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Development and Evaluation of Eberconazole Loaded Niosomes †

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Abstract: The invasive fungal infection requires a long treatment schedule; however, treatment becomes more cumbersome due to the development of resistance. Most anti-fungal moieties show systemic toxicity upon oral administration, leading to delivery of anti-fungal moiety via topical route. Eberconazole (EBZ) is a BCS class II drug that has poor solubility and high permeability. It is a broad-spectrum imidazole derivative, which acts as both fungicidal and fungistatic by inhibiting ergosterol synthesis. Various topical creams of EBZ are available in the market, but the lack of a proper dosing schedule and rapid removal leads to poor bioavailability. Niosomes are vesicular carriers that can entrap both hydrophilic and lipophilic drugs. Niosomal formulations have been prepared using Span20 (nonionic surfactant) and cholesterol by thin-film hydration (TFH) technique. During the pre-formulation studies, purity of EBZ was ascertained using FT-IR and melting point studies, while standard calibration curve was prepared using UV-visible spectroscopy. The prepared niosomal formulations were characterized for their morphology, entrapment efficiency, particle size, and zeta-potential. The formulation has shown 86 ± 0.85% entrapment efficiency while noisome appeared ring-like structure during its microscopic evaluation. Further evaluations of its in vitro and in vivo release studies will be performed in the future for its efficacy and anti-fungal activity.

Keywords: Eberconazole; niosomes; sustained release; anti-fungal

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1. Introduction

Fungal infections or mycosis are prevalent infections, but few systemic infections are lethal to hosts. Every year around one billion people get affected by fungal infections worldwide. They mainly infect either superficially (pityriasis Versicolor), subcutaneously (eumycetoma), and systemically (cryptococcosis) in the different body regions [1]. These infections spread via air, such as breath in fungal spores and direct contact with skin or some open wounds or cuts in the host. It has also been observed that patients with weak immunity are highly susceptible to fungal infections. Many people with illnesses such as HIV/AIDS and people taking chemotherapy or steroids decrease their immunity and increase the chances of opportunistic fungal infections [2]. In the current scenario, the COVID-19 pandemic has shaken the world. The ongoing treatment leads to high demand for steroids, which directly decreases the immunity and increases opportunistic fungal infections such as black fungus (Mucormycosis), white fungus (candida infections), etc. [3]. Some common symptoms of fungal infections are skin rash, itching, lump, and discoloration of the skin. An anti-fungal agent, also known as an antimycotic agent, is a fungicide or fungistatic used to treat and prevent mycosis such as ringworm, candidiasis, etc. Different classes of antifungal agents are polyenes (Amphotericin B, Nystatin etc.), azoles, imidazoles (Bifonazole, Butoconazole, Clotrimazole etc.), triazoles (Fluconazole, Itraconazole, Posaconazole etc.) and echinocandins (Caspofungin, Micafungin, etc.) and other

agents. The significant side effects of anti-fungal agents are hepatotoxicity, anaphylaxis, etc. However, a long-term treatment regimen led to resistance toward anti-fungal agents [4–6].

Niosomal formulations are vesicular bilayer carriers that can entrap hydrophobic and hydrophilic drugs in their outer hydrophobic and inner aqueous core [7]. In vesicular systems, liposomes and niosomes are unilamellar or multilamellar spheroidal structures composed of amphiphilic molecules assembled into bilayers. They are considered primitive cell models, cell-like bioreactors, and matrices for encapsulation. Niosomes get greater attention than liposomes due to their high physical and chemical stability with low-cost nonionic surfactants than phospholipids [8]. These vesicles are composed of nonionic surfactants, cholesterol, and charge-inducing agents. These vesicles are non-immunogenic, biocompatible, and biodegradable moieties which may be considered as suitable drug delivery carriers for sustained and controlled release application. Its unique bilayer structure help to enhance the solubility and permeability of poorly soluble therapeutic moieties [9].

Its outer layer provides an amenable surface for modification with different ligands, which helps in targeted delivery. Many different class nonionic surfactants are available, such as alkyl ethers and alkyl glyceryl ethers, sorbitan fatty acid esters, polyoxyethylene fatty acid esters, and block copolymers. An ample number of methods are available to prepare niosomes, among which thin-film hydration is the most common technique [7,10].

