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ORIGINAL RESEARCH

Transdermal delivery of ethosomes as a novel vesicular carrier for paroxetine hydrochloride: *In vitro* evaluation and *In vivo* study

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ABSTRACT

The aim of the present work was to develop and characterisation of stable ethosomal formulation as a carrier for transdermal delivery of paroxetine hydrochloride. To prepare this ethosome, different concentrations of soya lecithin and ethanol were taken. Vesicular size, polydispersity index, zeta potential, entrapment effiency were determined by photon correlation spectroscopy and ultracentrifugation techniques. The *in vitro* permeation study across human cadaver skin was done. Stability study was done on optimised F2 formulation. The vesicle size decreases as an increase in the concentration of ethanol. Entrapment efficiency increases with the increase in concentration of soya lecithin. The ethosome exhibit entrapment efficiency of 40-64%. *In vitro* permeation study across human skin ethosome F2 formulation showed higher transdermal flux 29.64% μg/cm^2/hr. The release mechanism of in vitro permeation shows zero order drug release from the formulation. *In vivo* pharmacodyanamic study F2 formulation showed significant immobility as compared to control group. Stability study results revealed no significant change found in vesicle size distribution and polydispersity index. Our result indicates that the developed ethosomal system may be potential and safe for the delivery of paroxetine hydrochloride through skin.

Keywords: Ethosome, Transdermal, Paroxetine hydrochloride, Soya lecithin, Cholesterol.

1. INTRODUCTION

In recent years the attraction of lipid vesicle use in delivery system for skin treatment is increasing (1, 2). Paroxetine hydrochloride (PXH) is a selective serotonin reuptake inhibitor. Commonly available in tablets and capsule dosage form, but its oral administration has numbers of side effects as well as it undergoes extensive hepatic metabolism. Variation in plasma concentration and long term therapy leads to severe side effects (3).

To overcome these difficulties such as extensive hepatic first pass metabolism transdermal delivery is beneficial (4). The useful of transdermal release has been reported for some antidepressants (5, 6). It has previously reported that significant increase delivery of drugs across the skin would be done by using an ethosomes as novel permeation enhancing carrier (7-10). Ethosomes are soft, malleable vesicles used for delivery of drugs to reach the deep skin (11). Solubility and high encapsulation efficiency values for large range of

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lipophilic drugs can be obtained due to presence of ethanol. The vesicles with soft flexible characteristics are formed due to the presence of ethanol, which permit them to go through more simply into the deeper layers of the skin (12). The present aim focuses on the preparation and characterization of ethosomal formulation for PXH transdermal delivery. The aim of the present study was to develop stable ethosomes carrier for transdermal delivery of PXH. The effect of ethanol and soya lecithin on the permeation of PXH through the human skin was evaluated.

2. MATERIALS AND METHODS

2.1 Material

Paroxetine hydrochloride (PXH) was a gift sample from Zyduscadila Healthcare Ltd. Soya lecithin was purchased from Research Lab Mumbai. Ethanol was purchased from Loba chemical Mumbai. Cholesterol was purchased from Research Lab Fine Chem Industries, Mumbai. PEG-400 was purchased from Dipa Laboratory Chemicals. All materials and solvents used in this study are of analytical grade.

2.2 Preparation of ethosomes

Soya lecithin, cholesterol and PXH were dissolved in ethanol then addition of propylene glycol. Double distilled water was added slowly with a fine stream in above ethanol dispersion with constant mixing at 700 rpm on a magnetic stirrer, in a well-sealed glass container. Mixing was continued for an additional 5 min. The system was kept at 30^{0}C throughout the preparation and was then left to cool at room temperature. (7, 8). The compositions of all ethosomal formulations are shown in Table. 1.

Table 1. Composition of ethosomal formulations.

