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Research Article

Intranasal Microemulsion of Sildenafil Citrate: *In Vitro* Evaluation and *In Vivo* Pharmacokinetic Study in Rabbits

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Abstract. The purpose of the present study was to prepare intranasal delivery system of sildenafil citrate and estimate its relative bioavailability after nasal administration in rabbits to attain rapid onset of action with good efficacy at lower doses. Sildenafil citrate saturated solubility was determined in different solvents, cosolvents, and microemulsion systems. For nasal application, sildenafil citrate was formulated in two different systems: the first was a cosolvent system (S3) of benzyl alcohol/ethanol/water/Transcutol/taurodeoxy cholate/Tween 20 (0.5:16.8:47.7:15.9:1:18.1% *w*/*w*). The second was a microemulsion system (ME6) containing Oleic acid: Labrasol/Transcutol/water (8.33:33.3:16.66:41.66% *w*/*w*). The prepared systems were characterized in relation to their clarity, particle size, viscosity, pH, and nasal ciliotoxicity. *In vivo* pharmacokinetic performance of the selected system ME6 (with no nasal ciliotoxicity) was evaluated in a group of six rabbits in a randomized crossover study and compared to the marketed oral tablets. The targeted solubility (>20 mg/ml) of sildenafil citrate was achieved with cosolvent systems S1, S3, and S5 and with microemulsion systems ME3–ME6. The saturated solubility of sildenafil citrate in cosolvent system S3 and microemulsion system ME6 were 22.98±1.26 and 23.79±1.16 mg/ml, respectively. Microemulsion formulation ME6 showed shorter t_{max} (0.75 h) and higher $AUC_{(0-\infty)}$ (1,412.42 ng h/ml) compared to the oral tablets which showed t_{max} equals 1.25 h and $AUC_{(0-\infty)}$ of 1,251.14 ng h/ml after administration to rabbits at dose level of 5 mg/kg. The relative bioavailability was 112.89%. In conclusion, the nasal absorption of sildenafil citrate microemulsion was found to be fast, indicating the potential of nasal delivery instead of the conventional oral administration of such drug.

KEY WORDS: bioavailability; intranasal; microemulsion; nasal; sidenafil citrate; solubilization.

INTRODUCTION

Erectile dysfunction (ED) is estimated to affect up to 50% of men between the ages of 40 and 70 years (1,2). ED is frequently associated with depression, increased anxiety, and poor self-esteem and compromises interpersonal relationships (3). In diabetes mellitus, ED occurs at an earlier age and has a higher prevalence. ED is a side effect commonly associated with use of the selective serotonin uptake inhibitors to treat depression (4). To induce penile erection, relaxation of the smooth muscle cells of the corpus cavernosum and associated arterioles is required. A major component of this relaxation process is mediated by nitric oxide stimulation of cyclic guanosine monophosphate (cGMP). In response to sexual stimulation, the release of nitric oxide by nerve endings and endothelial cells increases the levels of cGMP to induce the erection. cGMP is readily hydrolyzed by phosphodiesterase 5 (PDE5) resulting in restoration of quiescent muscle tone and detumescence (5). Sildenafil citrate is selective inhibitor of PDE5 with IC_{50} values of 3.9 nM (6). It is rapidly absorbed after oral administration with absolute bioavailability 40%. A high-fat meal delays the onset of action for sildenafil (7). A sublingual preparation of sildenafil has been developed, and this will not be affected by food. An initial study with sublingual sildenafil (20 mg) has shown that the mean onset of action was 15.5 min and lasted for an average of 40 min with 13/20 of subjects with ED achieving erections (8). Nasal delivery has been paid attention as an alternative dosage form. Nasal mucosa offers the possibility for simple and comfortable drug administration. Nasal mucosa possesses a total thickness of only about 100 μm and consists of various cell types, which are ciliated and nonciliated columnar cells, goblet, and basal cells (9). The nasal respiratory region, which extends backward by approximately 6–8 cm to the nasopharynx, is the primary site for drug absorption. The mucosa in this region consists of an epithelium resting on a basement membrane connected with two to four layers of submucosa (10). Epithelial cells in the nasal mucosa are generally in close apposition, and adjacent cells are extremely adherent. Cohesion at the apices of epithelial cells occurs in specialized regions collectively referred to as the junctional complex. This structure controls the diffusion of ions and molecules between cells and constitutes a barrier to the intercellular movement of macromolecules across the epithelium (11). A lipophilic component in the formulation seems to be advantageous, but in case of nasal administration, incompatibilities

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can be indicated by impairment of ciliary movement. Use of a microemulsion may minimize these side effects by administering a lipophilic component in a transparent, water-continuous system (12,13).

