Journal of Young Pharmacists, 2024; 16(4):772-780.
https://www.jyoungpharm.org

Original Article

Bioavailability Enhancement of Nebivolol Hydrochloride Loaded Transferosomes *via* Transdermal Route: Pharmacokinetic and Pharmacodynamic Evaluation in Rats

Saritha Dunaka, Krishnaveni Janapareddi*

Department of Pharmaceutics, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, Telangana, INDIA.

ABSTRACT

Background: Nebivolol hydrochloride is a beta blocker used to treat hypertension but has low oral bioavailability (12%) because of first-pass metabolism by CYP3A enzymes. Hence to improve bioavailability, nebivolol hydrochloride loaded transferosomes delivery via transdermal route is studied. **Materials and Methods:** Transferosomes were developed by thin film hydration method and evaluated for physicochemical properties. Study state flux was determined by permeability studies on excised rat abdominal skin using Franz diffusion cells. To evaluate the efficacy of transferosomes Pharmacokinetic and Pharmacodynamic studies were carried out on Wistar male rats. **Results:** Optimized transferosomal formulation TF2D comprises soya lecithin, tween80 and drug in the proportion 90:10:5 with 5% DMSO as permeation enhancer. The optimized formulation showed vesicle size 141.9 nm, PDI 0.143, Zeta potential -39.1 mV, entrapment efficiency 98.5% and steady state flux 97.4 µg/cm^2/hr. The steady state flux of the TF2D was 4.23 times the flux of drug suspension. Scanning electron microscopic images displayed sphere shaped vesicles. FTIR studies confirm the compatibility between drug and formulation additives. Antihypertensive activity of TF2D was significantly high compared to oral drug suspension and the effect was sustained up to 48 hr. The bioavailability of TF2D was significantly high at $p<0.0001$ (4.13 folds) in comparison with oral drug suspension. The histopathological study on the rat skin confirmed the safety of the transferosomal formulation of nebivolol. **Conclusion:** We conclude that introducing transferosomes as vesicular drug carriers via transdermal route could significantly enhance the bioavailability of nebivolol hydrochloride.

Keywords: Nebivolol hydrochloride, Transdermal drug delivery, Transferosomes, Permeability studies, Pharmacodynamic activity, Pharmacokinetic activity.

Correspondence:
Prof. Krishnaveni Janapareddi
Department of Pharmaceutics, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, Telangana, INDIA.
Email: jkrishnaveni@kakatiya.ac.in

Received: 16-06-2024;
Revised: 03-08-2024;
Accepted: 04-09-2024.

INTRODUCTION

Hypertension is a chronic medical condition characterized by persistent rise in arterial blood pressure. Nebivolol hydrochloride is a drug used to treat hypertension. However, it undergoes hepatic first-pass metabolism by CYP450 enzymes resulting in low oral bioavailability (12%). To improve bioavailability there is a need to develop an alternative route and delivery system that can effectively deliver nebivolol into systemic circulation. Several approaches have been studied by researchers. One promising approach is the development of transdermal delivery systems.[1,2]

Transdermal delivery of drugs gained importance as it avoids first-pass metabolism and exposure of drugs to drastic gastrointestinal environment. Apart from bioavailability enhancement, transdermal delivery of drugs maintains plasma levels consistent for longer periods.[3] The stratum corneum, the topmost layer of skin, is the biggest barrier which limits the drug permeation across the skin. The most promising approach for enhancement of drug permeation is nanocarrier based systems for transdermal drug delivery. Transferosomes are one of the nanocarriers, which are ultradeformable and overcome the disadvantages of poor penetration and stability.[2-4]

Transferosomal nanocarrier concept is introduced by Cevc in 1991.[5] These are known as ultra-deformable vesicles. Transferosomes are primarily composed of phospholipids and surfactant as edge activator. Surfactant is responsible for the membrane's ultra flexible nature.[6] Due to their deformable or elastic nature, when applied on the skin they can easily penetrate into the deeper layers of skin and deliver drug into systemic circulation.[6,7]

The present investigation aims to develop Nebivolol hydrochloride loaded transferosomes for transdermal delivery and evaluate its

DOI: 10.5530/jyp.2024.16.97

Copyright Information :
Copyright Author (s) 2024 Distributed under Creative Commons CC-BY 4.0

Publishing Partner : Manuscript Technomedia. [www.mstechnomedia.com]

ex vivo permeability, Pharmacodynamic and pharmacokinetic activity in rats comparison to oral drug suspension.

