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Design and characterization of delavirdine ethosomal drug delivery to enhance the bioavailability via topical drug delivery

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***Abstract*---**The increasing demand for efficient administration and delivery of pharmaceutical dosage forms possessing the attributes namely minimum side effects, improved patient compliance has resulted in the formulation of novel drug delivery system. Ethosomes are very effective since they enhance the penetration of drugs via skin to several times whose compound to the simple creams, elixirs and liposomal carriers. Hence, there is an absolute necessity to formulate ethosomes of the selected drugs, In the presant work ethosomal formulations of, delaviridine, was prepared for obtaining the objective of improving skin permeability and bioavailability. The prepared formulations gave good percentage yield and size distribution. The best formulation of ethosomes of each respected drug were incorporated into carbopol based gel systems for controlled release and the invitro studies proven that the formulations followed zero order release mechanisms. Compatability studies were performed for the materials selected and results are observed o be possitive. Stability studies indicated that the formulations were stable at low temperatures but can undergo rapid deformation at higher temperatures.

***Keywords*---**delaviridine, ethosomes, drug delivery.

Introduction

Transdermal drug delivery system (TDDS) showed promising result in comparison to oral drug delivery system as it eliminates gastrointestinal interferences and first pass metabolism of the drug but the main drawback of TDDS is it encounters the barrier properties of the Stratum Corneum i.e. only the lipophilic drugs having molecular weight < 500 Da can pass through it. To improve the permeation of drugs through the skin various mechanisms have been investigated, including use of chemical or physical enhancers, such as iontophoresis, sonophoresis, etc. Liposomes, niosomes, transferosomes and ethosomes also have been reported to enhance permeability of drug through the stratum corneum barrier.

Ethosomes are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water. Ethosomes are soft vesicles made of phospholipids and ethanol (in higher quantity) and water. Ethosomes can entrap drug molecule with various physicochemical characteristics i.e. of hydrophilic, lipophilic, or amphiphilic. The size range of ethosomes may vary from tens of nanometers to microns (μ).

Ethosomes Composition

Ethosomes are vesicular carrier comprise of hydroalcoholic or hydro/alcoholic/glycolic phospholipid in which the concentration of alcohols or their combination is relatively high. Typically, Ethosomes may contain phospholipids with various chemical structures like phosphatidylcholine (PC), hydrogenated PC, phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylglycerol (PPG), phosphatidylinositol (PI), hydrogenated PC, alcohol (ethanol or isopropyl alcohol), water and propylene glycol (or other glycols). Such a composition enables delivery of high concentration of active ingredients through skin. Drug delivery can be modulated by altering alcohol: water or alcohol-polyol: water ratio. Some preferred phospholipids are soya phospholipids such as Phospholipon 90 (PL-90). It is usually employed in a range of 0.5-10% w/w. Cholesterol at concentrations ranging between 0.1 1% can also be added to the preparation. Examples of alcohols, which can be used, include ethanol and isopropyl alcohol. Among glycols, propylene glycol and Transcutol are generally used. In addition, non-ionic surfactants (PEG-alkyl ethers) can be combined with the phospholipids in these preparations. Cationic lipids like cocoamide, POE alkyl amines, dodecylamine, cetrimide etc. can be added too. The concentration of alcohol in the final product may range from 20 to 50%. The concentration of the non-aqueous phase (alcohol and glycol combination) may range between 22 to 70%.

Methodology

Formulation Design

Preparation of Sonicated Delaviridine Ethosomes (by Cold method)

Making slight modification to the Touitou et al method Delaviridine Ethosomes were prepared[13]. The Delaviridine Ethosomal system comprised of 20-50% of ethanol, 10% of propylene glycol, 2-5% of phospholipids, 0.005g of cholesterol and an aqueous part of 100%w/w. At room temperature, 0.025g of Delaviridine was added to ethanol in a covered vessel along with propylene glycol and dissolved by stirring it vigorously. At 30 ^{0}C mixture was heated using separate vessel and then drop wise it was added to the mixture in the centre of the vessel by stirring it at 700rpm for 5min in a vessel which was covered. Then by using extrusion[30] method or sonication[30] method the particle size of Ethosomal formulation was reduced to the desirable extent. At last the Ethosomal formulation was kept under refrigeration. The following process was used to prepare Ethosomes spontaneously.

Preparation of Unsonicated Delaviridine Ethosomes

By the minor modification to Touitou et.al. Method, unsonicated Delavirdine Ethosomes were prepared. The Ethosomes consisted of 2 – 5% phospholipids, 20-40% ethanol, 0.005 % of cholesterol, 0.025gm of Delaviridine and distilled water quantity sufficient 100% w/w at room temperature. The mixture was heated to 30^{0}c and continuous stirring with 700RPM for 5 minutes is added drop wise in a closed i.e. minutes vessel for 5 min.

Ethosomal Formulations

Characterization of Ethosomes and Liposomes

Size and Shape Analysis

For the determination of Ethosomes, average size microscopic analysis was done. A sample of Ethosomes was taken and it was diluted by using distilled water. For the examination at 45×15 X microscope on a glass slide diluted drop is taken which is then covered with a cover slip. By using calibrated eyepiece micrometer with stage micrometer, the diameter of 150 vesicles was determined. The formula used for calculating average diameter was

Average diameter = nd /n
n= number of vesicles d= diameter of vesicles

The vesicle size was decreased by Sonication method because size of these vesicles were unable to be analyzed using the microscopic method at a magnification of 15*45 X. So the Sonicated vesicles were analyzed under a special microscope which has a connection with software and under 400 and 800 magnification its microphotographs were taken. Further few microphotographs were selected and analyzed for size by using a particular software “particle size

analysis "which was developed by BIOVIS. This special software is working on microphotograph images with standard dimension[14, 15].

Scanning Electron Microscopy

Determination of morphological characters like its texture of smoothness, round structure and aggregate gel formation of Delaviridine Ethosomes was done using scanning electron microscopyp[16, 17, 18].

Entrapment Efficiency

A 10 ml aliquot of Delaviridine Ethosomal suspension was ultra-centrifuged to determine entrapment efficiency. For every 2minutes gap all the samples were vortexes for 5minutes each cycle at least of 2 cycles were done. Then from all the samples which have been vortexed a quantity of 1.5ml is taken and Ethosomal formulations which are fresh and untreated were put into different tubes of centrifuge. The centrifugation of the samples was done at a rate of 20,000 rpm for duration of 3 hours. Separation of the supernatant layer was done and diluted with a suitable quantity of water and then absorbencies for the respective concentration of the drug were found at 295nm in both un-vortexed and vortexed samples[19].