Advantages of microemulsion include its ease of preparation due to spontaneous formation, thermodynamic stability, transparent and elegant appearance, increased drug loading, enhanced penetration through the biological membranes, increased bioavailability (14,15), and less inter- and intra-individual variability in drug pharmacokinetics (16). These advantages make microemulsions attractive drug delivery systems.

The advantages of nasal route have been suggested as follows: rapid absorption, higher bioavailability allowing lower doses, fast onset of therapeutic action, avoidance of liver or gastrointestinal metabolism, avoidance of irritation of the gastrointestinal membrane, reduced risk of overdose, non-invasive administration, ease of convenience and self-medication, and improved patient compliance (17). Many authors concluded that the nasal route has been found to give improved bioavailability compared to the oral route as in case of metoclopramide HCl (18), propranolol (19,20), and midazolam (21). So, it would be valuable to develop a sildenafil nasal delivery system to increase its bioavailability and hence decrease the administered dose and also to attain rapid onset of action.

This study aimed to develop a new sildenafil intranasal formula and to study its relative bioavailability compared to the conventional oral tablets after intranasal administration to rabbits.

MATERIALS AND METHODS

Materials

Sildenafil citrate was a gift from Sigma Co, Egypt; hydrochloric acid (HCl), citric acid, propylene glycol, polyethylene glycol 400 (PEG 400), ethanol, isopropyl myristate (IPM), dioxane, benzyl alcohol, and isobutanol were obtained from Fisher Scientific (Fair Lawn, NJ, USA); miglyol, Labrasol, labrafil® M, and Transcutol were kindly obtained from (Gattefoss, St. Priest, France); oleic acid, taurodeoxycholate, and Tween 20 were purchased from Sigma Co. (St. Louis, MO, USA), dimethyl isosorbide (DMI) was obtained from (Aldrich Chemical Company, Milwaukee, WI, USA).

Preparation of Sildenafil Citrate Cosolvent Systems

Determination of Sildenafil Citrate Saturated Solubility in Different Solvents

Excess amounts of sildenafil citrate were mixed with 2 ml of various solvents viz. 0.1 N HCl, 10% citric acid solution, propylene glycol, PEG 400, 10% hydroalcoholic solution, 50% aqueous PEG 400 solution, DMI, and distilled water in separate vials. The vials were well closed and left to be shaken at 25°C for 24 h in thermostatically controlled shaking water bath. The suspensions were centrifuged at 5,000 rpm for 10 min. The contents of each vial were filtered through a 0.45-μm filter, and the supernatant was assayed for the drug content using high performance liquid chromatography (HPLC) method.

Determination of Sildenafil Citrate Solubility in Different Cosolvent Systems

Different cosolvent systems were used to determine the maximum solubility of sildenafil citrate by the same method mentioned in the previous step. Systems S1 to S5 were prepared according to the composition listed in Table I.

Preparation of Sildenafil Citrate Microemulsion Systems

Determination of Saturated Solubility of Sildenafil Citrate in Different Oils

This step was performed as previously discussed in order to select the suitable oil which has a good solubilizing capacity for sildenafil citrate. This oil can be used as the oil phase in microemulsion system. The oils selected were isopropyl myristate, miglyol, labrafil® M, and oleic acid.

Table I. Composition of Different Cosolvent Formulations (%, *w*/*w*) and Saturated Solubility of Sildenafil Citrate in these Solvent Mixtures at 25°C

	S1	S2	S3	S4	S5
Composition	% *w*/*w*	% *w*/*w*	% *w*/*w*	% *w*/*w*	% *w*/*w*
Benzyl alcohol	17.3	0.5	0.5	0.5	0.5
Distilled H_2O	47.7	47.7	47.7	47.7	47.7
Transcutol	15.9	15.9	15.9	15.9	15.9
Taurodeoxycholate	1.0	1.0	1.0	1.0	–
Tween 20	18.1	18.1	18.1	18.1	19.1
PEG 400	–	16.8	–	–	–
Ethanol	–	–	16.8	11.8	–
Isobutanol	–	–	–	5	–
DMI	–	–	–	–	16.8
Labrasol	–	–	–	–	10
Saturated solubility (mg/ml)±SD	34.90±2.25	18.88±1.20	22.98±1.26	19.35±1.99	20.05±2.13

Formulation of Different Microemulsion Systems and Determination of Solubility of Sildenafil Citrate in These Systems

The pseudoternary phase diagrams of the oil (oleic acid), distilled H_2O and surfactant (Labrasol), and cosurfactant (Transcutol, plurol or DMI) mixtures were constructed at room temperature (25°C). Appropriate quantities of oleic acid, Labrasol, and cosurfactant were stirred at high rate until it formed clear solution, then water was added dropwise to each mixture under vigorous stirring. The microemulsions were left to attain equilibrium. The resulting microemulsions were tightly sealed and stored at 25°C for further evaluation. The composition of each microemulsion system is shown in Table II.

