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In-vitro efficacies of topical microemulsions of clotrimazole and ketoconazole; and *in-vivo* performance of clotrimazole microemulsion

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ABSTRACT

Topical microemulsions comprising clotrimazole (CTZ) (1%) or ketoconazole (KTZ) (2%), clove oil (6%), tween-20 (25–30%) and aqueous phase (water/buffers) were prepared. Microemulsions were characterized for droplet size, polydispersity, pH value and tested for physical stress and thermodynamic stability. Effective mean diameter of CTZ and KTZ microemulsion droplets ranges between 30 and 50 nm. It was observed that, acidic external phase (pH 1.2, 3.0 and 4.1) for CTZ and KTZ microemulsion requires less emulsifier than the external phase of higher pH (5, 6). Microemulsions were physically stable and there was no substantial change in droplet size or polydispersity under stress condition of centrifugation, freeze-thaw cycle and high temperature. *In-vitro* efficacy of CTZ and KTZ microemulsions was tested against *Candida albicans* (ATCC 10231), and was found better than corresponding marketed creams. Microemulsions were found safe and non-irritant when investigated using female Wistar albino rats. *In-vivo* antifungal efficacy of CTZ microemulsion was studied in rats and compared with its cream. After three days of treatment the scabbing of skin lesion was started, and after five days the scabs became hard and started to erode from the skin. *In-vivo* observations suggested that CTZ microemulsion can be a successful approach for treatment of topical fungal infection.

1. Introduction

Candidiasis is a common fungal infection caused by Candida species. *Candida albicans* is the predominant yeast amongst pathogenic Candida species [1]. Generally, the topical candidiasis are oral, vaginal, rectal and cutaneous [1–3]. Treatment of topical infections is preferred by locally applying the medicinal product at the site of infection. Topical application of drug products for local treatment avoids/minimizes the unnecessary systemic exposure of other organs. Numerous formulations are available in the market for topical treatment of local candidiasis. Clotrimazole (CTZ), ketoconazole (KTZ) and miconazole, are used for the treatment of local candidiasis.

CTZ is a commonly used antifungal agent and included in the list of essential medicines by world health organization. It is available in several conventional dosage forms such as topical cream (Lotrimin AF) vaginal cream (Gyne-Lotrimin), vaginal tablets (Mycelex-G, Gyne-Lotrimin), lotions (Lotrimin AF), solutions (Lotrimin AF) and troche/lozenge (Mycelex). CTZ is a poorly water soluble broad spectrum antifungal agent used for local treatment of cutaneous and mucosal infections. Poor solubility of CTZ hampers its availability and may also limit its effectiveness [4]. KTZ is a broad spectrum antifungal agent. Several topical formulations of KTZ are available in the market including Nizoral® (shampoo), Xolegel® (gel), Extina® (foam), Ketozol® (cream). KTZ cream, 2% (Kuric™) is indicated for the topical treatment of cutaneous candidiasis caused by *Candida spp*.

The efficacy of drug substances has been improved by using several state of the art techniques. In addition to chemical/physicochemical modifications (e.g. salts, pro-drugs, polymorphs) in drug molecules; the formulation approaches for solubility, dissolution and permeability enhancement are frequently tested to improve the efficacy or bioavailability of drugs [5–7]. Amongst the

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formulation approaches, the microemulsion is a promising one, since it improves solubility and permeability of drug substances [8,9]. Solubility and dissolution of CTZ was improved by formulating it in nanoemulsion drug delivery system comprising Capryol® 90, Solutol® HS 15 and Gelucire® 44/14. The solubility of CTZ was enhanced up to 25 mg/ml and the 100% drug was released within 15 min in various dissolution media [10]. CTZ nanoemulsion showed better antimalarial activity in *Plasmodium berghei* infected mice than CTZ suspension [11]. Nawaz et al. reported that almond oil (2%) and tween-80 (0.5%) synergistically enhanced the permeability of CTZ through rabbit skin in an *in-vitro* investigation [12]. Self-nanoemulsified drug delivery systems comprising oleic acid, coconut oil, tween-20, polyethylene glycol-200 and n-butanol were prepared to increase the solubility and dissolution of CTZ [13]. Transdermal spray of CTZ comprising eutectic mixture (camphor and menthol, 1:1) improves its transport through skin and antifungal efficacy by improving the permeation of CTZ and increasing zone of inhibition [14]. CTZ loaded oleic acid vesicles showed prolonged release (up to 5 days) of drug from its vesicular formulation [15]. Transdermal delivery/skin permeation of CTZ was enhanced through proniosomal gel formulation. The proneosomal gel also showed higher anticandidial activity and it was presumed that the proneosomal formulation may have enhanced drug penetration through fungal cell wall [16]. Hashem et al. reported that microemulsion system of CTZ (isopropyl myristate, tween-80, n-butanol and water) has higher *in-vitro* anti-fungal activity against *Candida albicans* than conventional cream [4]. Microemulsions of KTZ were also developed in various compositions. Efficacy of KTZ was increased in these microemulsions. Badawi et al., reported that KTZ degraded severely in the microemulsion prepared in Labrafil M 2125, while it was more stable in microemulsions prepared using Isopropyl myristate [17].

