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Evaluation of cell viability of formulated fluconazole nanoemulgel by cytotoxicity analysis

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Abstract: *Oral Candidiasis, also known as oral thrush among other names is candidiasis that occurs in the oral cavity. Candida albicans is the most common species responsible for the disease. Fluconazole has a broad spectrum of activity and is currently the drug of choice for controlling oropharyngeal candidiasis especially in patients suffering from AIDS. The aim of the present study was to evaluate the in vitro cytotoxicity and cell viability of fluconazole nanoemulgel formulated for the treatment of oral candidiasis. In vitro Vero cell line based cytotoxicity analysis was performed using MTT assay. It was observed that Fluconazole loaded nanoemulgel (FCZ-NE) showed dose dependent cytotoxicity for entire concentration range. Fluconazole loaded nanoemulgel (FCZ-NE) exhibited cell viability of 100% to 76% for concentration range of 2xCmax to 20xCmax, whereas fluconazole gel (FCZ) exhibited cell viability of 99% to 67% for the same concentration range. The results suggested that prepared Fluconazole loaded nanoemulgel (FCZ-NE) exhibited good cell viability (%) in vitro thus establishing its safety profile.*

Key words : *Cytotoxicity, Fluconazole nanoemulgel, Candidiasis*

I. Introduction

Fungal infections, that can infect any body part either locally, a few being nails and oral cavity or can be systemic, are reported to affect more than billion individuals every year, with the numbers increasing [1, 2].

As per reports, inspite of many fugal species being pathogenic, candida (esp. Candida albicans) is responsible for majority of the infections caused by fungi. Among the different infections caused by candida, oral candidiasis id the most common fungal infection, The disease has attracted a lot attention over a past few years due

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to excessive use of corticosteroids, antibiotics which are broad spectrum for prolonged periods of time, immunosuppressant's as well as due to prevalence of AIDS Around 20%to 75% of the population is reported to be asymptomatic carrier of the candida infection [3].

In order to treat infections due to manifestation by candida, a number of antifungal medications , a few of which include fluconazole, ketoconazole, itraconazole as well as clotrimazole are available. However, among them, fluconazole is reported to be the most effective drug for treating oropharyngeal candidiasis [4, 5].

Fluconazole is commercially marketed as tablets and capsules for oral administration, as gel for cutaneous application and as IV formulation. The dose strengths of the drug are 50, 100, 150, and 200 mg in tablet form and 200 or 400 mg in IV formulation [6].

All the dosage forms of fluconazole are associated with disadvantages. The administration of the drug orally is associated with many serious systemic side effects like gastric distress, abdominal pain and pre systemic metabolism where as the gel meant for topical application to the skin has limited penetrability. The IV dosage form is associated with patient noncompliance. Besides this, the drug is a BCS class II drug with low solubility (< 1 mg/ml) thereby resulting in its limited absorption [7] .In the light of the above facts and in order to overcome the disadvantages associated with the commercially available dosage forms of the drug, development of alternative approaches encompassing the use of new carrier systems is the need of the hour [8] . One such approach is use of nanoemulsions and nanoemulsion based gels, commonly referred to as nanoemulgels, for the improving the effectiveness of administered drugs [9].

Nanoemulsion, are composed of excipients which are approved by FDA for human consumption as GRAS excipients (generally recognized as safe) [10] . Protection of drugs liable to hydrolytic and/or enzymatic degradation, nanosize of drugs resulting in enhancement of solubility of poorly soluble drugs, capacity to dissolve hydrophobic therapeutics, improved permeation across mucosa, ease of formulation as well as thermodynamic stability are few important advantages of nanoemulsion based drug delivery systems as compared to conventional dosage forms [11]

Since no nanoemulsion based gel for delivery of fluconazole is available, a nanoemulgel formulation loaded with fluconazole was formulated for enhancing its solubility, penetration and to decrease systemic side effects, targeted delivery to infection site thereby improving treatment efficacy as well as patient compliance [12].

The aim of the present study was to evaluate the *in vitro* cytotoxicity and cell viability of fluconazole nanoemulgel formulated for the treatment of oral candidiasis.