Eberconazole (EBZ) is a topical, broad-spectrum imidazole derivative, effective in dermatophytosis, candidiasis, and pityriasis treatment. It was first introduced in Spain by salvat laboratories in 2005 for cutaneous fungal infections [11]. It also shows high activity against most triazole-resistant yeasts (Candida krusei and Candida glabrata) and fluconazole-resistant Candida albicans. It has also been shown to be effective against Gram-positive bacteria. Thomas et al. have reported its anti-inflammatory properties, making it unique among other imidazole derivatives and treating inflamed dermatophytic infections. It is a BCS class II drug and, due to its poor solubility, it requires high dosing, leading to toxicity. The available formulation for EBZ is a topical cream that has shown significantly high activity against superficial fungal infections [12].

In this study, EBZ loaded niosomal formulations were developed for the sustained delivery against fungal infections. EBZ loaded niosomes were formulated using Span20 and Cholesterol by thin-film hydration technique. The prepared niosomal formulations were characterized for their morphology, particle size, zeta-potential, entrapment efficiency, and functional group analysis through FTIR studies.

2. Materials and Methods

2.1. Materials

Span20, Cholesterol, and Triton-X were purchased from Sigma-Aldrich. Eberconazole (EBZ) was provided as a gift sample from Mylan labs (Telangana), dialysis bag (MWCO 12000DA) was purchased from hi-media. A solvent such as ethanol and chloroform were purchased from qualigens. Millipore water was used throughout the experimental study with a molar conductivity of 18 mΩ.

2.2. Methods

2.2.1. Pre-Formulation Studies

Preformulation studies were performed for the identification of EBZ using melting point and FTIR (fourier transform infrared) spectroscopy.

2.2.2. Preparation of Blank and Drug-Loaded Niosomal Vesicles

The niosomes were prepared by a thin-film hydration technique. Accurately weighed cholesterol and span20 were taken in a 3:1 molar ratio and dissolved in 10 mL chloroform and methanol mixture (8:2). The above solution was subjected to the rotary evaporator

(IKA-HB10) at 80 rpm and 40 °C. The thin film was formed after the complete evaporation of the organic solvent. Then, the film was hydrated with 10 mL of Milli-Q water above gel liquid transition temperature at 70 °C by vortex mixing for 25 min. The drug-loaded niosome was also prepared by mixing EBZ in chloroform and methanol, along with cholesterol and Span20 [13].

2.3. Characterization Methods

Morphological Characterization using Leica Microscope: This study was carried out to obtain structure and images of the prepared niosome. This prepared niosome was taken over a glass slide, and a coverslip was mounted over it. The prepared slide was finally visualized under 10X and 40X magnification using a Leica microscope [14].

Particle size analysis (PSA): Particle size analysis was carried using Malvern Zetasizer nano series (Nano-S90) instrument based on the principle of photon correlation spectroscopy. 3 mL of prepared niosome was taken in a quartz cuvette and exposed to laser light diffraction at an angle of 90° for particle size analysis [15].

Zeta potential: To measure the zeta potential, prepared niosomal solution was diluted, injected into folded capillary supported with platinum electrodes, and placed in the sample holder of Zetasizer (Malvern) for analysis [15].

FT-IR analysis: FT-IR studies of different niosomal systems were performed using the KBr disc method. Further, this study was done to analyze the various functional groups present in EBZ, blank niosome, and EBZ loaded niosomes [16].

Entrapment efficiency: The entrapment efficiency of EBZ loaded niosomal formulation was determined by lysis of niosomal vesicles using Triton-X. After the lysis, EBZ loaded niosomal formulations were centrifuged at 10,000×*g* for 30 min at 4 °C using a refrigerated centrifuge (Eppendorf, 5415 R, Germany) to analyze the entrapment efficiency. After the centrifugation, the free drug concentration in the supernatant was determined by measuring absorbance at 208 nm using a UV-visible spectrophotometer (Shimadzu, UV 2450 PC, Kyoto, Japan). The percentage of drug entrapment in niosomes was calculated using the following formula. [17]

$$\%Drug\ Entraped = \frac{(Total\ drug - Drug\ in\ supernatant)}{Total\ Drug} \times 100$$

3. Results and Discussion

3.1. Pre Formulation Studies

A melting point experiment has been done to determine the purity of the sample. The smaller melting point range generally governs the purity of the sample. Here the melting point determined by the OptiMelt (automated melting point system) instrument is found to be 183.4 °C. Reports are available for melting point appears in the range of 183–184.5 °C. As the result of melting point study appeared within the defined range, and hence it may be concluded that the drug is pure [18].

