey and their constituents against various dermatophytes. J.Microbiol. 2007, 45: 460-465.
24. Matan N, Rimkeeree H, Mawson AJ, Chompreeda P, Haruthaithanasan V, Parker M: Antimicrobial activity ofcinnamon and clove oils under modifiedatmosphere conditions. J. Food. Microbiol. 2006, 107: 180–185.10.1016/j.ijfoodmicro.2005.07.007.

25. Narang JK, Narang RS, Singh B, Kahlon SS, George J, Dogra A. Comparative efficacy of tea tree oil nanoemulgel and clove oil nanoemulgel against *Candida albicans*. Int. J. Pharma.Investig. 2018, 8: 50-2.