II. Materials and Methods

Dulbecco's Modified Eagle's medium (DMEM, High Glucose), Fetal bovine Serum (FBS, South American Origin), Antibiotic-Antimycotic mixture (10x), 3-(4, 5-dimethylthiazol-2- yl)-2, 5- diphenyltetrazolium bromide

(MTT), Dimethyl Sulfoxide (DMSO) and Trypsin-EDTA were purchased from Sigma Aldrich. Plasticwares were purchased from Tarsons, Mumbai while glasswares were purchased from Borosil, India.

Cell line maintenance

Vero (ATCC CCL-81) kidney epithelial cell line was selected for *in vitro* analysis of the prepared fluconazole loaded nanoemulgel. Cells were maintained in humidified CO_2 (5%) incubators at 37ºC with DMEM media supplemented with 10 % FBS. To avoid any contamination, 1x Antibiotic-Antimycotic solution (comprising of Penicillin, Streptomycin and Amphotercin-B) was added. Upon reaching to ~75% confluency level, cells were detached using Trypsisn-EDTA and passaged after cell counting using haemocytometer by Trypan-Blue staining method.

Cytotoxicity Assay

In vitro cytotoxicity analysis was done by, 3-(4, 5-dimethylthiazol-2- yl)-2, 5- diphenyltetrazolium bromide (MTT) assay. Each well of 96 well microtitre plate was loaded with $1x10^5$ cells, which were left for seeding overnight. The cells after attachment were washed using Dulbecco's phosphate buffer saline and were treated with DMEM with drug formulations. Fluconazole loaded nanoemulgel (FCZ-NE) and fluconazole gel (FCZ) were evaluated for cytotoxicity for the concentration range varying from $C_{max}/2 - 20xC_{max}$ values for fluconazole (C_{max} for fluconazole = 6.72 µg/mL) where C_{max} value is the reported maximum plasma concentration value of fluconazole. Equivalent volume of placebo nanoemulgel without drug was also studied for possible cytotoxicity. Cells were treated for 24 h after which MTT dye (5mg/ml) was added to each well for 3 h. Media was then removed and replaced with 100 µl DMSO to dissolve the formazon crystals which were developed by mitochondrial enzymes of the living cells. Absorbance was then taken using ELISA plate reader at 570 nm. Assay was performed in triplicates and Cell viability (%) was calculated using equation:

$$Cell\ viabilty\ (\%) = \frac{Absorbance\ of\ treated\ cells}{Absorbance\ of\ untreated\ cells} * 100$$

Results were represented in Cell Viability (%)±Standard Deviation values.

III. Results

In vitro Vero cell line based cytotoxicity analysis was performed using MTT assay. It was observed that Fluconazole loaded nanoemulgel (FCZ-NE) showed dose dependent cytotoxicity for entire concentration range. FCZ-NE showed almost 100% cell viability up to $2xC_{max}$ concentration range whereas FCZ gel showed around 96% cell viability at $2xC_{max}$ concentration. Further, FCZ-NE exhibited higher cell viability % of around 96%, 90%, 81% and 76% for $4xC_{max}$, $8xC_{max}$, $10xC_{max}$ and $20xC_{max}$ concentration respectively, however, FCZ showed comparatively higher cytotoxicity with cell viability of 92%, 84%, 73% and 67% respectively. (Table :1, Figure: 1).

Table 1: Cell Viability (%) of vero cells against concentration of drugs.

		% Cell Viability of Vero Cells (±SD)		
Conc of Drug	**µg/mL**	**Fluconazole Gel**	**Fluconazole Nanoemulgel**	**Placebo**
Cmax/2	3.36	99±1.32	100±1.37	100±1.63
Cmax	6.72	99±1.68	100±1.32	100±1.45
2xCmax	13.44	96±1.93	100±1.13	100±1.76
4xCmax	26.88	92±2.32	96±2.02	98±1.32
8xCmax	53.76	84±2.11	90±1.68	93±2.11
10xCmax	67.2	73±1.94	81±1.87	88±2.34
20xCmax	134.4	67±2.01	76±2.31	80±2.12