MATERIALS AND METHODS

Nebivolol hydrochloride is procured from Cadila Pharmaceuticals Limited, Ankleshwar, Bharuch. Soya lecithin is from Tokyo Chemical Industry, Japan. Tween 80 is purchased from Sigma Aldrich, Bangalore, India. All solvents employed are of Merck's HPLC quality.

Estimation of nebivolol hydrochloride by UV method

Nebivolol hydrochloride (10 mg) weighed into 10 mL volumetric flask, dissolved in sufficient quantity of methanol. The volume was made up to 10 mL using phosphate buffer pH 7.4. Dilutions were made with buffer to obtain required concentrations. The absorbance was measured at 282 nm using a UV-visible spectrophotometer.[8]

Estimation of nebivolol hydrochloride by HPLC method

Stock solution of nebivolol hydrochloride (1 mg/mL) was prepared with mobile phase. Dilutions were made from stock solution to get concentrations 0.25, 0.5, 1, 2, 4, 6 and 8 μg/mL. About 20 μL was injected into HPLC column. Calibration curve was constructed. The column used was, Merk C18, (250 x 4.6 mm). Mobile phase was composed of acetonitrile, methanol, orthophosphoric acid (0.1 N) in the ratio 80:20:10. Detection was at 282 nm and at a flow rate of 1 mL/min.[9]

Preparation of transferosomes

Film hydration technique was employed for preparing transferosomes using lipid and surfactant as edge activator. Chloroform and methanol mixture was used as solvent.[10,11] Lipid and surfactant were weighed into a boiling tube. The drug dissolved in solvent mixture was added to lipid, surfactant mixture and vortex mixed. The clear solution formed was transferred into round bottom flask and evaporated on a rotary flash evaporator at 45°C, 80 rpm under reduced pressure to form a thin, dried film on the wall of the flask. Phosphate buffer of pH 6.8 was used to hydrate the film. The resulting preparation was probe sonicated for 5 min at 33% amplitude (Bandelin Electronic GmbH & Co.KG, Berlin) to get transferosomal formulation.

Permeation enhancer (Dimethyl sulfoxide) was added to the selected formulation TF2 and the influence on flux was studied.[12]

Carbopol gel (1.5%) was prepared by adding Carbopol 934 into distilled water under continuous stirring and neutralized with triethanolamine.[10]

Characterization

Vesicle size, Zeta potential and Polydispersity Index (PDI)

Mean vesicle size, zeta potential and PDI of transferosomes were measured using Zetasizer (Nano-ZS 90, Malvern Instruments, U.K). The measurements were carried out on 50 times diluted sample and an average of three readings was taken.[13,14]

Determination of drug Content

The transferosomal formulation equivalent to 10 mg of nebivolol was taken in 10 mL volumetric flask and made up to 10 mL with methanol, bath sonicated for 30 min and filtered through 0.45 μm filter. The filtrate was suitably diluted with methanol and analyzed by UV method.[15,16]

Determination of entrapment efficiency

It is determined by ultra centrifugation method. About 2 mL of transferosomal formulation was taken in centrisort tubes and centrifuged for 30 min at 13,000 rpm. After centrifugation, the buoyant was filtered and analyzed by UV method after suitable dilutions. The entrapment efficiency was computed using following formula.[16]

$$EE\ (\%) = \frac{\text{Drug in formulation} - \text{Drug in buoyant (free drug)}}{\text{Drug in formulation}} * 100$$

FTIR study

FTIR spectrum of pure drug was obtained by KBr pellet method. Optimized transferosomal formulation FTIR spectrum was obtained by Attenuated Total Reflectance method. Both spectra were examined over a wavelength of 4000-400 cm^{-1}.[14]

Surface Morphology

The optimized formulation TF2D was subjected to Scanning electron microscopy (SEM JEOL-JSM-6510, Japan). A drop of transferosomal formulation was placed on a glass stub using double adhesive tape, air-dried and visualized under SEM equipped with a digital camera.[14,15]

Measurement of pH and viscosity

The pH was determined using digital pH meter. The viscosity of transferosomal gel was noted by using Brookfield viscometer with spindle No. 64 at 20 rpm and the corresponding dial reading was noted.[10,16]

Ex vivo Permeation studies

The rat was sacrificed by cervical dislocation method. The abdomen skin of rat was trimmed and excised. The excised skin was immersed in 60°C water for 45 sec and the epidermis was detached using blunt forceps.[17-19]

Vertical Franz diffusion cells were employed in permeation studies. The isolated rat epidermis was hydrated by placing it in