The efficiency of entrapment was calculated accordingly: Entrapment efficiency=T-C/T ×100

Where T denotes detected total drug from the vortexed supernatant sample. C denotes un vortexed detected drug supernatant sample and amount of drug entrapped

Delaviridine Ethosomal gel

Pre-formulation studies

Before the design and development of Delaviridine Ethosomal gel formulation, pre- formulation studies were performed on various formulations namely stability, partition coefficient, melting point, diffusion rate constant on the drug Delaviridine.

Partition coefficient

For the determination of substance lipophilic nature, water- oil partition coefficient is used. This in turn is further useful to predict whether substance is capable in crossing the biological membrane. The most popular method of finding partition coefficient is the shake flask method[2, 3].

Procedure

In to 5 ml of n-Octanol, a small quantity of Delaviridine was added to get a saturated solution. It was passed through filter paper to obtain a clear solution. A 3 ml portion was mixed with 2ml of fresh n- Octanol. Taking 15ml of water at

37°c 5ml of prepared solution of Delaviridine in n-Octanol was mixed well for 24hrs on cryostat temperature shaker bath. Using UV Spectrophotometric method, the drug contents in the separated phases namely water and n-Octanol phases is estimated by making suitable dilutions and by employing the formula its partition coefficient (Kp) is found.

Kp = Corg / Caq

Where
Corg is drug content in organic phase
Caq is drug content in aqueous phase

Melting point: Melting point (MP) of Delaviridine pure drug was determined by the closed capillary tube method. The temperature at which the drug melted was noted. The melting points are tabulated in table.

Compatibility Studies

For the investigation and prediction of any physicochemical interactions between varied components of a formulation, IR spectroscopy can be used and thus can be useful in selecting chemically compatible excipients which are suitable.

Preparation of Ethosomal gel of Delaviridine

In the Carbopol gel of various compositions (1%, 1.5%, 2% w/w) the best achieved Ethosomal suspension, of formula was selected and was incorporated specific amounts of Carbopol 934 powder which were added slowly to ultra-pure water and kept for 20mins at a temperature of 100°C. Then later on, drop wise Triethanolamine was added into it. Then incorporation of an accurate quantity of formula which contains Delaviridine (1.5% w/w) was done in the gel base and then water of sufficient quantity was added with continuous stirring to the other ingredients of the formulation until a formulation of homogenous nature was obtained which were christened as (G-1, G-2, G-3).By the use of 1.5%w/w Carbopol, a gel constituting free Delaviridine was prepared with the aid of similar method

Evaluation Studies

In a mortar one part of sample and three parts of potassium bromide were taken and triturating was done. Using hydraulic press, a small quantity of sample which was triturated was put into machine. Then it was compressed at a pressure of 10kg/cm^2. In Bruker IR spectrophotometer the pellet was scanned from 4000/cm to 400/cm then its comparison was done with the original spectrum. To find if there is any shifting in functional peaks and no involvement of functional group, IR spectra was checked thoroughly and even compared with the original one. From the observations it is clearly found that there are no interactions between the drugs, mixtures and selected carriers. Hence compatibility was seen between the selected Delaviridine and carrier without mutual interactions.

Polymer skin compatibility

The skin irritation was done to investigate the polymer compatibility with the skin. The following procedure was adapted to healthy albino rats of average weight between 230- 250mg and the skin irritation test was conducted. 0.5% of aqueous solution was used a standard irritant.Polymeric solution were spread over 1 cm^2 on each rat on its dorsal surface on left side and on right dorsal side. 0.5% formalin was applied. After 24hr, alcohol swabs were used to remove the polymers followed by identification for the presence of edema/erythema and data was tabulated in a table[4, 5, 6, 7]

Scanning electron microscopy study for filter membrane-vesicle interaction

A filter membrane with a pore size of 50nm was applied with a vesicle suspension of (0.2ml) and kept into a diffusion unit. Upper side is exposed to air whereas the (Phosphate saline buffer solution) PBS of pH 6.5 was in contact with the lower side. The removal of the filters was done after an hour and preparation was done for SEM studies by keeping it overnight in Karnovsky's fixative for fixation at 4 degree C and then by using ethanol graded solution (100% 95%,90%,70%,50%,30%v/v in water) It was dehydrated. At last, they were put a coating of gold and its examination was done in SEM.

Skin permeation studies

By the use of a pair of scissors, fur of animal under study is removed carefully (<2mm) and then by the use of a scalpel the abdominal skin and the underlying connective tissue was separated from each other. For the removal of fats and subcutaneous tissue the dermis of skin which was excised was kept on an aluminium foil. When the temperature of the cell was maintained at ±32 ^{0}c where the cell permeation is 1.0 cm^2 the receptor volume was 10ml. In the compartment of the receptor PBS (10ml of pH 6.5) was present. In between donor and receptor the excised skin was moulded. 0.1ml Ethosomal formulation is applied on the skin surface and at 1,2,4,8,12,16,20 and 24 hrs interval 0.5ml of sample is taken from the diffusion cell through sampling port and assay is performed by using the HPLC.

Stability studies

By storing particles at 4+/-^{0}C the determination of their stability was done. After duration of about 180 days the zeta potential, entrapment efficiency and particle size vesicles is estimated by appropriate method mentioned above.

SEM and TEM study for skin-Vesicle interaction

Animals ultra-skin sections were taken (Austria, Ultracut and Vienna) and placed on form vacated grids and then under transmission electron microscope its examination was done. For analysis by SEM the dehydrated sections of skin were placed on the stubs by the use of adhesive tapes and then gold palladium coat is coated by coater called fine coat ion sputter. Examination of section is done by electron microscope scanning.

Fluorescence microscopy study of skin-Vesicle interaction

By following the protocol which is used for SEM and TEM fluorescence microscopy was carried out. For the micro cytotoxicity assay 5micrometer thin sections of paraffin using microtome are cut. At 37^0c and 5% CO2, 2mmol/L L-glutamine, 100 U/ml penicillin, 100mg/ml streptomycin and 10% foetal calf serum containing solution which is called as Dulbecco's modified Eagle is used to examined T-lymphoid cell lines (MT-2 cells). 50% of absorbance decrease at 540nm is due to the cytotoxicity dose (CD50).