Code	Paroxetine HCl%	Soya lecithin%	Cholesterol%	Ethanol	Propylene glycol
F1	2%	1%	2%	30%	0.5%
F2	2%	1%	2%	35%	0.5%
F3	2%	1.5%	1%	30%	0.5%
F4	2%	1.5%	1%	35%	0.5%
F5	2%	2%	2%	30%	0.5%
F6	2%	2%	1%	35%	0.5%

2.3 Characterisation of ethosome

2.3.1 Vesicles size distribution, polydispersity index (PI) and zeta potential

The vesicle size distribution, polydispersity index and zeta potential of vesicles were determined using photon correlation spectroscopy (Beckmann counter, Delsa Nano, USA). The formulation were diluted by 1/4th distilled water before measurement and measured three times at a scattering angle of 90^0. The polydispersity index (PI) was used as a measurement of the width of the size distribution. PI less than 0.4 indicates a homogenous and monodisperse population. Zeta potential was measured as the particle electrophoretic mobility means of laser microelectrophoresis in a thermostated cell.

2.3.2 Entrapment efficiency (EE)

The entrapment capacity of PXH by ethosomal vesicles was determined by ultracentrifugation. The drug containing ethosomal formulations were kept overnight at 4°C and centrifuge in ultracentrifuge (Tarsons) 12000 rpm for 30 min. The supernatant was removed and drug amount was determined by using UV-Visible spectrophotometer at 294 nm in both the sediment and the supernatant. The entrapment capacity was calculated as follows, [(*T*-S) /*T*] *100, where *T* is the total amount of drug that is detected both in the supernatant and sediment, and S is the amount of drug found in the supernatant (13).

2.3.3 In vitro permeation study

2.3.3.1 Preparation of cadaver skin

The human cadaver skin sample provided from Government Medical College and Hospital, Aurangabad. The human cadaver skin contained same subcutaneous fat which was carefully trimmed and then rinse with normal saline, prepared skin was wrapped in aluminium foil and stored at -20 °C until use (14).

2.3.3.2 Procedure

Franz diffusion cell was used to carry out the *in vitro* skin permeation study having an effective diffusion area 0.785 cm^2 and having a receptor compartment volume of 15 ml. The cadaver skin was brought to room temp and mounted with the donor compartment dry and open to the atmosphere. Initially, the donor compartment was empty and receiver compartment was filled with phosphate buffer pH 7.4. The receiver fluid was stirred with magnetic bead with the speed of 100 rpm and the temperature was maintained at 37±1°C. The phosphate buffer pH 7.4 was replaced with the fresh buffer at every 30 min. No peak was found that after 3 hours indicating complete stabilisation of skin. A 5 ml formulation was placed into the donor compartment and sealed with paraffin film to provide occlusive condition. The samples were withdrawn at regular interval for 10 hrs filtered through 0.45 µm. membrane filter and analysed for drug amount by UV-Visible spectrophotometer at 294 nm.

2.3.4 Permeation data analysis

The cumulative amount of penetrant, *Q* (g/cm^2), which permeated the skin per unit surface area was plotted against time. The linear portion of the plot was taken as being the steady-state flux, (*J*s). The permeability coefficient (*K*p) was calculated as (15),

$K_p = J_s/C_v$

Where *C*v is the concentration of penetrant in the donor solution.

2.3.5 In vivo pharmacodynamic study

Approval to carry out pharmacodynamics studies was obtained (Institutional Animals Ethical Committee, approved the protocol). Forced Swim test (FST) was used to evaluate the antidepressant effect of the optimized F2 formulation. Rats of either sex weighing 250–300 g were kept under standard laboratory conditions (temperature 23-30°C).The rats were kept with free access to standard laboratory diet. Approximately 14 cm^2 of the abdominal side of rats skin was shaved on the in each group except group treated with the marketed tablet formulation.

Rats were divided randomly into three groups each containing six animals. Group 1 was considered as a control. Group 2 was treated with oral tablets of PXH containing 1.40 mg/day and administered without anaesthesia by using simple poly-ethylene tube. Group 3 was treated with the optimized F2 formulation applied transdermally containing 2 mg/day (equivalent to 0.60 mg/day) drug.