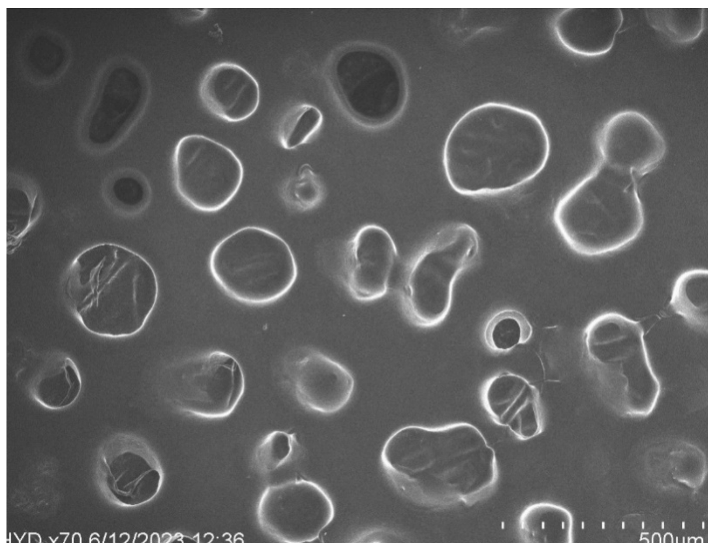

Figure 1: SEM image of optimized formulation (TF2D).

Phosphate buffer saline of pH 7.4 for 2 hr before use. PBS (pH 7.4) was filled in the receptor cell and the epidermal layer was placed between the receiver and donor cell with stratum corneum confronted upwards and the apparatus was fixed using clamp. Formulation containing equivalent to 5 mg of nebivolol was applied onto the skin. The apparatus was placed on a magnetic stirrer and stirred at 200 rpm at room temperature. Samples were withdrawn at time intervals of 0, 1, 2, 3, 4, 6, 8, 12 and 24 hr from the receiver cell and replaced with fresh buffer. After filtration, samples were analyzed by UV. The following equation was used to calculate the cumulative amount of drug permeated through the epidermal layer.[17,18]

$$Q_n = [C_n V + \sum_{i=1}^{n-1} C_i S]$$

$Q_{n=}$Cumulative quantity of drug permeated at n^{th} time,

C=Drug concentration (µg/mL) determined at n^{th} sampling interval,

V=Receiver cell volume,

$\Sigma C_i S$=Sum of sample concentrations at sampling points 1 to n-1 multiplied by sample volume (S).

A graph was constructed between the cumulative drug permeated (µg) versus time (hr). The flux at steady state J_{ss} (µg/h/cm^2), permeability coefficient K_p (cm/hr) and Enhancement Ratio (ER) are figured by the following equations.[19,20]

Jss=Cumulative amount of drug permeated/Area of diffusion cell

Kp=Steady state flux (Jss)/Concentration of drug in donor cell

ER=Jss of transferosomal formulation /Jss of drug suspension

Statistical significance was done by one-way ANOVA using GraphPad prism software (Version 9.5.1).

In vivo Studies

The optimized formulations were assessed for pharmacokinetic characteristics and pharmacodynamic activity in comparison with the oral route in a rat model.

Male Wister rats weighing around 200 to 250 g were procured from Vyas Labs, Hyderabad. The protocols of animal experiments were authorized by CPCSEA and the Institutional Animal Ethical Committee (IAEC), Kakatiya University (KU) vide no IAEC/08/UCPSc/KU/2022. The rats were housed in polypropylene cages filled with sterile paddy husks, at a temperature of 22±2°C a 12 hr light-dark cycle and 55-65% humidity. A day before the study the dorsal side hair of rat was removed using electrical trimmer and marked 2cm^2 area and the animals were kept overnight fasting with free access to water.[18,19]

Non-invasive Blood Pressure Method (Tail cuff method)

Anti-hypertensive activity in rats was conducted using Non-Invasive blood pressure system (NIBP 200 A; Biopic System, Inc., Goleta, CA, USA). A pneumatic pulse sensor equipped with a cuff was fastened to the tail. Before study, the rats were trained for two days in rat restrainer to stay calm and non-aggressive. The Wistar male rats were divided into 7 groups each housing six animals. Group A kept as normal control, groups B to G hypertension was induced by adding fructose in drinking water (10%) for 2 weeks.[21-23] Two weeks later, systolic BP was measured and rats with a minimum mean systolic BP of 145- 150 mm of Hg were selected. Fructose water was removed and normal water was kept during treatment period. Group B was positive control