Drug uptake studies

In 24 well plates (Corning Inc) MT-2 cells uptake the drug (1*106 cells/ml) is done by which an RPMI medium of 100 micro-L was added. After incubating with 100 micro litre of solution of drug with cells, drug content is evaluated by HPLC assay and marketed after evaluated by using PBS (pH 7.4).

HPLC Assay

MT-2 cell and Skin permeation can be determined by allowing the mobile phase flow at the rate of 1ml/min into the receptor compartment, where the mobile phase of HPLC contain 70:20:10 ratio of methanol: distilled water: acetonitrile respectively. The size of the C18 column was about 4.6*150mm By the use of UV detector (SPDM10A VP diode) the column effluent was monitored at 271nm. The squared correlation coefficient was 0.9968 and 1.0%-2.3% was the standard curve variance coefficient.

Results And Discussion

Preformulation studies of Delaviridine

These studies were performed as per the details mentioned under this heading in the Chapter- Materials and Methods in order that the actual conditions and parameters required for the actual formulation of Delaviridine Ethosomes and Liposomes could be frozen. The data obtained has been presented below. IR spectra were compared and checked for any shifting in functional peaks and non-involvement of functional groups. From the spectra it is clear that there is no interaction between the selected carriers, drug and mixtures. Hence the selected carrier was found to be compatible in entrapping the selected Delaviridine with carriers without any mutual interactions. Following the above DSC spectrums, it is observed that there is a minor change in the melting point energies of pure drug, polymers than in the optimized formulation which are negligible.

Characterization of Ethosomes and Liposomes

Since the physical characterization is meant for physical integrity of the dosage form, the results were pooled at one place. Discussion on the results described for Sonicated and unsonicated Ethosomes formulation and Liposomes under the same heading as table which presented below.

Size and Shape Analysis

Microscopic analysis was performed under different magnification to visualize the vesicular structure, lamellarity and to determine the size of Ethosomal and Liposomal preparations.

Ethosmes with Sonication

Sonication method was adopted to reduce the vesicular size by giving high level energy to the lipid suspension. The type of Sonicator used was probe Sonicator in order to produce high energy to a small aliquot of the lipid suspension. The selected Ethosomal formulations were subjected to Sonication by the probe Sonicator. The photomicrograph revealed reduce the particle size of the Ethosomes after being subjected Sonication. The result of size and shape revealed consistency with the observations of Jain NK et al.,

Ethosmes Without Sonication

Ethosomes were prepared comprising of the formula containing 2-5% Phospholipids, 20-40% ethanol and quantity sufficient water were found to appear as multilamellar vesicles. It was observed that the Ethosomal lamellae were evenly spaced to the core. This confirms that the vesicular structure of high ethanolic concentration were present.This Liposomal formulation was observed to be longer than that of Ethosomal formulation which were characterize to be multilamellar and gained type too. Size distribution and average vesicular size analysis were done as per the procedure mentioned under methodology.

The maximum entrapment efficiency of Ethosomal vesicles as determined by ultracentrifugation was 73.70% for Ethosomal formulation containing 20% ethanol (EF1) which was almost double to the formulation containing 40% ethanol (EF6). As the ethanol concentration increased from 20% to 40% w/w, there was decrease in the entrapment efficiency and with further increase in the ethanol concentration (>30% w/w) the vesicle membrane becomes more permeable that lead to further decrease in the entrapment efficiency. Results of entrapment efficiency also suggest that 2% phospholipids are optimal concentration for entrapment efficiency and hence increased in concentration of phospholipids reduces the entrapment efficiency of vesicles. These results are further supported by observations made by Jain NK et al14, Entrapment efficiency of Ethosomal formulations is significantly different which are reported.

In-Vitro Skin Permeation Study

The objectives in the development of in-vitro diffusion tests are to show the release rate and extent of drug from the dosage form. The in-vitro skin permeation study of Delaviridine from Ethosomal formulation was studied using Franz diffusion cell and method described in methodology chapter. The release data was obtained for all the Ethosomal formulations. Spectrometric results were obtained and given consideration to sampling loss, to calculate actual cumulative drug diffused was calculated since the volume of receptor cell was only 20 ml. The obtained diffused amount of drug was extrapolated to diffusion by unit surface

area of rat skin. These cumulative values were plotted as a function of time and steady state transdermal flux was calculated from the slop of linear portion.

Entrapment Efficiency

The maximum entrapment efficiency of Ethosomal vesicles as determined by ultracentrifugation was 73.70% for Ethosomal formulation containing 20% ethanol (EF1) which was almost double to the formulation containing 40% ethanol (EF6). As the ethanol concentration increased from 20% to 40% w/w, there was decrease in the entrapment efficiency and with further increase in the ethanol concentration (>30% w/w) the vesicle membrane becomes more permeable that lead to further decrease in the entrapment efficiency. Results of entrapment efficiency also suggest that 2% phospholipids are optimal concentration for entrapment efficiency and hence increased in concentration of phospholipids reduces the entrapment efficiency of vesicles. These results are further supported by observations made by Jain NK et al14,

In-Vitro Skin Permeation Study

In-vitro skin permeation study or in-vitro diffusion study have been extensively studied, developed and used as an indirect measurement of drug solubility, especially in preliminary assessments of formulation factors and manufacturing methods that are likely to influence bioavailability.

Evaluation of ethosomal gel

In-Vitro Release Studies

Based on the in-vitro cumulative %drug release comparative studies of Delaviridine formulations it was established that EF2 (Sonicated Ethosomes) released maximum drug of 93.7%, EF3(Sonicated Ethosomes)89.3%, EF1(Sonicated Ethosomes) 88.4%, EF4 (unsonicated Ethosomes) 87.11%, EF5 (unsonicated Ethosomes) 78.2%, EF6 (unsonicated Ethosomes) 75.5%, EF gel 74.35%, liposomes 72.44% followed by marketed drug FD 47.26%. EF2 was adjudged the best based on in-vitro % drug release parameter which was almost two times the marketed drug.

In-Vivo Studies

Estimation of Delavirdine in Plasma Samples by Rp-HPLC

Chromatographic conditions
Instrument: waters-2690/95 equipped with waters PDA-96 detector with EMPOWER-II software.
Mobile phase: acetonitrile, 50 mM sodium dihydrogen phosphate (60:40, v/v)
Stationary phase/Column: Hypersil BDS (250mm x 4.6mm), 5 µm (Thermo Technologies, USA).
Column temperature: 40^{O}C.
Flow rate: 1.0 mL min^{-1}
Detector wavelength: 295 nm

Injection volume: 50 µL
Standard: Rescriptor-tablets-200mg.