The solubilization capacity of each microemulsion for sildenafil citrate was investigated. Increasing amounts of sildenafil citrate were added portion-wise to 2 ml of each microemulsion system with stirring after each addition to form a clear and transparent liquid till excess solid does not dissolve. The mixture was stirred at 25°C for 24 h. The solubility of the drug was determined as mentioned before, but filtration was done without centrifugation.

Measurement of pH, Viscosity, and Droplet Size

The pH of cosolvent systems and microemulsions were measured using pH meter (Model C G 820, Schott Gerate, Germany). The viscosities of the cosolvent systems and microemulsions were evaluated at 25°C using a viscometer (Brookfield Viscometer, model LVT, USA). The mean diameter of the droplets of the microemulsions was measured at 25°C using image analysis software. Samples of sildenafil microemulsions were examined microscopically for particle size analysis at a magnification of ×10 and ×40 with a Leica image analyzer Model Q 550IW equipped with Leica DM LB microscope (Cambridge, England), which consists of binocular microscope equipped with a computerized digital camera. A drop of microemulsion preparation was placed on a microscope slide and then was examined. The mean diameter of the microemulsion droplets was measured after storage for 3 months at 25°C.

Nasal Ciliotoxicity

Nasal ciliotoxicity studies were carried out using *in situ* toad palate model with minor modification (22). The upper palate of the male toads (20–30 g, Animal house of College of Pharmacy, Cairo University, Egypt) was exposed and treated with 0.5 ml of tested microemulsion containing sildenafil citrate (20 mg/ml) or with solvent system containing sildenafil citrate (20 mg/ml) for 30 min then rinsed with saline. The palate was dissected out, and the mucocilia was examined with an optical microscope (Leica Imaging Systems, Cambridge, England). Saline was used as a control.

***In Vivo* Nasal Absorption**

Animal Handling and Drug Administration

Six male New Zealand white rabbits weighing 2.30± 0.12 kg were housed individually in stainless steel cages, fed a commercial laboratory rabbit diet, and allowed free access of water. The rabbits were fasted for 18 h prior to and during the pharmacokinetic study. The animals were conscious throughout the duration of the experiments and were held in rabbit restrainers during blood sampling. In a crossover study with 1 week apart as a wash-out period, the selected sildenafil microemulsion was administered intranasally to each rabbit in a dose of 5 mg/kg divided equally in each of the two nostrils using a micropipette inserted 1 cm into the nostril. Sildenafil citrate tablet (Viagra® 50 mg) was crushed and suspended in 5 ml of saline. The equivalent volume containing 5 mg/kg was administered orally via gastric intubation for each fasted rabbit. For all animal studies, the experimental procedures conformed to the ethical principles of the Center of Applied Research and Advanced Studies, Faculty of Pharmacy, Cairo University on the use of the animals. All animals were treated according to the principles of laboratory animal care (National Institutes of Health publication #86-32) (23).

Sample Collection and Analysis

After administration of the different formulations, blood samples (1.5 ml) were collected at time intervals of 0.00, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, and 7.00 h from the central auricular artery of the rabbits. Blood samples were allowed to clot and then centrifuged at 3,000 rpm for 10 min. The obtained serum samples were deep-frozen at −20°C, pending HPLC analysis. The plasma analysis of sildenafil was performed using a well validated HPLC-UV method after liquid–liquid extraction. Aliquots of 0.5 ml plasma were pipetted into 4-ml centrifuge tubes, 100 µl of internal standard (Glimepiride) was added and vortexed for 10 s. Then, 100 µl (1.0 M) hydrochloric acid was added and then vortexed for 30 s. Ethyl acetate (4.0 ml) was added and vortexed for 30 s, then centrifuged for 10 min at 4,000 rpm. The organic layer was decanted into clean

Table II. Composition of Different Microemulsion Systems (%, *w*/*w*) and Saturated Solubility of Sildenafil Citrates in these Systems at 25°C