2.3.6 Force swim test

Rats were forced to swim in a cylindrical glass tank (60 cm height X 30 cm in diameter) containing water after the administration of doses. The water was filled up to 40 cm height so they were swim without touching their hind limb or tail to the bottom of the tank. On the 1st day of experiments, rats were forced to swim for 10 min. After 24 h, rats were re-exposed to forced swim for 5 min and animals were judged for immobility, climbing, and swimming. After a 5-min swim test, the rat was removed from the cylinder, dried and then returned to its home cage. (16)

2.3.7 Vesicle stability study

Stability of optimised F2 formulation was kept at room temp for 3 weeks. Vesicle size and polydispersity index were measured at 1, 2, 3, 7, 14 and 21 days and mean value is used for the analysed of the data.

3. RESULT AND DISCUSSION

3.1 Vesicle size, polydispersity index and zeta potential

The vesicle mean diameters for all formulations are shown in Table 2. The result of photon correlation spectroscopy shows narrow peak for all formulations, which indicating that the size of vesicle population is comparatively uniform in size. In accordance with other researcher, this decrease in the mean diameter of the vesicle is due to the presence of ethanol. Higher concentration of ethanol produced lower vesicle size. Probably the ethanol causes the modification of the net charge of the system and confer it some degree of stearic stabilization (10). Polydispersity index of formulations is shown in Table 2. Compare to all formulations F2 formulation showed a less polydispersity index is 0.23 indicates a homogeneous population of ethosome vesicle that may finally lead to decrease in mean particle size. In the formulation the concentration of ethanol increases from 30-35% the significant decrease in vesicle size. On the other hand, it was observed that the increase in soya lecithin concentration resulted in an increase in mean particle size. Small vesicle size is formed with the F2 formulation having a 1% of soya lecithin and 35% ethanol. Twice fold increase in soya lecithin concentration (1%-2%) resulted in two fold increase in ethosomes size (from 501.33nm ±1.92 - 1314.33nm±3.5). The charge of vesicles is an important parameter that can influence both stability and skin vesicle interaction. The zeta potential value of all formulations shown in Table 2. The concentration of ethanol increase from 30-35% v/v resulting in an increase in zeta potential values. Polydispersity index was determined as a measure of homogeneity in formulation. Polydispersity index ≤ 0.3 indicate homogeneous population of ethosome vesicle in formulation Ps.

Table 2. Particle size, polydispersity index, zeta potential, flux and permeability coefficient of formulations.

Code	Particle Size (nm)	Polydispersity Index	Zeta potential (mV)	Flux (µg/cm^2/hr)	Permeability coefficient (x10^{-3})(cm/hr)
F1	697±0.04	0.473±0.004	-22.26	12.94	1.2
F2	501.33±1.92	0.25±0.02	-16.10	29.64	2.9
F3	1126. 33±450	1.06±1.004	-25.48	24.87	2.4
F4	1125.66±4.04	0.691±2.004	-24.93	19.46	1.9
F5	1314.33±3.5	0.622±2.004	-28.46	16.40	1.6
F6	1249.33±4.5	0.957±0.0052	-16.81	24.07	2.4

Values are expressed in mean ±SD, n=3

3.2 Entrapment efficiency

The entrapment efficiency of all formulations shown in Figure 1. Entrapment efficiency of formulation containing of 1% soya lecithin and 30% ethanol (F1) was found to be 60%, which significantly increased to 64% when the amount of ethanol increases to 35% (F2) keeping the concentration

of soya lecithin constant. Ethosomes formulation prepared with 1.5% soya lecithin and 30% ethanol (F3) exhibited 40% entrapment efficiency, which was increased to 45% (F4) respectively; keep the amount of soya lecithin constant. Formulation prepared with 2% soya lecithin and 30% ethanol (F5) showed 42% entrapment efficiency, which was increased to 61% when the concentration of ethanol increased to 35% (F6) respectively. These data supported by previous findings that solubility and high encapsulation efficiency values for large range of lipophilic drugs can be obtained due to presence of ethanol (13). From these results entrapment efficiency of formulation was observed due to increase in ethanol concentration.