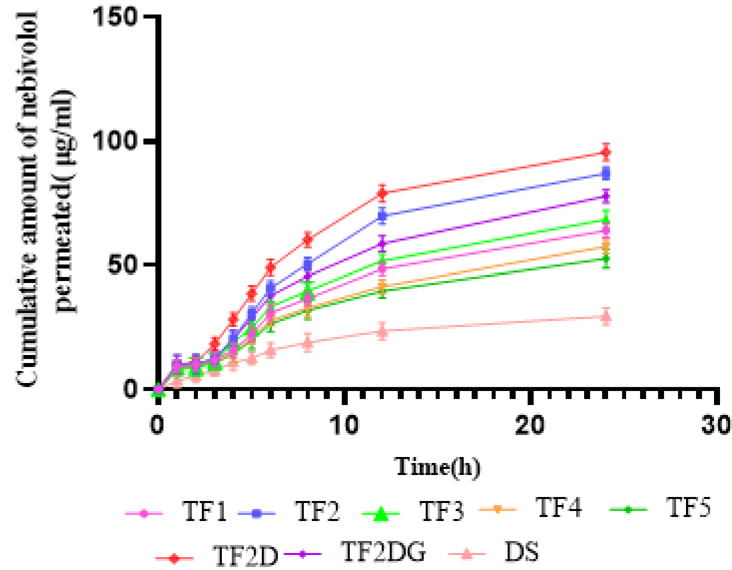

Figure 2: *Ex vivo* permeation profiles of transferosomal formulations (mean±SD, *n*=3).

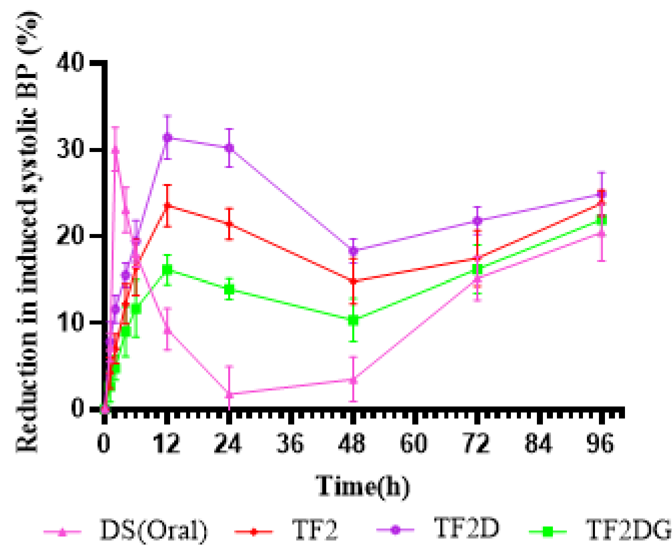

Figure 3: Percent reduction in SBP of oral drug suspension and transferosomal formulation (mean±SD, *n*=3).

Table 1: Composition and characterization of Transferosomal formulations (mean±SD, *n*=3).

Formulation code	Soya lecithin: Tween80 (mg/mL)	Vesicle size (nm)	PDI	Zeta potential (mV)	Content (%)	Entrapment efficiency (%)
TF1	95:5	190.2±2.235	0.253±0.12	-24.5±1.26	98.5±0.74	91.5±0.63
TF2	90:10	148.6±1.276	0.179±0.07	-33.5±0.96	99.2±0.96	97.9±0.57
TF3	85:15	186.9±1.355	0.235±0.03	-30.3±0.74	98.6±0.82	95.6±0.61
TF4	80:20	200.6±1.514	0.325±0.09	-19.8±0.81	97.5±0.79	93.1±0.54
TF5	75:25	222.5±2.765	0.315±0.07	-32.4±1.32	97.6±0.91	89.8±0.95
TF2D	90:10+Dmso (5%)	141.9±1.756	0.143±0.05	-39.1±0.85	99.6±0.56	98.5±0.64

TF: Transferosome Formulation; Note: All the transferosome formulations contain 5 mg of Nebivolol hydrochloride in 1 mL.

and received no treatment. Groups C&D were treated with drug suspension and marketed formulation in suspension form (Nebicard® 5mg) respectively through oral route at a dose of 5 mg/kg. Groups E, F and G received formulations TF2, TF2D and TF2DG respectively through transdermal route at a dose of 5 mg/kg. The rats were kept in the restrainer at time points of 1, 2, 4, 6, 8, 12, 24, 48, 72 and 96 hr. Using NIBP, lab chart software, the systolic BP and heart rate were recorded. The average of three successive readings was recorded for each rat.[24-26]