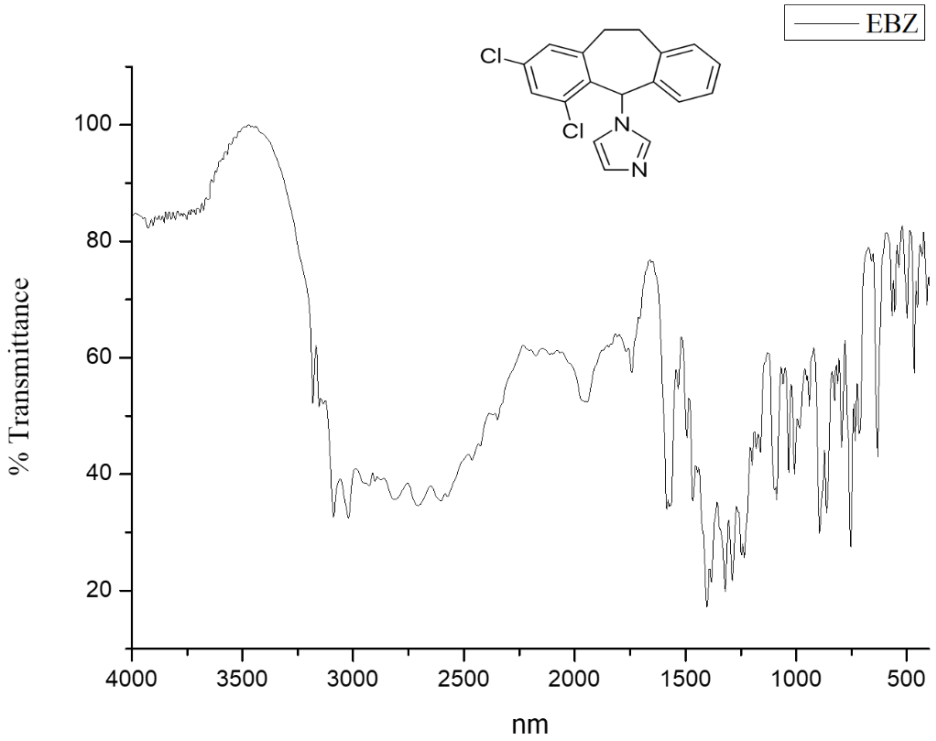

Figure 1. FTIR graph of Eberconazole.

3.2. FTIR Study

FTIR of EBZ shows two characteristic peaks at 1284 cm^{-1} & 632 cm^{-1} for the alkyl halide and halo compound (chloro compound). The IR peak at 2110 cm^{-1} corresponds to R-N=C stretching vibration, while the IR peak at 1963 cm^{-1} corresponds to C=C stretching vibration for unsaturation of carbon in an aromatic ring. Multiple small characteristic peaks in the region of 1030–1230 cm^{-1} may be due to C-N stretching [18].

3.3. Standard Plot

The standard stock solution of EBZ was prepared by dissolving 10 mg of EBZ in 10 mL of the water-methanol system (7:3). To prepare standard calibration curve, different concentrations of EBZ were prepared in the range of 2–12 µg/mL by diluting with a water-methanol system (7:3). The absorbance of the various dilutions of EBZ was measured at 208 nm using a UV-Visible spectrophotometer (Shimadzu UV-2450). The regression coefficient of the EBZ under the selected concentration range was found as $r^2 = 0.9959$. The graph of the standard plot has been depicted in Figure 2.