	ME1	ME 2	ME 3	ME 4	ME 5	ME 6	ME 7
Composition	% *w*/*w*	% *w*/*w*	% *w*/*w*	% *w*/*w*	% *w*/*w*	% *w*/*w*	% *w*/*w*
Oleic acid	4	4	8.33	8.33	8.33	8.33	10
LabrasolLabrasol	40	30	40	33.33	40	33.33	20
Transcutol	10	10	10	16.67	10	16.67	20
DMI	20	26	20	16.67	–	–	–
Distilled H_2O	26	30	21.67	25	41.67	41.67	50
Saturated solubility (mg/ml)±SD	16.83±1.78	18.44±1.70	23.58±2.34	22.30±2.48	21.12±2.11	23.79±1.16	15.36±1.10

centrifuge tubes, then evaporated using vacuum concentrator (Eppendorf Concentrator 5301, Germany) till dryness at 45°C. The residue was reconstituted with 100 μL of mobile phase, and then 25 μl was injected into the HPLC system using autosampler (SIL-10A Shimadzu, Japan). The stationary phase used was Discovery C_{18} (Supelco; 250×4.6 mm, 5 μm particle size). The guard column was Thermo C_{18} (5×4.0 mm, 5 μm particle size). The mobile phase consisted of acetonitrile and (0.05 M) phosphoric acid (50%, 50%, *v/v*), adjusted at pH 4.00 with phosphoric acid before filtration. The mobile phase was delivered into the HPLC apparatus at flow rate of 0.5 ml/min (isocratic pump, Model LC-20AD, Shimadzu, Japan). The detection wave length was 220 nm (ultraviolet variable wavelength detector, Model SPD-20A Shimadzu, Japan). All assays were performed at ambient temperature.

Pharmacokinetic Analysis

Pharmacokinetic analysis was performed by means of a model independent method **(**noncompartmentally) using a Kinetica™ 2000 computer program. The elimination rate constant (Lz) was obtained as the slope of the linear regression of the log-transformed plasma concentration values *versus* time data in the terminal phase. The elimination half-life ($t_{1/2}$) was calculated as 0.693/Lz. The area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-t}+C_t/Lz$, where C_t is the last measurable concentration.

RESULTS

Cosolvent Systems

The saturated solubility of sildenafil citrate in different solvents at 25°C presented in Table III could be arranged in an ascending order as follows: 1.40±0.17, 3.20±0.11, 3.31±0.22, 3.61±0.25, 4.05±0.23, 4.79±0.40, and 6.89±0.62 mg/ml for 10% citric acid, distilled water, propylene glycol, 10% ethanol in water, PEG 400, 50% PEG 400 in water, and 0.1 N HCl, respectively. The maximum solubility was achieved in DMI (9.98±0.79 mg/ml).

The solubilities of sildenafil citrate in different cosolvent mixtures at 25°C **(**Table I**)** were 34.90±2.25, 18.88±1.20, 22.98±1.26, 19.35±1.99, and 20.05±2.13 for S1, S2, S3, S4, and S5, respectively.

Table III. Saturated Solubility of Sildenafil Citrate in Different Solvents at 25°C

Solvent	Saturated solubility (mg/ml)±S.D
Distilled water	3.20±0.11
0.1 N HCl	6.89±0.62
10% Citric acid	1.40±0.17
Propylene glycol	3.31±0.22
PEG 400	4.05±0.23
10% ethanol in water	3.61±0.25
50% PEG 400 in water	4.79±0.40
DMI	9.98±0.79

Table IV. Saturated Solubility of Sildenafil Citrate in Various Oils at 25°C

Oil	Saturated solubility (mg/ml)
IPM	3.18±0.33
Miglyol	4.23±0.39
Labrafil	2.34±0.21
Oleic acid	6.77±0.54

Microemulsion Systems

Solubilities of sildenafil citrate in different oils at 25°C were presented in Table IV. They were 3.18±0.33, 4.23±0.39, and 2.34±0.21 mg/ml for IPM, miglyol, and labrafil, respectively. Oleic acid showed the highest solubility (6.77±0.54 mg/ml) compared to other oils so it was chosen as the oil phase for microemulsion preparation.

The formation of microemulsion systems (ME1–ME7) was observed at room temperature. During the addition of water to the selected oily mixtures (mixture of oleic acid, Labrasol, Transcutol in addition to DMI in all formulations, a continuous transition from water in oil systems (W/O) to oil in water (O/W) systems was observed, a transparent, one-phase and low viscous system was obtained. The O/W microemulsions formed are shown in three-component triangular diagram (Fig. 1).

