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In-Vivo Pharmacokinetics Study of Exemestane Hydrochloride Self-nanoemulsifying Drug Delivery Systems via Oral Route

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Abstract: Exemestane HCl (EXM) is a novel irreversible steroidal aromatase inhibitor for the adjuvant treatment of hormonally responsive breast cancer in postmenopausal women. Poor aqueous solubility of EXM is the biggest hurdle for developing solid oral dosage forms. That's why the current study aims to evaluate the pharmacokinetics of formulating the EXM loaded self nano emulsifying drug delivery (SNEDDs) system. SNEDDs were formulated using Labrafac CC (20% w/v), Tween 80 (27%w/v), and Triacetin (54%w/v) as oil, surfactant, and co-surfactant, respectively, by water titration method. A comparative Pharmacokinetics study of EXM suspension and EXM SNEDDS was performed using a female waster rate. The developed formulation had a 37.65± 5.08 nm size and a 21.57±0.73 sec of self-emulsification time. Cmax of EXM suspension and EXM SNEDDS was found to be 122.49±8.27 and 194.86 ± 14.75 ng/mL, respectively. AUC0-720 of EMX SNEDDS was 1.71 times higher compared to EXM suspension, indicating that lipid nanoparticles improve the drug concentration in the plasma. So we conclude that SNEDDS improves the pharmacokinetic of EXM, which subsequently improves oral bioavailability.

Keywords: exemestane hydrochloride; SNEDDS; bioavailability.

1. Introduction

Exemestane HCl (EXM) is BCS class II drug for hormonally sensitive breast cancer as adjuvant therapy. EXM is practically insoluble in water and a highly lipophilic drug. Due to this, EXM is limited oral bioavailability (42 %) with higher plasma protein binding (90%). EXM is highly metabolized by the liver's metabolic enzymes [1]. Low water solubility is a prime challenge for formulation scientists. That's why, For the improvement of the solubility of EXM, various approaches were explored by different researchers, such as solid dispersion [2,3], cyclodextrin complexation [4], micelles [5], nanosuspension [6], polymeric nanoparticles [7] etc.,.

Different approaches to solubility enhancement have their merits with demerits. As in cancer therapy, one of the key aspects is lymph node targeting [8,9]. It is well reported that tumor cells are spreading through the lymphatic systems [10]. There are different ways to target

lymph nodes [11,12]. In the oral route, triglyceride-based Nanostructured lipid systems are highly explored by many researchers [13-15]. From that, self-nano emulsifying drug delivery system (SNEDDS) [16] is widely explored for the oral delivery of lipophilic anti-cancer and anti-HIV drugs and is also commercially available such as Ritonavir and Saquinavir loaded SNEDDS [17-19]. SNEDDs are pro-nanoemulsion, a pre-mix of the oil, surfactant, and co-surfactant. In the body, pre-mix is converted into the 5-100 nm fine droplet of nanoemulsion (NE) by diluting the stomach or body fluid. Developed NE is solubilized drug encapsulated inside the oil to improve the rate and extent of absorption [17-19].

Current research work aims to evaluate the pharmacokinetics of developed EXM-loaded SNEDDS via the oral route. EXM SNEDDS was prepared by the water titration method, and future size reduction was made using the ultrasonication technique. Developed SNEDDS were characterized for size, PDI, and self-emulsification time. After satisfactory results, the Pharmacokinetics of developed EXM SNEDDS was performed. EXM suspension was formulated for comparison purposes, and the pharmacokinetics study was perfumed. Different Pharmacokinetics parameters such as Cmax, Tmax, and AUC were calculated for better comparison between EXM SNEDDS and EXM Suspension.

2. Materials and Methods

2.1. Materials.

EXM was received as a gifted sample from Astron Research Center, Ahmedabad, India. Labrafac^{TM} cc was gifted by GATTEFOSSE (France). Tween-80 was procured from S.D. Fine-Chem. Ltd., Mumbai, India. Triacetin was procured from the Central Drug House, New Delhi, India.

2.2. Methods.

2.2.1. Calibration curve of EXM by HPLC.

A standard solution of EXM (100µg/ml) was prepared in methanol. From the standard stock solution, different aliquots ranging from 0.5 to 5 µg/mL of EXM were prepared with appropriate dilution with methanol. A sample of 20µl was manually injected into Shimadzu (Columbia, MD) RP-HPLC instrument (LC-2010C_{HT}). Phenomenex (Torrance, CA) C_{18} column (250 mm × 4.6 mm id, 5 µm particle size) and LC-solution software were used. Acetonitrile: Methanol (40:60) was used as a mobile phase at a flow rate of 1 mL/min.