Animals

Male New Zealand whit rabbits weighing between 1.5 to 2.0 kg were used for *in vivo* studies of orally administered Stavudine. Animals were housed at 25 ± 1^0C in air conditioned room at a relative humidity of 60 ± 5% and were provided with water and standard rabbit feed obtained from Sanzyme pvt. ltd. Hyderabad. Animals were fasted for 24 hrs prior to the administration of the pellet formulation, but had free access to water. The formulations and standards are administered/applied to the animals, About 2 ml of blood was collected at 0, 1, 2, 4, 6, 8, 12, 16, 20 and 24 hrs after formulation administration from marginal ear vein in to heparinized tubes and plasma was separated immediately by centrifugation and frozen at – 20^0C. The plasma samples were analyzed for stavudine by HPLC methods as described earlier.

Conclusion

In the present work ethosomal formulations of the selected drugs rilpivirine, delaviridine, zanamivir were prepared for obtaining the objective of improving skin permeability and bioavailability. The prepared formulations gave good percentage yield and size distribution. The best formulation of ethosomes of each respected drug were incorporated into carbopol based gel systems for controlled release. And the invitro studies proven that the formulations followed zero order release mechanisms. Compatibility studies were performed for the materials selected and results are observed o be possitive. Stability studies indicated that the formulations were stable at low temperatures but can undergo rapid deformation at higher temperatures.

References

1. Allen. LV, Popovich. NG, Ansel. HC. “Ansel’s Pharmaceutical Dosage Forms and Drug Delivery Systems, 8th Edition, Lippincott Williams &Wilkins, 2005.pp. 298-315.
2. Akiladevi D, Basak D. Ethosomes: A Non-Invasive approach for transdermal drug delivery. Int. J. Curr. Pharm. Res, 2010; 2(4, 14): 1-4.
3. Anon. “Transdermal delivery systems-general drug release standards. Pharmacopeial Forum”, 1980; 14: 3860-3865.
4. Banker. G. S and Rhodes. C.T. “Modern pharmaceutics, third edition”, New York, Marcel Dekkar, Inc., 1990.
5. Barry B. “Transdermal Drug Delivery”. In Ed: Aulton M E, Pharmaceutics: The Science of Dosage Form Design, Churchill Livingston.2002 pp: 499-533.
6. Bendas, E.R., Tadros, M.I., 2007. Enhanced transdermal delivery of salbutamol sulfate via ethosomes. AAPS PharmSciTech. 8, E1–E8.
7. Bhalaria MK, Naik S, Misra AN. Ethosomes: a novel delivery system for anti-fungal drugs in the treatment of topical fungal disease. Ind J Exp Biol 2009(47):368–75

8. Bhasin V, Yadav H, Markandeywar T, Murthy RSR. Ethosomes: The Novel Vesicles for Transdermal Drug Delivery. IJPI's Journal of Pharmaceutics and Cosmetology. 2011;2(7):68-80.
9. Chourasia, M. K., Lifeng K and Sui, Y. C (2011). Nanosized ethosomes bearing ketoprofen for improved transdermal delivery. Results in pharma sciences. 1(1):60-67.
10. Esposito, E., Eneo, M and Rita, C (2004). Ethosomes and liposomes as topical vehicles for azelaic acid: a preformulation study. International Journal of Cosmetic Science. 26(5):270-271.
11. Finnin. BC, Morgan. TM "Transdermal penetration". J Pharm Sci. Oct 1999; 88 (10):955-958.
12. Godin, B., Touitou, E., 2004. Mechanism of bacitracin permeation enhancement through the skin and cellular membranes from an ethosomal carrier. J. Control. Release 94, 365–379.
13. Jain, S., Tiwary, A.K., Sapra, B., Jain, N.K., 2007. Formulation and evaluation ofethosomes for transdermal delivery of lamivudine. AAPS PharmSciTech. 21, E1–E9.
14. Laib. S, Routh. AF. "Fabrication of Colloidosomes at low temperature for the encapsulation of thermally sensitive compounds". J. Colloid & Interface Sci. 2008; 317: 121-129.
15. Nikalje A. P and Tiwari S. (2012). Ethosomes: A novel tool for transdermal drug delivery. International Journal of Research in Pharmacy and Science. 2(1):1-20.
16. Nikalje P, Tiwari S. Ethosomes: A Novel Tool for Transdermal Drug Delivery. Int J Res Pharm Sci . 2012;2(1):1-20.
17. Pathan IB, Nandure H, Syed SM. Transdermal delivery of ethosomes as a novel vesicular carrier for paroxetine hydrochloride: in vitro evaluation and in vivo study. Marmara Pharm J 2016;20:1–6.
18. Rakesh, R., and Anoop, K, R (2012). Formulation and optimization of nano-sized ethosomes for enhanced transdermal delivery of cromolyn sodium. Journal of pharmacy & bioallied sciences 4(4): 333.
19. Swarnlata. S, Rahul. R, Chanchal. D.K, Shailendra. S: "Colloidosomes an advanced vesicular system in drug delivery". Asian J.Sci. Research 2011; 4(1): 1 – 15.
20. Subheet J, Ashok KT, Bharti S, Jain NK. Formulation and evaluation of ethosomes for transdermal delivery of lamivudine. AAPS Pharmscitech 2007;8(4):E1–9.
21. Sarat C., Shirwaikar A., Sarala D. A., Vipin K (2012). Comparative Evaluation of Sonicated and Un-Sonicated Ethosomes Containing Ketoconazole. International Journal of advances in Pharmacy, Biology and Chemistry. 1(1):15-20.
22. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes—novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J Control Release 2000;65:403–18.
23. T. Jun-Bo, Y. Zhuang-Qun, H. Xi-Jing, X. Ying, S. Yong, X. Zhe, C. Tao, Effect ofethosomalminoxidil on dermal delivery and hair cycle of C57BL/6mice, J. Dermatol. Sci. 45 (2007) 135–137.
24. Vaibhav D, Dinesh M, Manoj N, Vikas J, Jain NK. Enhanced transdermal delivery of an anti-HIV agent via ethanolic liposomes. Nanomed Nanotechnol Biol Med 2010;6(2):590–6.

25. Verma P, Pathak K. Therapeutic and cosmeceutical potential of ethosomes: An overview. J Adv Pharm Tech Res. 2010;1(3):274-82.