The solubilities of sildenafil citrate in different microemulsion systems at 25°C are shown in Table II. The required solubility of sildenafil citrate (>20 mg/ml) was achieved in microemulsion systems ME3–ME6 which contained 8.33% oleic acid; there was no statistically significant difference in

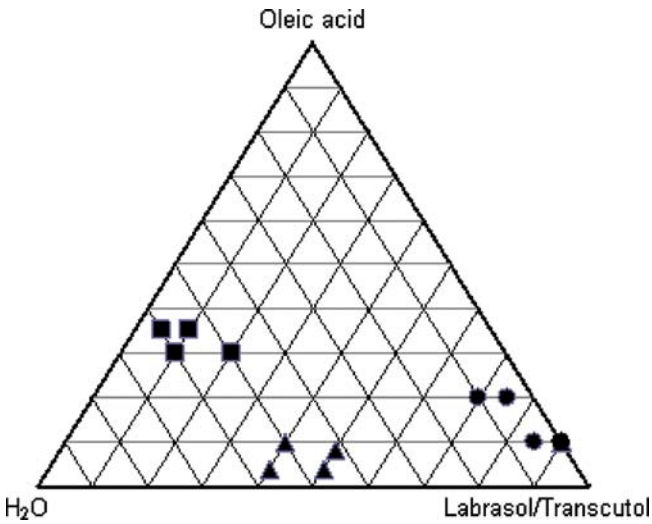

Fig. 1. Pseudo ternary phase diagram of a microemulsion system composed of oil (oleic acid), surfactant (Labrasol), cosurfactant (Transcutol), and water. *Closed triangles* microemulsion, *closed circles* clear gel, and *closed squares* emulsion

Table V. Droplet Size, Apparent Viscosity, pH, and Clarity of the Selected Microemulsion and Solvent Vehicles (Mean±SD, n=3)

Formulation	Droplet size (nm)	Viscosity (mpa•s)	pH	Clarity
ME6	37.5±1.9	472±33	6.0±0.5	Clear
ME6 (stored for 3 months at 25°C)	38.2±2.2	475±45	6.0±0.5	Clear
S3	–	265±23	4.5±0.5	Clear

solubilization for sildenafil citrate among these four microemulsion formulations ($p<0.05$).

pH, Viscosity, and Droplet Size

All the prepared systems were transparent and clear even after storage for 3 months. The pH, viscositi of cosolvent system S3 and microemulsion system ME6, and the droplet size of ME6 are shown in Table V. pH of ME6 was 6.0±0.5 compared to 4.5±0.5 for the solvent system S3. The droplet size of ME6 containing sildenafil citrate was not significantly affected by incorporation of the drug when compared to the droplet size of microemulsion prepared with no drug. No significant droplet size change was found when these preparations were stored at 25°C for 3 months.

Nasal Ciliotoxicity

Optical microscope results showed that there were a great number of cilia with fast rate beating on the edge of mucosa that was treated with microemulsion formulation ME6. The same effect was observed when treating the mucosa with saline. In contrast, the effect of solvent system S3 on ciliary movement was inhibitory, and no ciliary movement was observed as shown in Fig. 2.

In Vivo Nasal Absorption

The mean percentage recovery of sildenafil from quality control low and quality control high spiked quality control samples was 95.33±3.16 and 99.3±2.65 (CV%=2.96 and 2.12, respectively), and the mean correlation coefficient of the standard curves was 0.9969. Within-day accuracy and precision of the method and the reproducibility of the assay (tables not shown) revealed that the mean CV% was <3.62.

The pharmacokinetics of sildenafil citrate was determined for the selected microemulsion ME6 composed of Oleic acid/Labrasol/Transcutol/H_2O (8.33:33.33:16.67:41.67%) and was compared to the peroral tablets. The sildenafil citrate content was consistent in both formulations to achieve a dose of 5 mg/kg.

The mean serum drug concentration–time profiles after administration of the oral tablets as well as the nasal sildenafil microemulsion are illustrated in Fig. 3.

From the plasma time profile, it is clear that nearly twofolds higher serum drug levels were achieved in case of the nasal microemulsion compared to the oral tablet. The C_{max} values were 713.16±22.98 ng/ml for the nasal microemulsion, while it was 346.50±18.72 ng/ml for the oral tablets as listed in Table VI. Statistical analysis revealed that the C_{max} was significantly higher in case of the nasal microemulsion system. Concerning the rate of absorption, the results showed that the nasal microemulsion had significantly shorter t_{max} values of 0.75±0.00 h compared to 1.25±0.00 h for the oral tablets at $P<0.05$. Moreover, the $AUC_{0-\infty}$ values were 1,412.42±25.87 and 1,251.14±30.19 ng h/ml for the nasal microemulsion and oral tablets, respectively. These values corresponded to relative bioavailability values of 112.89%.