The objectives of present investigations were to develop and characterize microemulsions of CTZ and KTZ for topical application. Another objective was to improve and compare the *in-vitro* and *in-vivo* antifungal efficacy of developed formulations against marketed products.

2. Materials and methods

Ketoconazole (KTZ) (PubChem CID: 3823) and Clotrimazole (CTZ) (PubChem CID: 2812) were kindly gifted by Tabuk Pharmaceutical Mfg. Co. KTZ was manufactured by Halcyon labs, batch no. HKTZ00312. Clove oil B.P. (PubChem CID: 12658395) was purchased from Alan Pharma United Kingdom. Tween-20 (PubChem CID: 443314) was purchased from Avonchem Ltd., United Kingdom. The MilliQ water was prepared through MilliQ Direct8. Kenazole® (2%) cream (batch no. 23238) manufactured by Pharma International Co. Amman-Jordan, was obtained from pharmacy shop at local market. Canesten® cream (1%) (Batch no. BHPJKBK), manufactured by Kern Pharma, S.L.-Spain, was obtained from pharmacy shop at local market. The standard strain is *Candida albicans* ATCC 10231. Diethyl ether was of Sigma-Aldrich, Product of Germany. Animal feed was manufactured by Grain Silos and Flour Mills Organization, Riyadh, Saudi Arabia.

2.1. Preparation of placebo formulations

Placebo formulations were prepared to check the physical stability and suitability of o/w system at different compositions of clove oil and emulsifier (tween-20). In placebo formulations, the clove oil content was kept constant while the emulsifier and external phase compositions were varying. The amount of clove oil taken was sufficient to solubilize the drug (CTZ or KTZ) and the emulsifier percentage was investigated to achieve stable clear transparent microemulsion. Ratio of clove oil to tween-20 in placebo formulations PL1, PL2, PL3, PL4 and PL5 was 1:1, 1:2, 1:3, 1:4 and 1:5; respectively. Five placebo formulations (PL1 to PL5) were prepared by mixing the clove oil (6%) with tween-20 over vortex (Vortex Gene®-2, Model no. 560, Scientific Industries Inc., Bohamia, N.Y. USA) in different ratio and then titrating the composition with water. The microemulsion compositions for loading drug substances were selected from placebo formulations after screening the suitable ratio of clove oil and emulsifier. Selection of placebo formulation was based on the clarity, droplets size and physical stability.

2.2. Optimization of sonication time

Effect of bath sonication and probe sonication on microemulsion droplets size and size distribution was optimized. Seven o/w microemulsions of clove oil (PL6-PL12) were prepared. Compositions of PL6-PL12 were similar to PL5 (clove oil 6%, tween-20 30% and water 64%). The clove oil and emulsifier (tween 20) were mixed with the aid of vortexer. The external phase (water) was added drop wise to the mixture of clove oil and emulsifier, while holding it on the vortexer. Formulation PL6 was kept aside without further treatment. The PL7 and PL8 were sonicated on sonication bath (Nexul ultrasonic cleaner, Kodo Technical Research Co. Ltd. Korea)) for 5 and 10 min respectively. Formulations PL9, PL10, PL11 and PL12 were first sonicated on sonication bath for 10 min and then they were ultrasonicated using a probe sonicator [model VCX130 (Sonics Ultracell™), Sonics and Materials Inc. (USA)] (frequency 20 kHz, power 130W) for 25, 50, 100 and 200 pulses; respectively. Each pulse was of 1 s sonication and 1 s pause, at amplitude of 60%. Effective mean diameter and polydispersity of PL6-PL12 were measured using 90 plus particle size analyzer (Brookhaven Instrument Corporation).

2.3. Preparation of drug loaded microemulsions

Oil in water microemulsions of CTZ (1%) and KTZ (2%) were prepared by simple titration method. The turbid compositions were also subjected to bath sonication and or probe sonication. KTZ (2%) loaded in the placebo composition PL4 and CTZ (1%) was loaded in placebo composition PL5 (see Table 1). Different external phases (water and buffer of different pH value) were used to prepare different microemulsion formulations of CTZ (1%) and KTZ (2%) (Table 1). The drug (CTZ or KTZ) was dissolve in the clove oil with the help of vortexer and or bath sonicator; and then emulsifier (tween-20) was added to the clove oil and drug mixture. The composition of drug, clove oil and emulsifier was mixed properly with the help of vortexer and or bath sonicator; and then external phase (water or buffer of different pH) was added drop wise while vortexing the mixture. This biphasic composition was kept in sonication bath for 10 min and or exposed to the probe sonication (two cycles of 25 pulses each, each pulse of 1 s sonication and 1 sec pause, at amplitude of 60%). The clear transparent compositions CF9, CF12, KF3, and KF6 were selected for further studies.

2.4. Characterization of microemulsions

Microemulsions of CTZ (CF9, CF12) and KTZ (KF3, KF6) were characterized for droplet size and size distribution, pH value and physical thermodynamic stability.

2.4.1. Droplets size and polydispersity

Droplets size and polydispersity of selected formulations CF9, CF12, KF3, and KF6 were determined. Measurement of effective mean diameter and polydispersity of developed microemulsions