% reduction in SBP is calculated by the following equation.

$$\textit{Percentage reduction in } \text{Systolic BP (R)} = \frac{\text{Induced SBP (before treatment)} - \text{SBP after treatment}}{\text{Induced SBP}} * 100$$

Pharmacokinetic study

Male Wister rats were made into five groups of six rats. Group A and B received drug suspension and marketed formulation in suspension form (Nebicard ®tablet 5 mg) via oral route. Groups C, D and E received formulations TF2, TF2D and TF2DG respectively through transdermal route. The groups were treated with nebivolol at a dose of 5 mg/kg. After treatment at time points of 0, 0.5, 1, 2, 3, 4, 8, 12 and 24 hr, about 0.5 mL of blood sample was drawn from the retro-orbital plexus of rat into EDTA coated vacutainers. The samples were centrifuged for 20 min at 5000 rpm. Plasma was pipetted and preserved at -20°C for further analysis by HPLC.[26,27]

Plasma sample of 100 µL was placed in a 2 mL Eppendorf tube, 100 µL of valsartan solution (0.5 g/mL) was added as Internal Standard (IS) and vortexed for 2 min. About 0.5 mL of acetonitrile was added and vortexed for 3 min and centrifuged at a speed of 5000 rpm for 30 min. The supernatant was pipetted and filtered using 0.22µm syringe filters. The sample was spiked into the HPLC column.[28,29]

Kinetica Software 2000 (Version 5.0, Inna phase Corporation, Philadelphia, PA) was used to determine pharmacokinetic parameters such as AUC_{total}, MRT, $t_{1/2}$, C_{max} and T_{max}. The statistical significance between the groups (*p*-value) was determined by Graph Pad Prism (Version 9.5.1).[27,30]

Skin irritation and histopathological studies

The male Wister rats were divided into groups A, B, C, D and E each containing three. The dorsal side hair of the rats was shaved and marked area of 2 cm^2. Groups A, B, C, D and E were treated with normal saline (control), 5% w/v Sodium lauryl sulfate (Positive control), formulation TF2, TF2D and TF2DG respectively at a dose of 5 mg/kg twice daily for 5 days. After treatment, the treated skin was visualized for oedema and redness. The skin was excised after sacrificing rat and preserved in a 10% formalin solution. Hematoxylin-eosin dye was used to produce specimen slides, which were examined under a light microscope for histopathological alterations.[19,24,26]

RESULTS

The calibration curves of UV method and HPLC method showed good linearity with R^2 values of 0.999 and 0.998.

The transferosomal formulations were prepared by varying between soya lecithin 75-95 w/w and tween 80 (5-25 w/w) using a mixture of chloroform and methanol (2:1 ratio) as solvent system.

The mean vesicle size, PDI, zeta potential and entrapment efficiency of transferosomes are shown in Table 1. Formulation TF2 containing soya lecithin, tween 80 in the ratio of 90:10 exhibited significantly small size (148.6 nm), PDI (0.179) and high steady state flux (89.9 µg/hr/cm^2). TF2 is modified by adding DMSO (5%) to enhance permeation. The formulation TF2D showed significant improvement ($p<0.05$) in permeation compared to TF2 formulation.[11,12] The transferosomal formulation TF2D was incorporated in Carbopol 934 gel (1.5%) to form Transferosomal Gel (TF2DG).

The pH of transferosomal formulations is between 6.22 to 6.56. The pH of transferosomal gel was 6.42 and viscosity was 183 mPa-s.

FTIR spectrum of optimized formulation retained peaks of specific functional groups such as 3199-3350 cm^{-1}(O-H), 1544-1621 cm^{-1} (C=C), indicating unaltered drug.

Table 2: *Ex vivo* permeation parameters of transferosomal formulations and Drug suspension (mean±SD, *n*=3).

FC	J_{ss} (µg/cm^2/hr)	Kp×10^{-3} (cm/h)	ER
TF1	59.6±1.4	11.6±1.8	2.56
TF2	89.9±1.2	17.9±2.3	3.80
TF3	67.8±2.2	13.5±2.1	2.94
TF4	52.3±1.8	10.4±1.9	2.26
TF5	50.3±1.6	10.07±1.5	2.17
TF2D	97.4±2.1	19.4±1.3	4.23
TF2DG (gel)	80.3±1.3	16.04±1.7	3.38
DS (Drug in CMC)	23.6±1.7	4.7±2.5	1

FC: Formulation Code; **Kp:** Permeation coefficient; **ER:** Enhancement ratio; **Jss:** steady state flux.