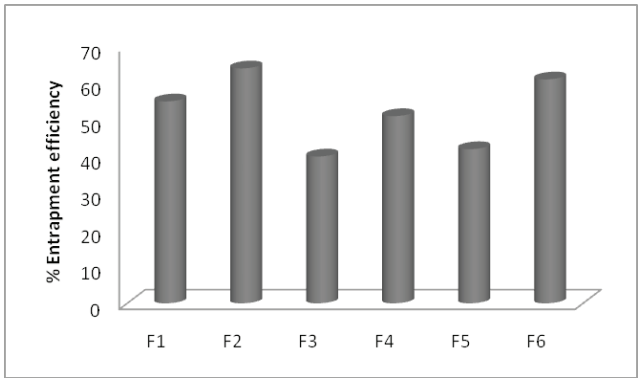

Figure 1. % Entrapment efficiency of all formulation.

3.3 *In vitro* permeation study

In vitro skin permeation experiment was performed using human cadaver skin showed that permeation was highest in F2 formulation as shown in Figure 2. Flux value of F2 formulation was significantly higher when compared with other formulation (P≤0.05) as shown in above Table 2. Highest flux value 29.64 of F2 as compared to other formulation. These may be due to small vesicle size and high entrapment efficiency along with high concentration of ethanol. These data supported by previous findings that ethanol interact with a lipid molecules of stratum corneum, resulting in a reduction in the Tm of stratum corneum, increase in there fluidity. The intercalation of ethanol due to the polar head group environment can result in an increase in membrane permeability (17). It may also suggest that mixing of phospholipids with the stratum corneum lipid of the intercellular layers enhances the permeability of the skin (18). F2 formulation was selected as an optimized formulation from the vesicle size distribution, polydispersity index, zeta potential, drug entrapment efficiency, and *in vitro* permeation study results and considered for further study.

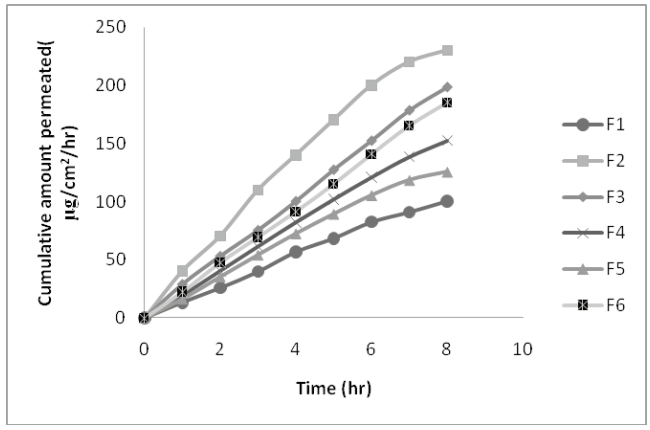

Figure 2. *In vitro* skin permeation profile of PXH through human cadaver skin.

3.4 *In vivo Pharmacodynamic* study

The pharmacodynamic activity of the ethosomes F2 formulation was compared with orally administered dose. The force swim test is most widely used pharmacodynamic activity model for assessing the antidepressant activity. The total immobility period would decrease if high concentrations of paroxetine hydrochloride reached target site.

3.5 Force swim test

Results of FST confirmed that there was significant reduction in the total immobility period in seconds by treating the rats by transdermal ethosomal F2 formulation. There was significant ($p<0.001$) differences in the total immobility period of control compared with paroxetine hydrochloride treated group as shown in Table 3. Ethosomes formulation significantly ($p<0.001$) reduced total immobility period by 33.73 sec than orally treated group.