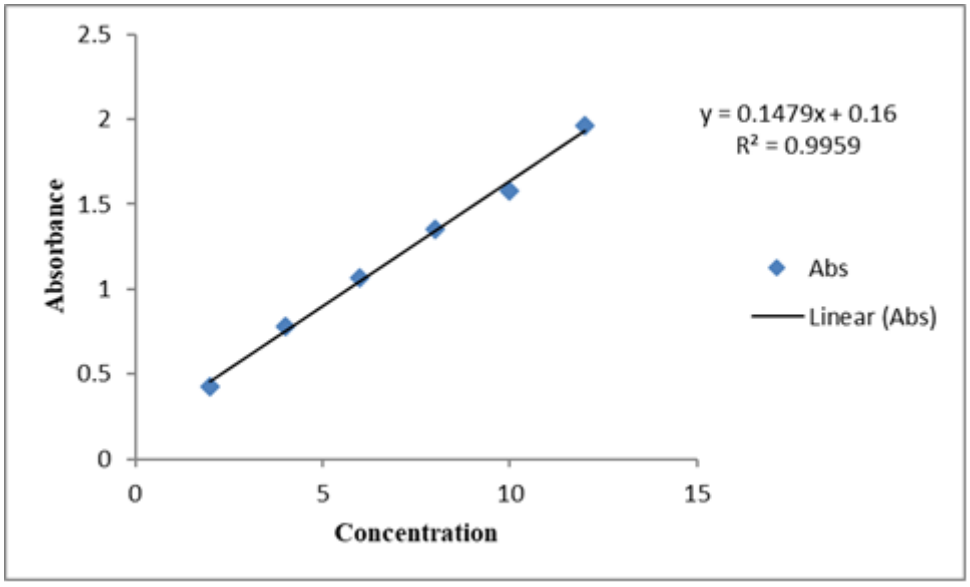

Figure 2. Standard calibration curve of EBZ.

3.4. Morphological Evaluation

Morphological evaluation of developed niosomal formulation was done using Leica microscopy (10x and 40x), which reveals the circular ring-like structure of niosomes. Morphological images of developed niosomes have been depicted in Figure 3. [19]

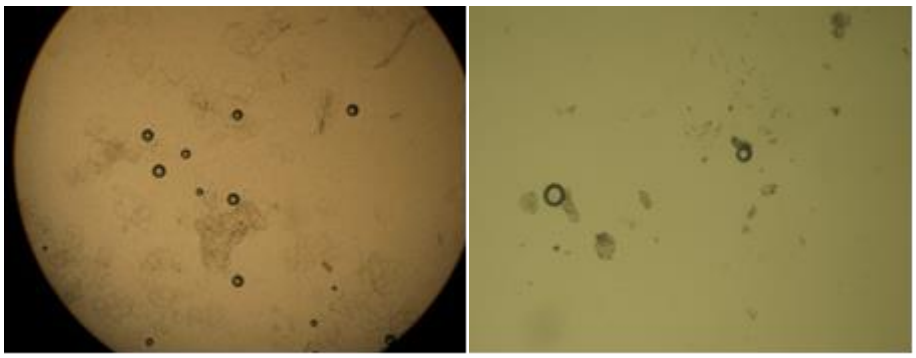

Figure 3. Leica microscopic image of niosomes at 10X and 40X.

3.5. Particle Size and Zeta-Potential

The particle size and zeta-potential were calculated for EBZ loaded niosomal formulation with the help of Malvern Zetasizer. The obtained particle size appeared in the range of 550–620 nm, and zeta-potential appeared in the range of −23.5 ± 1.89 mV to −25.5 ± 1.63 mV, respectively. The pictograph of particle size and zeta potential has been depicted in Figures 4 and 5, respectively. [10]