Tables

Table-1: Composition of different Sonicated Delaviridine Ethosomes and Liposomes

Ethosomal formulation	Lecithin (Soya lecithin %)	Ethanol (%)	Propylene glycol (%)	Drug (g)	Cholesterol(g)	Water
EF1	1	20	10	0.200	0.5	q.s
EF2	2	20	10	0.200	0.5	q.s
EF3	3	20	10	0.200	0.5	q.s
EF7 (Liposomes)	2	-	-	0.200	0.5	q.s

Table-2: Composition of different Unsonicated Ethosomes and liposomes

Ethosomal formulation	Lecithin (Soya lecithin %)	Ethanol (%)	Propylene glycol (%)	Drug (g)	Cholesterol(g)	Water
EF4	4	20	10	0.200	0.5	q.s
EF5	5	20	10	0.200	0.5	q.s
EF6	6	20	10	0.200	0.5	q.s
EF7 (Liposomes)	2	-	-	0.200	0.5	q.s

Table-3: Composition of different Ethosomal Gel formulation

Gel formulation	Delaviridine Ethosomal suspension(ml)	Carbopol (%)	Triethanolamine (ml)	Phosphate buffer (pH 7.4)
*G-4	0.025g	1.5	0.5	q.s
G-3	20	2	0.5	q.s
G-2	20	1.5	0.5	q.s
G-1	20	1	0.5	q.s

*G-4 Drug free gel.

Table 4. Size Distribution Of Delaviridine Ethosomal Formulations

	Size range					
S. No.	Eye piece micrometer	In micrometer	Average size (d)	No. of vesicles (n*)	% no. of vesicles	N × d
1	0-1	0.00-3.33	1.66	70	46.66	116.2
2	1-2	3.33-6066	4.996	67	44.66	334.665
3	2-3	6.66-9.99	8.325	7	4.66	58.275
4	3-4	9.99-13.32	11.655	4	2.66	46.62

5	4-5	13.32-16.65	14.985	2	1.33	29.97
				Sn =150		Snd = 585.73

Table 5. Drug entrapment efficiency of various formulations of Ethosomes:

S.N O	Sample code		Concentration	Dilution factor	Amount of drug T = CXDF	Entrapped drug E=T-U	%Entrapped drug %E=E/T x100
01	EF1	Total drug(T)	9.65	10	96.5	71.2	73.7
		Free drug (U)	2.53	10	25.3		
02	EF2	Total drug(T)	9.717	10	97.17	67.92	70.0
		Free drug (U)	2.92	10	29.2		
03	EF3	Total drug(T)	9.832	10	98.32	65.82	66.9
		Free drug (U)	3.25	10	32.5		
04	EF4	Total drug(T)	9.95	10	99.5	53.5	53.7
		Free drug (U)	4.6	10	46.0		
05	EF5	Total drug(T)	9.93	10	99.3	45.8	46.12
		Free drug (U)	5.35	10	53.5		
06	EF6	Total drug(T)	9.7	10	97.0	41.0	42.2
		Free drug (U)	5.6	10	56.0		
07	LP	Total drug(T)	9.94	10	99.4	34.4	34.60
		Free drug (U)	6.5	10	65.0		

Table 6. Drug release profile

S.NO	Time(min)	Cumulative %drug release (flux)
01	5	1.01
02	10	2.2
03	15	2.87
04	30	5.67
05	60	9.21
06	120	12.48
07	240	21.22
08	360	25.51
09	720	40.21
10	1440	71.35

Table: 7. Drug entrapment efficiency of various formulations of Ethosomes

S.N O	Sample code		Concentration	Dilution factor	Amount of drug T = CXDF	Entrapped drug E=T-U	%entrapped drug %E=E/T x100
01	EF1	Total drug(T)	9.65	10	96.5	71.2	73.7
		Free drug (U)	2.53	10	25.3		
02	EF2	Total drug(T)	9.717	10	97.17	67.92	70.0
		Free drug (U)	2.92	10	29.2		
03	EF3	Total drug(T)	9.832	10	98.32	65.82	66.9
		Free drug (U)	3.25	10	32.5		
04	EF4	Total drug(T)	9.95	10	99.5	53.5	53.7
		Free drug (U)	4.6	10	46.0		
05	EF5	Total drug(T)	9.93	10	99.3	45.8	46.12
		Free drug (U)	5.35	10	53.5		
06	EF6	Total drug(T)	9.7	10	97.0	41.0	42.2
		Free drug (U)	5.6	10	56.0		
07	LP	Total drug(T)	9.94	10	99.4	34.4	34.60
		Free drug (U)	6.5	10	65.0		

Table 8. Drug release profile

S.NO	Time(min)	Cumulative %drug release (flux)
01	5	1.01
02	10	2.2
03	15	2.87
04	30	5.67
05	60	9.21
06	120	12.48
07	240	21.22
08	360	25.51
09	720	40.21
10	1440	71.35

Table No- 9: In-vitro cumulative % drug release profile for Delaviridine Formulations

	Cumulative %drug release								
Time (min)	EF1	EF2	EF3	EF4	EF5	EF6	EF7 Liposomes	EF GEL	Marketed FD
5	5.42	6.26	4.57	3.6	2.31	1.46	0.75	1.37	0.533
10	13.5	15.95	11.8	10.08	9.42	8.4	6.26	2.9	0.908
15	27.15	30	26.04	25.2	24.4	22.97	19.68	3.24	1.76

30	35.68	41.68	33.02	32.04	31.24	30.01	28.48	6.177	6.51
60	41.68	53.3	39.6	38.7	37.02	35.68	33.02	10.31	15.06
120	55.5	57.7	53.3	52.4	49.7	47.1	42.6	14.75	19.87
240	62.22	64.4	60.44	58.6	55.5	53.5	49.7	22.75	25.26
360	68.5	70.6	67.1	65.7	64.4	62.22	57.77	27.51	33.01
720	78.2	82.6	77.3	75.5	73.3	70.6	67.1	42.44	40.69
1440	88.4	93.7	89.3	87.11	78.2	75.5	72.44	74.35	47.26

Table 10. In-vitro release studies (EF2-EF gel)

S.NO	Time(min)	EF2	EF gel
01	5	6.26	1.52
02	10	15.95	1.82
03	15	30	2.13
04	30	41.68	2.74
05	60	53.3	3.39
06	120	57.7	5.57
07	240	64.4	7.56
08	360	70.6	9.98
09	720	82.6	28.5
10	1440	93.7	44.27

Table 11. Plasma Concentration (ng/ml) of standard drug and prepared formulation EF2