Table 3. Results of FST study

Treatment group	Total immobility (sec)			Mean
Control	180	188	179	179.66±8.5
Paroxetine hydrochloride oral administration	140	150	148	148.33±7.63
F2 through transdermal route	112	107	125	114.66±9.29

Values are expressed in mean ±SD, n=3, P value<0.05 considered statistically significant

3.6 Vesicle stability study

The results of stability study of vesicles of ethosomes system are shown in Table 4. Size distribution study of system of ethosomes was investigated by using photon correlation spectroscopy. There was no significant change in mean vesicles size and polydispersity index of F2 formulation for a period of 4 weeks.

Table 4. Mean particles size and polydispersity index after 21 days of optimized formulation

Days	Mean diameter (nm)	Polydispersity index
1	501.33±1.92	0.252±0.012
2	501.99±0.036	0.255±0.018
3	502.36±0.01	0.258±0.025
7	502.7±0.026	0.60±0.015
14	503.78±0.03	0.605±0.024
21	505.58±0.025	0.610±0.026

Values are expressed in mean ±SD, n=3

4. CONCLUSION

Ethosomal vesicles with appropriate size and maximum drug entrapment efficiency can be prepared. F2 formulation showed highest transdermal flux across human skin was composed of 1% soya lecithin, 35% ethanol and 2% cholesterol. *In vivo* pharmacodyanamic study of optimised formulation showed significant values compared to control group. Therefore, it can be concluded from the result of the study that ethosome formulation is a potentially useful carrier for transdermal delivery of paroxetine hydrochloride

5. CONFLICT OF INTEREST

The authors report no conflict of interests. The author along are responsible for content and writing of paper.

6. ACKNOWLEDGEMENT

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Etozomların, Paroksetin Hidroklorür İçin Yeni Veziküler Taşıyıcı Olarak Transdermal Verilmesi: *In Vitro* Değerlendirme ve *In vivo* Çalışma

ÖZET

Bu çalışmanın amacı paroksetin hidroklorür'ün transdermal verilişi için taşıyıcı olarak, stabil bir etozomal formülasyon geliştirilmesi ve karakterizasyonudur. Bu etozomu hazırlamak için farklı derişimlerde lesitin ve etanol kullanılmıştır. Vezikül boyutu, polidispersite indeksi, zeta potansiyeli ve yükleme etkinliği foton korelasyon spektroskopisi ve ultrasantrifüj yöntemleri kullanılarak belirlenmiştir. İn *vitro* deriden geçiş çalışmaları insan kadavra derisi kullanılarak gerçekleştirilmiştir. Stabilite çalışması optimize edilen F2 formülasyonu üzerinde yapılmıştır. Vezikül boyutu artan etanol derişimi ile azalmıştır. Yükleme etkinliği ise artan soya lesitin derişimi ile artmıştır. Etozom %40-60 arasında yükleme kapasitesi göstermiştir. İnsan derisinden *in vitro* geçiş çalışmasında F2 rumuzlu etozom formülasyonu daha yüksek transdermal akı göstermiştir (%29.64 μg/cm^2/sa). *In vitro* geçişteki salım mekanizması formülasyondan sıfırıncı derece ilaç salımı olduğunu göstermiştir. *In vivo* farmakodinamik çalışmada ise F2 formülasyonu kontrol grubuyla karşılaştırıldığında belirgin bir immobilite göstermiştir. Stabilite çalışmalarının sonuçları, vezikül boyut dağılımında ve polidspersite indeksinde belirgin bir değişiklik olmadığını ortaya çıkarmıştır. Sonuçlarımız, geliştirilen etozomal sistemin, paroksetin hidroklorür'ün transdermal uygulanmasında kullanımının olası ve güvenli olabileceğini göstermiştir.

Anahtar kelimeler: Etozom, transdermal, paroksetin hidroklorür, soya lesitin, kolestrol

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