DISCUSSION

The saturated solubility study in different solvents showed that 10% citric acid gave the lowest drug solubility, while DMI gave the highest drug solubility. Although it has been reported that solubility determinations in various buffers showed a maximum solubility at acidic pH (24), sildenafil citrate showed the lowest solubility in 10% citric

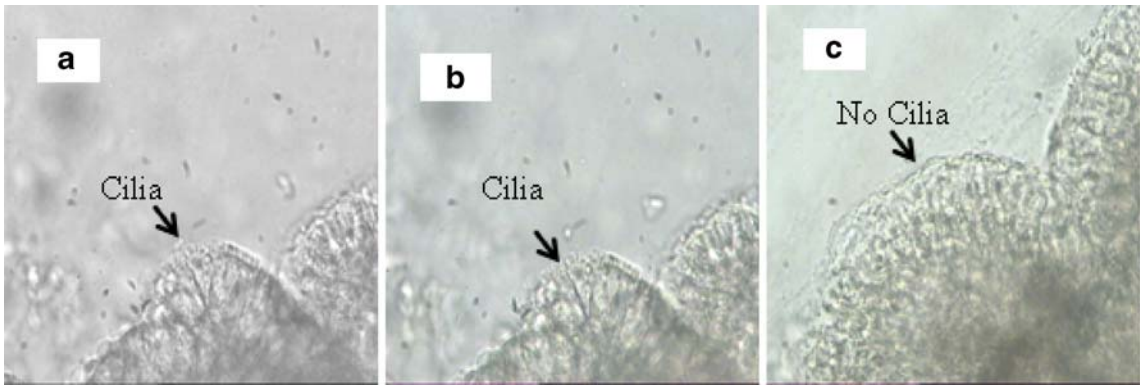

Fig. 2. Optical microscopic images of cilia on the mucosa half an hour after treatment with **a** negative control (saline), **b** microemulsion ME6, and **c** solvent system S3

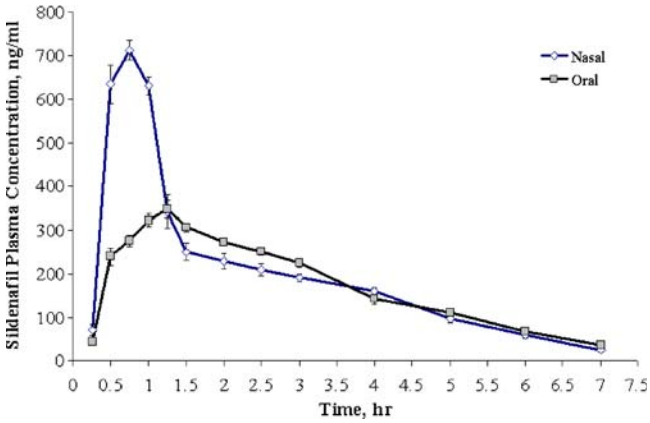

Fig. 3. Plasma concentration–time curve of sildenafil citrate after nasal administration of microemulsion ME6 and oral administration of tablets to rabbits in a dose of 5 mg/kg

acid solution. This may be explained due to the salting out effect exerted by citric acid. None of the tried solvents was satisfying in achieving the required solubility (20 mg/ml) to be used as nasal formulation.

The solubilities of sildenafil citrate in different co-solvent mixtures showed that S1 attained the maximum solubility of sildenafil citrate (34.90±2.25 mg/ml); however, this system contains high amount of benzyl alcohol (17.3%) which is not recommended in nasal route of administration as inhalation of benzyl alcohol may cause headache, vertigo, nausea, vomiting, and diarrhea. Overexposure may result in central nervous system depression and respiratory failure (25). Both S3 and S5 systems are considered suitable systems for sildenafil citrate solubility as the solubilities were 22.98±1.26 and 20.05±2.13 mg/ml, respectively. However, S5 contains high concentration of DMI (16.8%) which may be irritating if used nasally. Since S3 showed higher solubility with lower risk of nasal irritancy or toxicity, it was chosen for further studies.

In the preparation of microemulsion systems, oleic acid was chosen as the oil phase because it gave maximum solubility of sildenafil citrate. It was noted that the solubility of sildenafil citrate was improved by the use of microemulsion. Sildenafil citrate solubility reached about 23.79±1.16 mg/ml in ME6, an approximately sevenfold increase compared with intrinsic solubility in water (3.20±0.11 mg/ml) and fourfold increase compared to solubility in oleic acid (6.77±0.54 mg/ml).