Time(hrs)	STANDARD	EF2
0	0	0
0.5	11.8	12.6
1	32.9	49.2
2	62.37	80
4	84	93
8	22	88
12	4.2	65
16	0	48
24	0	22

Table 12. In Vivo Pharmacokinetic Parameters of standard drug and prepared formulation EF2

Parameters	Standard	Formulation EF2
C max	84 ng/ml	93 ng /ml
T max	4 hr	4 hr
$t_{½}$	2.62 hr	1.66 hr
K el	0.033/hr	0.097/hr
AUC (0-t)	544 ng-hr/ml	1511 ng-hr/ml
AUMC (0-t)	2016 ng-hr*hr/ml	14376 ng-hr*hr/ml
MRT	3.5 hr	11.0 hr

Figures

Figure 1: JEOL, JSM-6360A, Scanning Electron Microscope

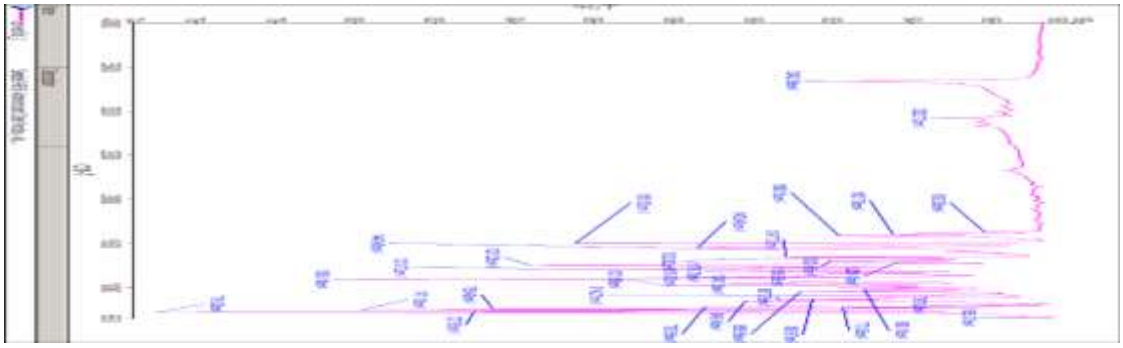

Fig 2. FTIR

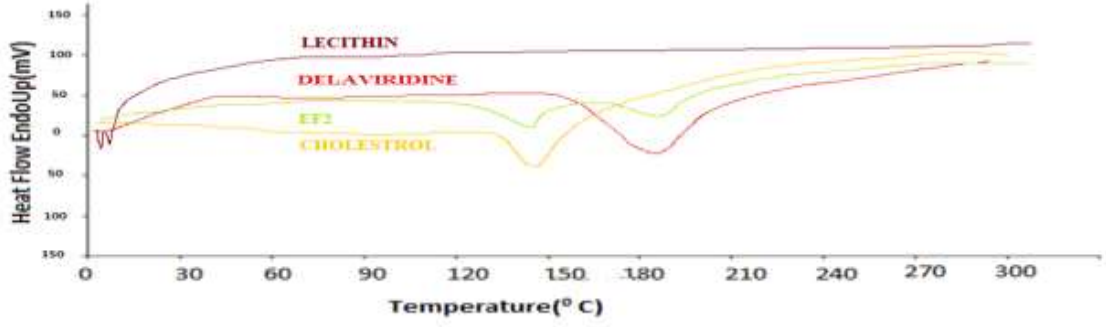

Fig 3. DSC

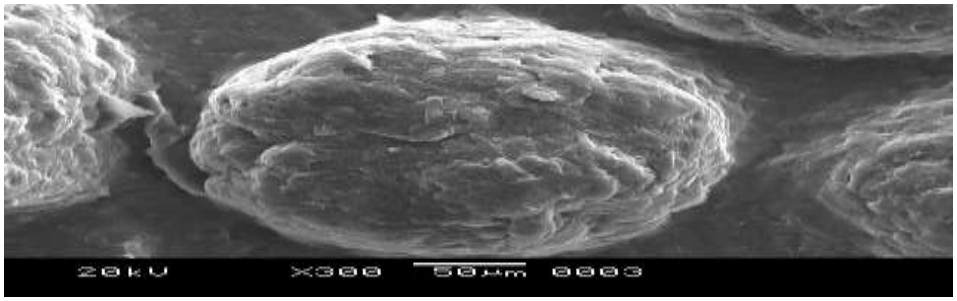

Fig 4. Scanning Electron Microscope (SEM)