2.2.2. Formulation of SNEDDS.

SNEDDS was formulated using the water titration method. First, EXM (equivalent to 250 mg) was dissolved in Labrafac Triacetin CC (2mL) in continuous stirring using a magnetic stirrer (700 RPM, 30 min). Premixed Smix (2.7 mL of Tween-80 and 5.4 mL Triacetin) was added dropwise into the oil-containing drugs (250 mg) and mixed using a magnetic stirrer. This mixture was ultrasonicated for 30 min for size reduction. Develop SNEDDS evaluated for self-emulsification, size, and PDI. *In-vivo* pharmacokinetics study was performed [20].

2.2.3. Evaluation of SNEDDS.

2.2.3.1. Self-emulsification capacity of SNEDDS.

The self-emulsification capacity of EXM SNEDDS was evaluated by measuring the time needed for the emulsification using a stopwatch. For that, EXM SNEDDs were diluted 100X with various solvents, i.e., water, 0.1 N HCl, and phosphate buffer. Diluted SNEDDs were kept for 8 h at room temperature to check their stability [13,21].

2.2.3.2. Globule size & Polydispersity index (PDI).

Globule size and PDI of developed EXM SNEDDs were evaluated using the Malvern Zetsizer (Model HAS 3000, 90° angle) at 25°C [22].

2.2.4. Pharmacokinetics study.

Pharmacokinetics studies in animals were approved and concerted in consonance with the precept of the institutional animal ethics committee (Registration No.: IAEC/SKPCPER /2011-01/33, under CPCSEA, Delhi, India). All animal studies were performed in the Central Animal Facility of S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Gujarat.

Female Wister rats (Weight range = 170 to 210 gm, 8-10 weeks old) were employed for the bioavailability study. Animals were divided into 2 groups (1stgroup: EXM suspension and 2nd group: EXM SNEDDS), and each group contained 3 animals. Each animal group received the equivalent of 18 mg/kg of EXM via the oral route. The dose was calculated based on body surface area. Animals were kept overnight fasting before the experiment.

Blood samples (0.5-1ml) were collected into sodium EDTA containing Eppendorf tube from the eye at 30, 60, 120, 240, 480, and 720 min intervals after administration from alternate rat. After that blood was immediately centrifugation at 10000 rpm for 10 min. Extraction of EXM from the plasma was done by the Protein precipitation method. Methanol was added to the plasma and centrifuged the result of the plasma at 10000 rpm for 10 min. After that supernatant was collected and stored at -20°C until analysis. Spike methods were used to determine EXM in the rat plasma using HPLC.

Pharmacokinetic software (PK Functions for Microsoft Excel, Pharsight Corporation, Mountain View, CA) was used to calculate different Pharmacokinetics parameters such as C_{max}, T_{max}, AUC_{0-720}, and $T_{1/2}$.

3. Results and Discussion

3.1. Analytical method development.

The developed analytical method was shown the linear regression in the range of 0.5–5 µg/mL concentration of EXM with the 0.997 R^2 value. The limit of detection and Limit of quantitation was found to be 0.08µg/mL and 0.24 ng/mL, respectively. RSD value of the developed method was found to be 0.423% which is lower than 1%. The result suggested that the developed method was sensitive and repeatable.

3.2. Formulation of SNEDDS.

EXM SNEDDS was successfully formulated with complete solubilization of drug.

3.3. Evaluation of SNEDDS.

3.3.1. Self-emulsification time.

Self-emulsification time was found to be 21.57±0.73 sec which is lower than 1 min. After emulsification with the water, 0.1 N HCL, and phosphate buffer pH 5.8, the formulation was optical clear without any sign of EXM precipitation, which indicated that surfactant and co-surfactant stabilized the NE in all body conditions [13,21].

3.3.2. Globule size & Polydispersity index (PDI).

Globules size and PDI of EXM SNEDDS was found to be 30.35±2.81 nm and 0.283±0.032 in 0.1 N HCl, respectively. Smaller globule size and PDI improved the rate and extent of EXM release from the NE, which improved the absorption of EXM. The smaller size of SNEDDs was due to the presence of Tween-80 and Triacetin. It was also reported that Tween-80 reduced the interfacial tension between two immiscible layers and stabilized NE droplets by steric stabilization. Triacetin (triglycerides) not only improves solubility but is also helpful for the improvement of bioavailability. It was reported that triglyceride solubilized the chylomicrons captured components, which were rich in lymphatic systems [13,21].