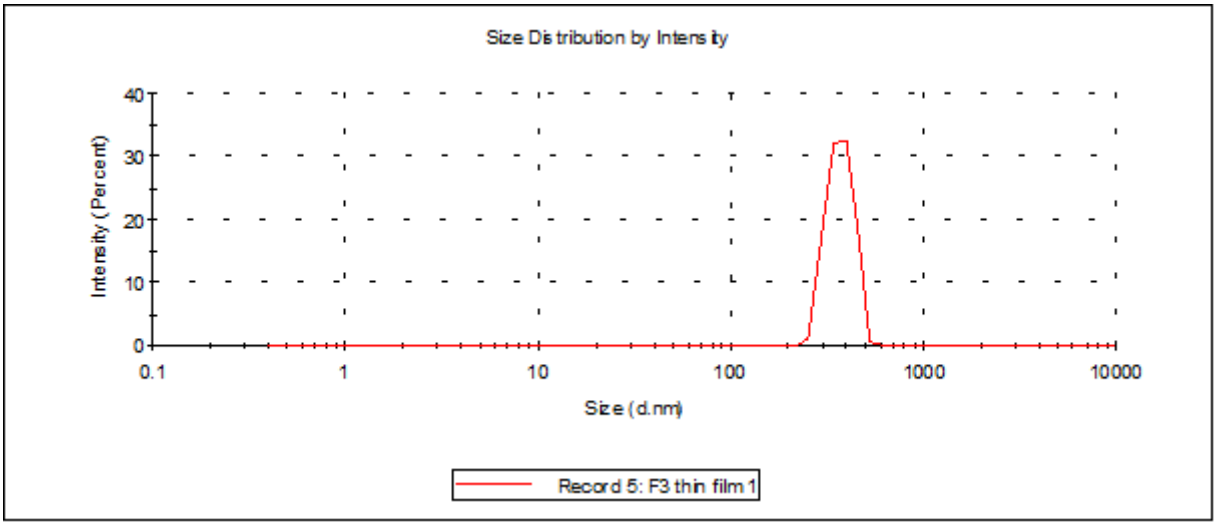

Figure 4. Graph representing particle size distribution of EBZ loaded niosomes.

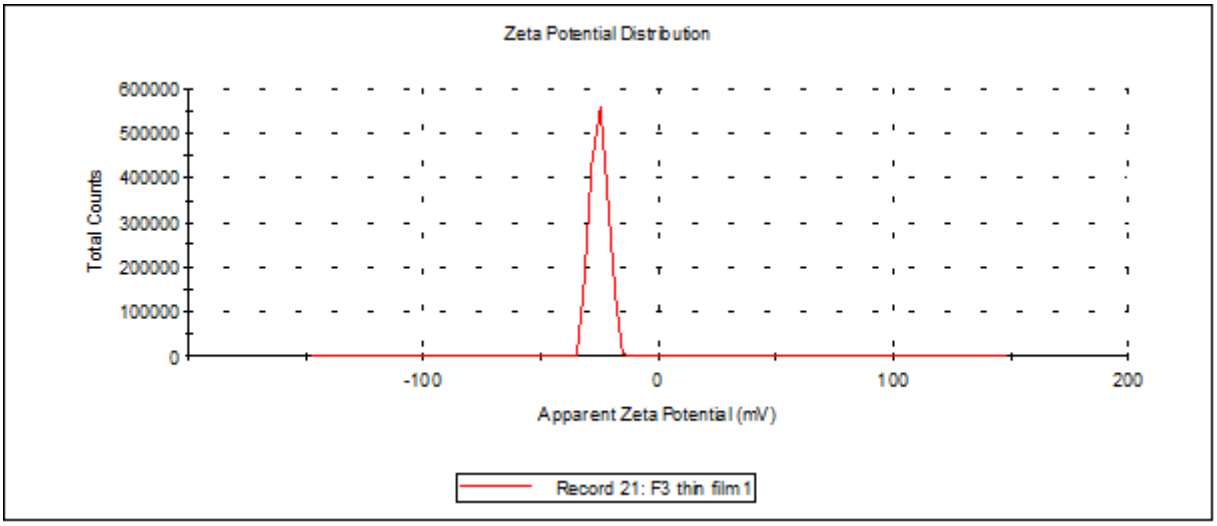

Figure 5. Graph representing zeta potential of EBZ loaded niosomes.

3.6. Entrapment Efficiency

The entrapment efficiency was performed using centrifugation method, and the obtained entrapment efficiency of EBZ in EBZ loaded niosomes was found to be 87.9%. Entrapment efficiency plays a significant role in the determination of the dose frequency of a particular formulation. It is generally assumed that the greater the loading efficiency of a particular therapeutic moiety in a given formulation, the lesser the dosing schedule will be if the drug releases from the system in a sustained or controlled release manner.

3.7. FTIR Studies

The drug loading was analyzed by comparing the functional group present among the EBZ, blank niosomes, and EBZ loaded niosomes. The combined FTIR spectra of EBZ, EBZ loaded niosome (EBZ-NS), and blank niosome (Blank-NS) has been shown in Figure 6.