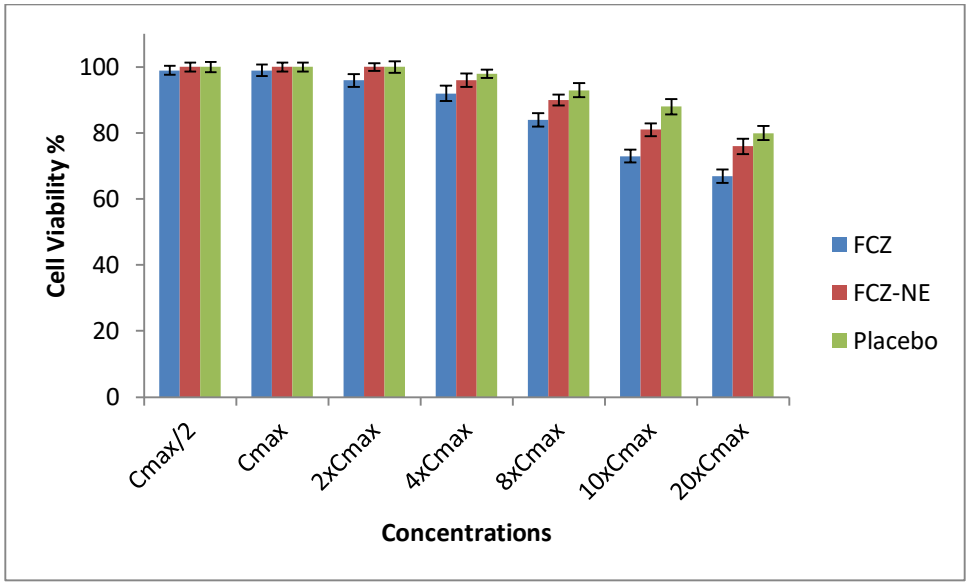

Figure 1: MTT assay for prepared Fluconazole loaded nanoemulgel (FCZ-NE), Fluconazole gel (FCZ) and placebo on Vero cell line.

IV. Discussion

In vitro cytotoxicity was evaluated for formulated fluconazole nanoemulgel on vero cell lines. Fluconazole loaded nanoemulgel exhibited around 100% cell viability on vero cells at approximate two times C_{max} value and almost 96% cell viability was achieved at near four times of Cmax value whereas fluconazole gel exhibited 96% and 92% cell viability at two times of Cmax value and four times of Cmax value respectively. Placebo nanoemulgel exhibited >90% cell viability $8xC_{max}$ values suggesting negligible cytotoxicity of the excipients used. These results clearly indicate that the nanoeulsion based gel formulation for the delivery of fluconazole was less toxic as compared to fluconazole gel. The reason attributed to this was that since the drug was encapsulated in the oil phase of the nanoemulsion forming the nanoemulgel with the help of a surfactant, which increased the cell survival chances of cells as compared to fluconazole gel. The results are in accordance with those reported by Nigam et al., in which the authors reported a low cytotoxicity of capsaicin encapsulated as nanoemulsions as compared to capsaicin aqueous solution [13]. The results were also in agreement with Shreaz et al, where fluconazole exhibited similar cytotoxicity trends, 28%-100% cell viability on H9c2 myoblasts cells for the concentration range of 640 µg/ml- 20 µg/ml [14]. In an another study by Mohammed et al., Fluconazole and Fluconazole nanogels showed >90% cell viability for concentration range upto 600 µg/ml on Human dermal fibroblast (HDF) cells [15]. These studies suggested that prepared Fluconazole loaded nanoemulgel (FCZ-NE) is exhibiting good cell viability (%) *in vitro*.

Furthermore, the use of non-ionic surfactant (Tween 80) in the formulation of nanoemulgel helped to reduce the toxicity associated with the use of surfactants [16].As per reports in literature, inclusion of charged surfactants in a formulation can result in toxicity as well potential to cause irritancy [17]. From the results of low toxicity and high cell viability exhibited by placebo as well as fluconazole loaded nanoemulgel on vero cell lines, it was concluded that nanoemulgel could be used as a suitable and safe carrier for the delivery of fluconazole to mammalian cells.

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