3.4. Pharmacokinetic studies.

A comparative study of optimized SNEDDS vs. drug suspension prepared in sodium CMC was performed. Plasma EXM concentration vs. Time curve was shown in figure 1.

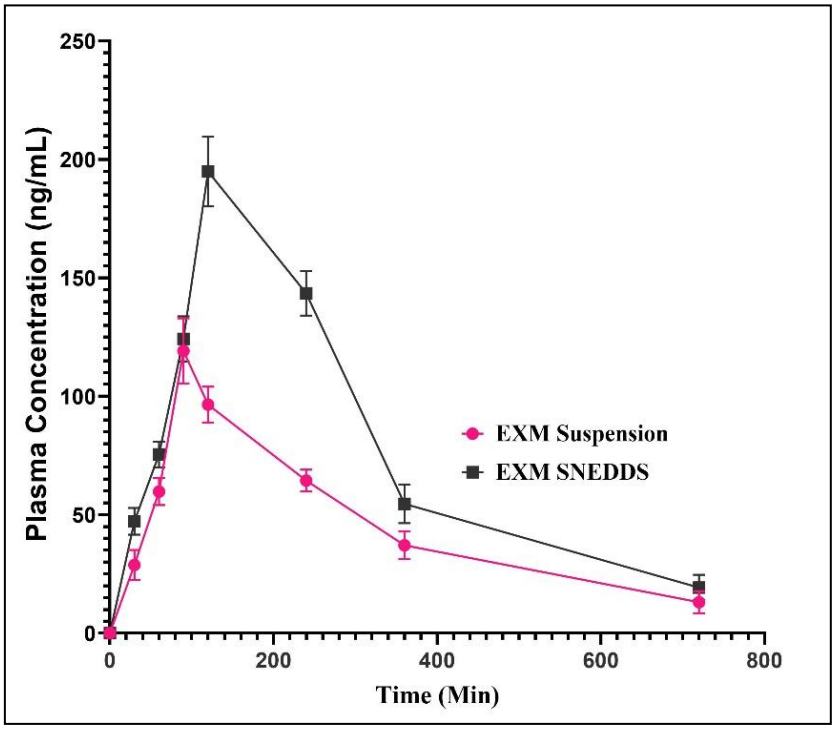

Figure 1. Plasma concentration vs. time curve of EXM.

The Cmax, Tmax, AUC_{0-720}, and $K_{elimination}$ consent were calculated using the pharmacokinetics curves. The Cmax of EXM Suspension and EXM SNEDDS was found to be 112.49±8.27 and 194.86±14.75, respectively. It was indicated that lipophilic components of SNEDDS enhanced the EXM concentration in plasma and increased the tmax [17-19]. The Tmax of EXM Suspension and EXM SNEDDS was found to be 90 and 120 min. Elimination consent was found to be 0.139±0.035 (EXM suspension) and 0.134±0.026 (EXM SNEDDS). Again, AUC0-720 of EXM Suspension and EXM SNEDDS was found to be 32566.12 ± 139.43 and 55812.79 ± 415.11, respectively [23,24]. It is reported that surfactants improve solubility and the same time, permeability also. The issue with lipophilic drugs is the inadequate solubility in the gastrointestinal fluid, which decreases the dissolution process and, ultimately, absorption from the gastrointestinal fluid. When it reaches the stomach, it is converted into a nanoemulsion within 21 sec, so EXM is available in soluble forms [23,24]. Labrafac CC is also reported for the enhancement of the bioavailability of lipophilic components [25]. Overall, EXM oral bioavailability was improved due to the smaller globule size and presence of lipophilic components with the surfactant.

4. Conclusions

The current study aims to evaluate the pharmacokinetics of EXM SNEDDS via the oral route. The developed SNEDDs are lower in size than PDI, and have good emulsification capacity with stability. Higher Cmax and AUC0-720 of EXM SNEDDS compared to EXM suspension indicated that the developed formulation improved the bioavailability of EXM. Further, Scale-up, pre-clinical pharmacodynamics, and toxicity studies will be required to evaluate the market potential of developed EXM SNEDDS.

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Conflicts of Interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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