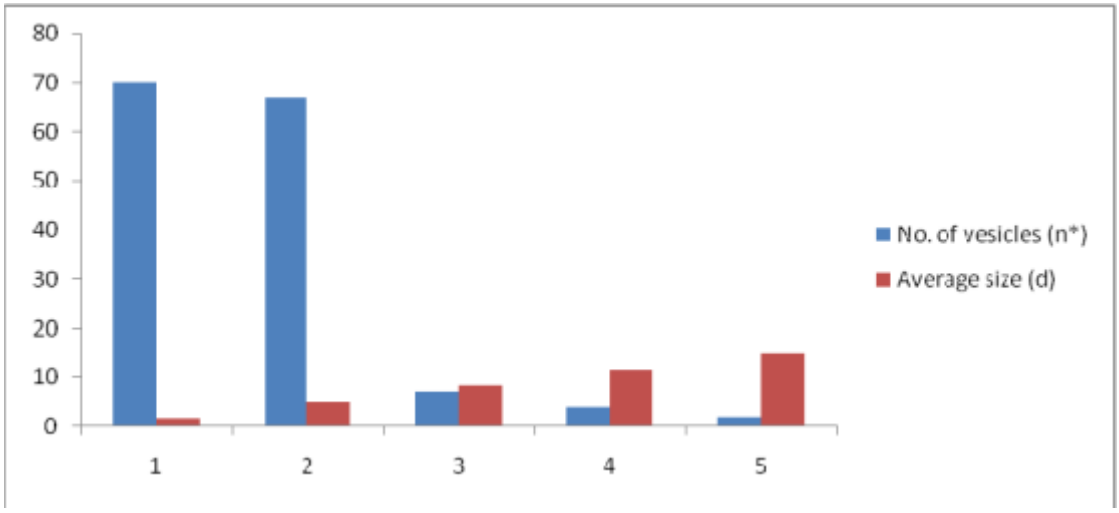

Fig 5. Size distribution of delaviridine ethosomal formulations

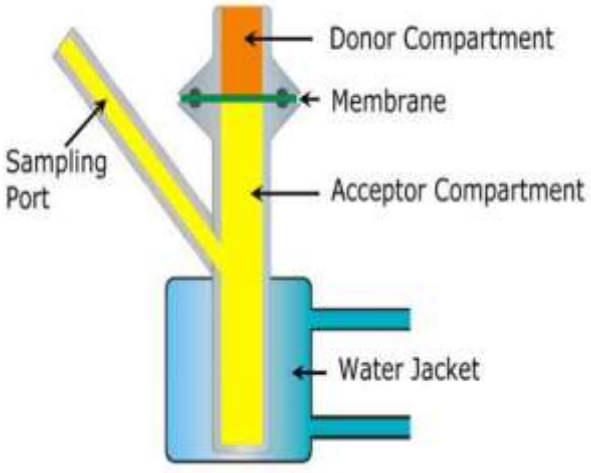

Figure:6 Franz diffusion cell

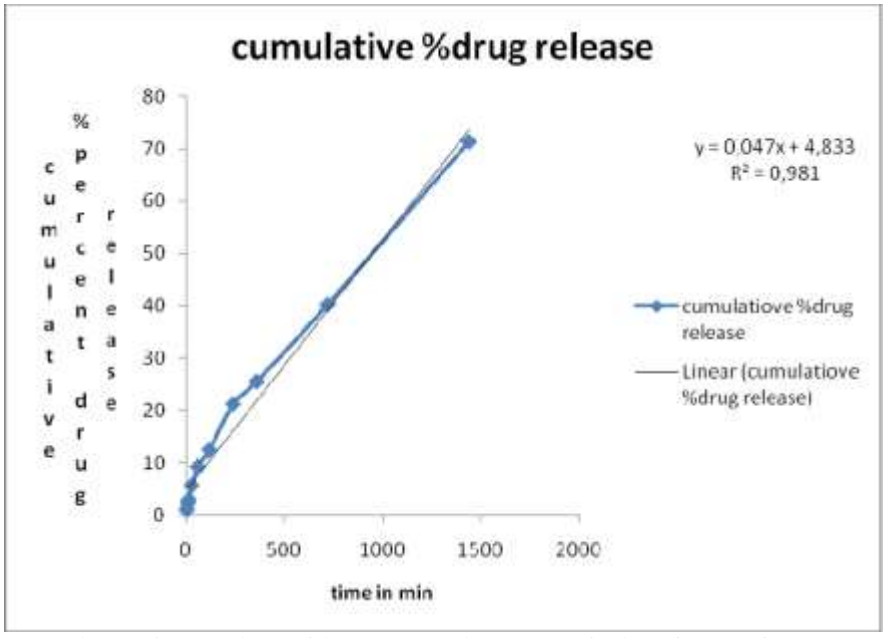

Figure 7: In-vitro skin permeation cumulative drug release

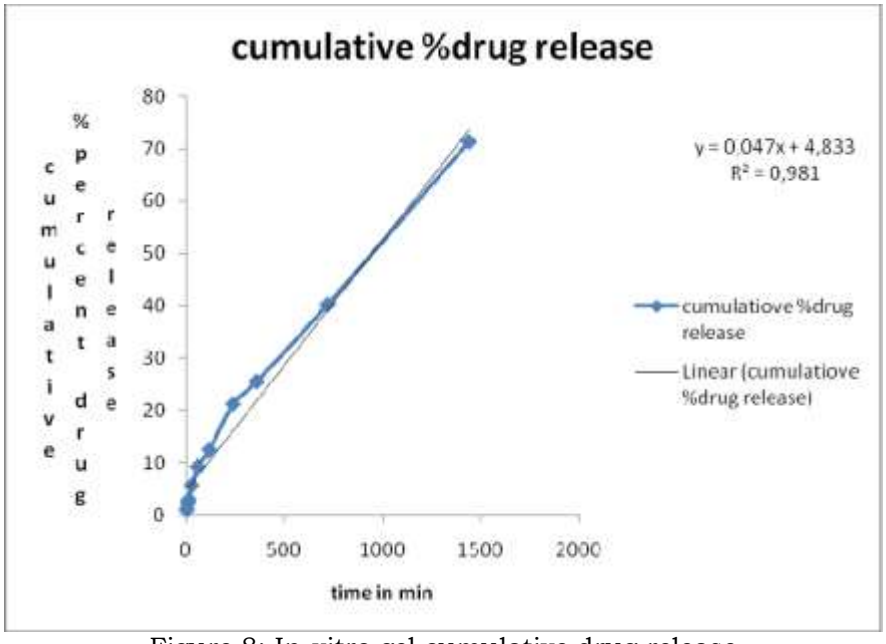

Figure 8: In-vitro gel cumulative drug release

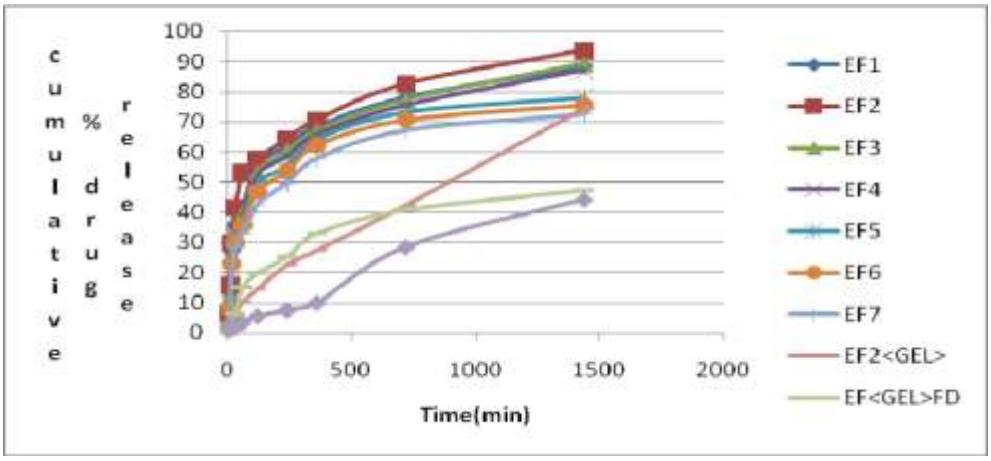