Table VI. Pharmacokinetic Parameters of Sildenafil Citrate after Nasal Administration of Microemulsion and Oral Administration of Tablets to Rabbits in a Dose of 5 mg/kg

Pharmacokinetic parameters	Nasal	Oral
C_{max} (ng/ml)	713.16±22.98	346.50±18.72
t_{max} (h)	0.75±0.00	1.25±0.00
$AUC_{0\text{-}7}$ (ng h/ml)	1369.11±23.60	1181.05±22.28
$AUC_{0-\infty}$ (ng h/ml)	1412.42±25.87	1251.14±30.19
Lz (h^{-1})	0.66±0.13	0.55±0.10
$t_{1/2}$ (h)	1.08±0.26	1.30±0.25
MRT (h)	2.46±0.08	3.04±0.16

ME6 was chosen for further investigations as it showed higher solubility of sildenafil citrate compared to other microemulsion formulations, and it contains the higher percent of water which is considered as a suitable carrier for intranasal administration. Although ME3 and ME4 gave high drug solubility, they were not chosen because they contain DMI which might be an irritant to nasal mucosa.

pH of ME6 was 6.0±0.5 compared to 4.5±0.5 for the solvent system S3 which indicates that less irritation effect of the microemulsion system is expected when applied nasally than the solvent system S3.

The viscosity of ME6 was 472±33 mpa•s, and there was no significant change when stored for 3 months at 25°C indicating physical stability.

The droplet size of ME6 containing sildenafil citrate was not affected significantly by incorporation of the drug. No significant droplet size change was found when these preparations were stored at 25°C for 3 months, indicating that sildenafil citrate-loaded microemulsion was physically stable.

The constituents of preparations intended for nasal delivery should not adversely affect the mucociliary clearance system. Therefore, a requirement in formulation development is absence of nasal mucosal irritation and no inhibition of ciliary movement. Results showed that there were a great number of cilia with fast-rate beating on the edge of mucosa when treated with ME6. In contrast, no ciliary movement was observed in solvent system S3.

Considering the solubilzation capacity, pH, viscosity, droplet size, and nasal ciliotoxicity, the microemulsion ME6 composed of oleic acid/Labrasol/Transcutol/H_2O (8.33:33.33:16.67:41.67%) seems to be an optimal formulation for nasal delivery of sildenafil citrate.

In the pharmacokinetic study of intranasal delivery of sildenafil citrate via a microemulsion system compared to the oral tablets, the plasma time profile showed that nearly twofolds higher serum drug levels were achieved in case of the nasal microemulsion. It showed significantly higher C_{max} and shorter t_{max}, with relative bioavailability 112.89%. Knowing that sildenafil is well absorbed orally (7), the significantly higher bioavailability of its intranasal microemulsion compared to its oral tablets could be attributed to escaping the first-pass metabolism attendant with peroral drug administration.

Theoretically, it was expected that the relative bioavailability of intranasally administered sildenafil will reach almost the double or more, but due to the limited mucosa contact time in the nose and extensive first-pass metabolism of the swallowed fraction of the dose, the relative bioavailability was 112.89%. But, on the other hand, focusing on the shorter t_{max} attained with the intranasal route of administration compared to the peroral administration, this can be considered an important pro.

CONCLUSION

In conclusion, intranasal sildenafil citrate formulated as a microemulsion composed of oleic acid/Labrasol/Transcutol/H_2O (8.33:33.33:16.67:41.67%) represents a safe and viable approach to achieving rapid-onset systemic drug levels and higher bioavailability through bypass of the liver metabolism for the management of erectile dysfunction.