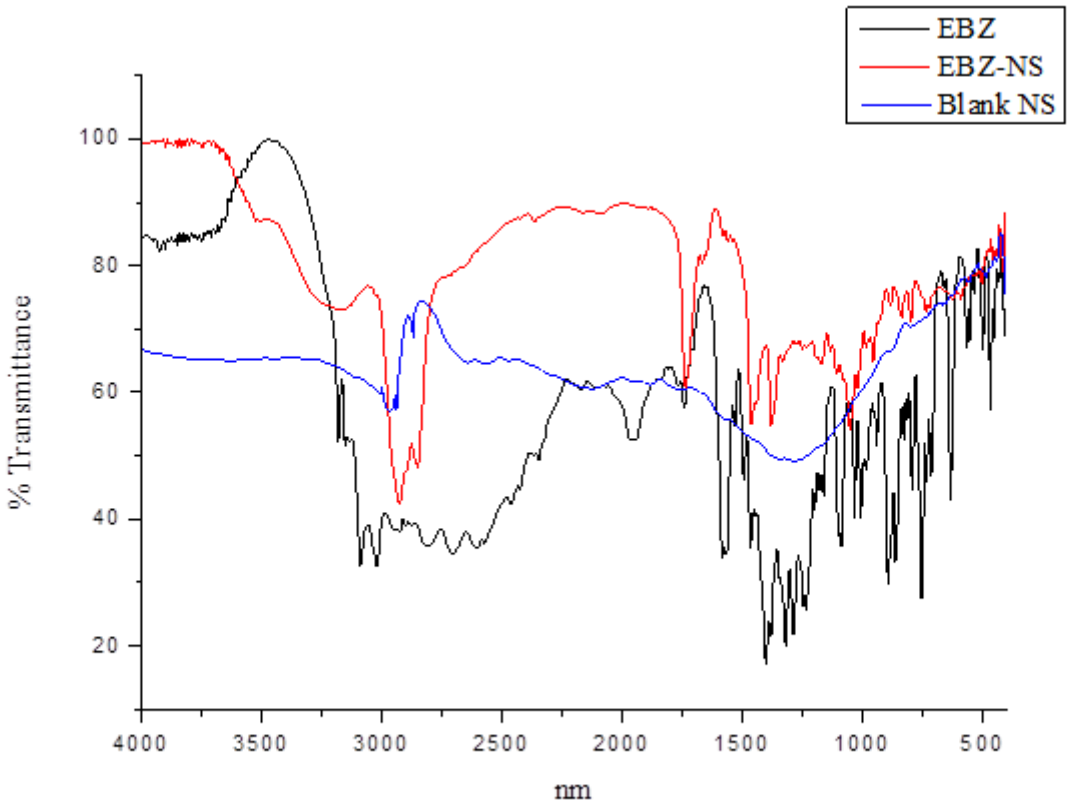

Figure 6. Combined spectra of EBZ, EBZ loaded niosomes, and blank niosomes.

The obtained results have been analyzed that there has been broadening of peak in EBZ loaded niosome at 3264 cm^{-1} which is very steep in EBZ and absent in blank niosomes. These results due to OH streching of Span 20 which is absent in EBZ. Few multiple peaks of EBZ appaered in the range of 2610 to 2805 cm^{-1} but same paek disappeared in EBZ loaded niosomes which may be strong amine salt streching. One broad peak at 1915 cm^{-1} may be due to aromatic C-H bending, which disappears in EBZ loaded niosome. The intensity of the C-N stretching peak at 1340 cm^{-1} in EBZ has been reduced in EBZ loaded niosomes, while it is absent in blank niosome which may suggest the entrapment of EBZ in niosomes. While multiple peaks in the range of 550 cm^{-1} to 850 cm^{-1} were characterised due to presence of C-Cl and it was also found to be reduced in EBZ loaded niosomes as compared to EBZ alone [18].

4. Conclusions

EBZ loaded niosomal formulation had been developed using thin-film hydration technology. These niosomal vesicles were then characterized based on particle size, zeta-potential, and drug entrapment efficiency. These vesicles were also observed under Leica microscopy revealed the spherical and ring like niosomal vesicles. Its negative zeta-potential of −25 mV suggests its better stability due to inter ionic repulsion, while its nano-size range may help in better penetration. Its high entrapment efficiency helps in dose reduction. EBZ loaded niosomal formulations are highly suitable for drug delivery in sustained and controlled release applications and may be a better alternative for the avaialble marketed preparation.

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

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