Figure 9: In-vitro cumulative % drug release

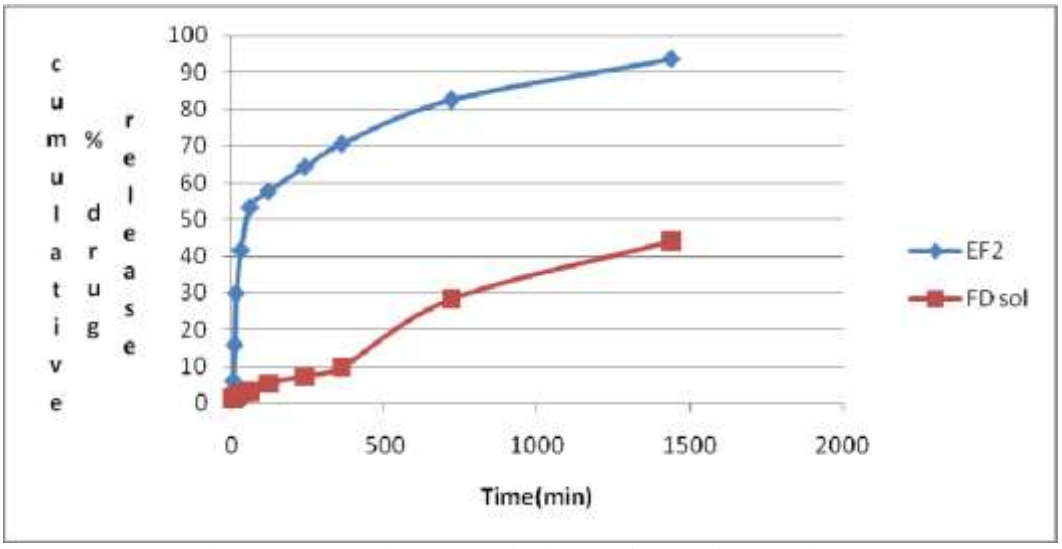

Figure 10: In-vitro cumulative % drug release of EF2

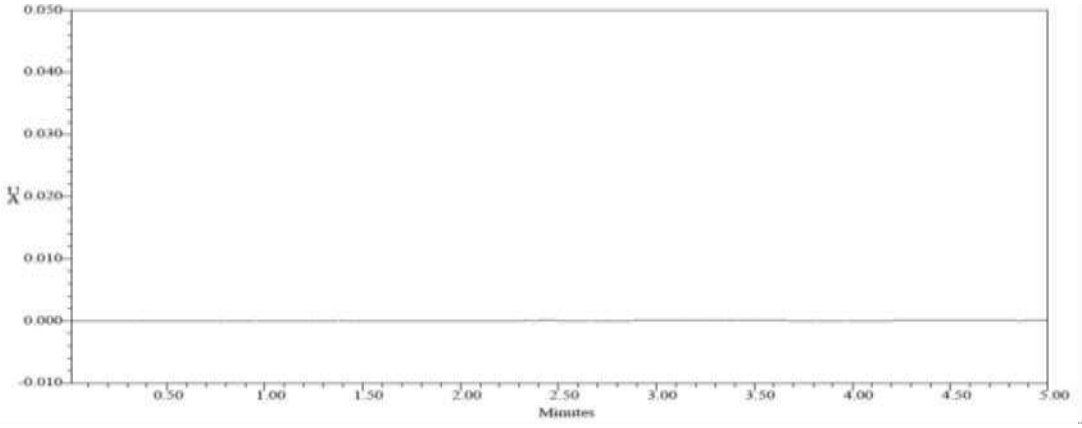

Figure 11: Blank run for Standard

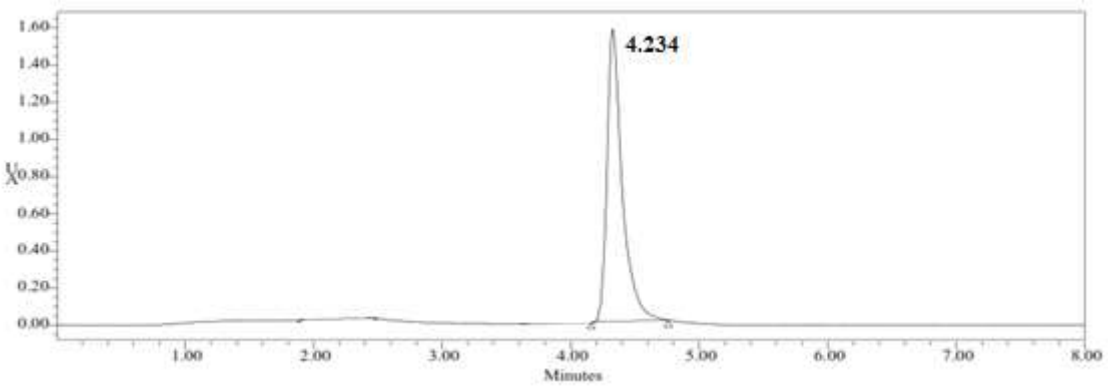

Figure 12: HPLC chromatogram of standard drug reference in rabbit plasma

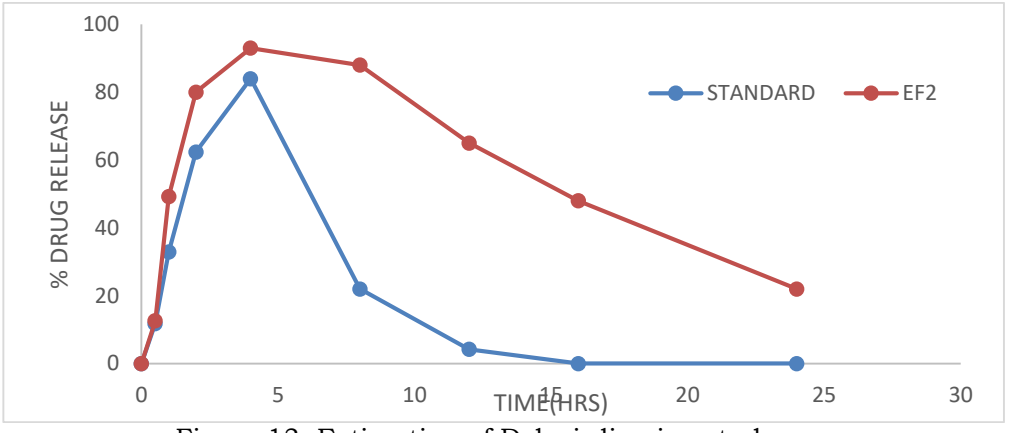

Figure 13: Estimation of Delavirdine in rat plasma