REFERENCES

1. Feldman HA, Goldstein I, Hatzichristou DG, Krane RJ, Mckinlay JB. Impotence and its medical and psychological correlates: results of the Massachusetts male aging study. J Urol. 1994;151:54–61.
2. Johannes CB, Araujo AB, Felman HA, Derby CA, Kleinman KP, McKinlay JB. Incidence of erectile dysfunction in men 40–69 years old: longitudinal results from the Massachusetts male aging study. J Urol. 2000;163:460–3.
3. NIH consensus development panel on impotence. Impotence. JAMA. 1993;270:83–90.
4. Rosen RC, Lane RM, Menza M. Effects of SSRIs on sexual function: a critical review. J Clin Psychopharmacol. 1999;19:67–85.
5. Doggrell S. Do vardenafil and tadalafil have advantages over sildenafil in the treatment of erectile dysfunction? Int J Impot Res. 2007;19:281–95.
6. Boolell M, Allen MJ, Ballard SA. Sildenafil: an orally active type 5 cyclic GMP-specific phosphodiesterase inhibitor for the treatment of penile erection dysfunction. Int J Impot Res. 1996;8:47–52.
7. Rajagopalan P, Mazzu A, Xia C, Dawkins R, Sundaresan P. Effect of high-fat breakfast and moderate-fat evening meal on pharmacokinetics of vardenafil, an oral phosphodiesterase-5 inhibitor for the treatment of erectile dysfunction. J Clin Pharmacol. 2003;43:260–67.
8. Deveci S, Peskircioglu L, Aygun C, Tekin MI, Dirim A, Ozkardes H. Sublingual sildenafil in the treatment of erectile dysfunction: faster onset of action with less dose. Int J Urol. 2004;11:989–92.
9. Richter T, Keipert S. *In vitro* permeation studies comparing bovine nasal mucosa, porcinecornea and artificial membrane: androstenedione in microemulsions and their components. Eur J Pharm Biopharm. 2004;58:137–43.
10. Su KSE, In "Encyclopedia of Pharmaceutical Technology", edited by J. Swarbrick, and J. C. Baylan, Marcel Dekker, Inc. 175–201 (1993).
11. Noach A, Hurni MA, De Boer AG, Breimer DD, In Drug Absorption Enhancement. Concept, Possibilities, Limitations and Trends, Ed.: Harwood Academic Publishers: Chur, Vol. 3, 1994, p. 291–324.
12. Haecker R, Kampfe E, Mattern C. Androgenic substitution for the ageing male by nasal administration of a precursor of testosterone. Geneve: Congress: Healthy Ageing of Male, 1998.
13. Sullivan DA, Wickham LA, Rocha EM. Androgens and dry eye in So••gren's syndrome. Ann NY Acad Sci. 1999;876:312–24.
14. Tenjarla SN. Microemulsions: An overview and pharmaceutical applications. Critical Reviews ™ in Therapeutic Drug Carrier Systems. 1999;16:461–521.
15. Lieberman HA, Rieger MM, Banker GS. Pharmaceutical dosage forms: Disperse systems, second ed, Vol 1. New York: Marcel Decker, 1996, pp. 211–281, 315–70.
16. Kovarik JM, Muller EA, Van Bree JB, Tetzloff W, Kathz K. Reduced inter- and intra-individual variability in cyclosporine pharmacokinetics from a microemulsion formulation. J Pharm Sci. 1994;83:444.
17. Behl CR, Pimplaskar HK, Sileno AP, deMeireles J, Romeo VD. Effects of physicochemical properties and other factors on systemic nasal drug delivery. Adv Drug Del Rev. 1998;29:89–116.
18. Zaki N, Awad G, Mortadaa N, Abd ElHady S. Enhanced bioavailability of metoclopramide HCl by intranasal administration of a mucoadhesive *in situ* gel with modulated rheological and mucociliary transport properties. Eur J Pharm Sci. 2007;32:296–307.
19. Hussain A, Foster T, Hirai S, Kashihara T, Batenhorst R, Jones M. Nasal absorption of propranolol in humans. J Pharm Sci. 1980;69:1240–43.
20. Chien YW. Nasal drug delivery systems. In: Swacrbrick J, editor. Novel drug delivery systems. New York: Marcel Dekker, 1992, pp. 139–96.
21. Knoester PD, Jonker DM, Van der Hoeven RTM, Vermeij TAC, Edelbroek PM, Brekelmans GJ, de Haan GJ. Pharmacokinetics and pharmacodynamics of midazolam administered as a concentrated intranasal spray. A study in healthy volunteers. Br J Clin Pharmacol. 2002;53:501–7.
22. Jiang XG, Cuii JB, Fang XL, Wei Y, Xi NZ. Toxicity of drugs on nasal mucocilia and the method of its evaluation. Acta Pharm. Sin. 1995;30:848–53.
23. Guide for the Care and Use of Laboratory Animals #86-23. Bethesda, MD: National Institutes of Health. (1985).
24. Hussain AA, Dittert LW. Method of administration of sildenafil to produce instantaneous response for the treatment of erectile dysfunction. United States Patent. 6,200,591 (2001).
25. Smolinske SC. Handbook of food, drug, and cosmetic excipients. Boca Raton, FL. CRC Press, 1992, pp. 47–54.