Table 3: Anti-hypertensive effect on rats after oral and transdermal administration of Nebivolol hydrochloride formulations (mean±SD, *n*=6).

Mean systolic blood pressure (mm of Hg)												
Groups (treatment)	Normal	Induced	1 hr	2 hr	4 hr	After treatment 6 hr	12 hr	24 hr	48 hr	72 hr	96 hr	%R (12 hr)
A(NC)	101.4±3.2	-	103.1±2.5	100.9±3.2	100.8±2.8	104.3±3.1	100.7±2.8	101.6±1.6	102.1±3.2	101.4±2.8	102.3±2.6	0.6
B(HC)	103.1±2.8	151.2±2.8	150.1±1.3	149.8±2.8	149.1±2.4	149.7±2.8	148.6±2.6	148.1±2.8	141.4±2.8	134.2±3.1	123.5±2.5	0.9
C(DS)oral	101.9±2.5	148.9±1.2	138.2±1.7	104.1±2.5*	114.2±2.6	122.2±1.5	135.1±2.4	146.3±2.6	143.7±2.6	126.4±2.5	118.5±1.6	9.2
D(MF) oral	102.1±2.3	148.1±1.6	134.6±2.2	103.6±2.2	105.8±1.5	116.7±1.6	129.5±3.2	142.9±1.7	139.8±1.6	129.7±2.2	122.2±2.8	12.6
E(TF2)	100.4±2.01	149.4±2.2	143.1±1.5	138.9±1.8	131.2±1.7	124.9±2.2	114.2±1.5***	117.4±2.4	127.3±1.8	123.3±1.8	113.8±3.2	23.5
F(TF2D)	101.2±1.74	149.2±2.5	137.5±2.3	131.9±1.6	126.1±1.4	120.2±2.4	102.3±2.5****	104.1±2.2	121.9±1.6	116.7±2.6	112.1±1.4	31.6
G(TF2DG)	103.1±1.6	150.1±1.4	146.1±1.8	142.9±1.4	136.6±1.9	132.6±1.2	125.9±1.6**	129.2±1.2	134.6±2.5	125.8±1.4	117.2±1.8	16.1

%R=Percentage reduction in mean Systolic blood pressure; NC: Normal control; HC: Hypertensive control; DS: Drug suspension; MF: Marketed formulation.**** Significant at $p<0.0001$, *** at $p<0.001$, ** at $p<0.01$ compared to oral drug suspension.

The SEM images of the optimized transferosomal formulation TF2D are shown in Figure 1. The well-defined spherical and oval shaped vesicles were observed in SEM images.

Permeation profiles of transferosomal formulations and drug suspension are shown in Figure 2 and data in Table 2. The optimal formulation of nebivolol was chosen based on maximum steady state flux and entrapment efficiency values. The formulation (TF2D) composed of soya lecithin, tween 80 and drug in the proportion 90:10: 5 with 5% DMSO as permeation enhancer showed highest flux (97.4±2.1 μg/cm^2/hr) and entrapment efficacy (98.5±0.64%). The optimized formulation TF2D showed significantly high steady-state flux compared with TF2 ($p<0.05$), TF2DG (gel) ($p<0.01$) and oral drug suspension ($p<0.0001$).

The antihypertensive activity of transferosomes was studied in comparison with oral drug suspension and the results are shown in Table 3. Peak activity of drug suspension was observed at 2 hr. The activity decreased gradually and reached to initial induced systolic BP within 24 hr. Whereas, the transdermal transferosomal formulations peak activity showed at 12 hr and sustained up to 48 hr. Percent reduction in Systolic BP versus time profiles is shown in Figure 3. The figure clearly shows the superiority of transferosomes in controlling systolic BP. In case of oral drug suspension, the effect was negligible in 24 hr. Antihypertensive activity of TF2D was significantly high in comparison with oral drug suspension ($p<0.0001$), TF2DG ($p<0.001$) and TF2 ($p<0.01$).

The plasma concentration versus time profiles of nebivolol hydrochloride formulations are shown in Figure 4. The results are given in Table 4. The C_{max} of the transdermal formulation containing DMSO (TF2D) was significantly high ($p<0.0001$) when compared to drug suspension. The AUC of TF2D was significantly high compared to TF2 ($p<0.05$), TF2DG ($p<0.01$) and oral drug suspension ($p<0.0001$).

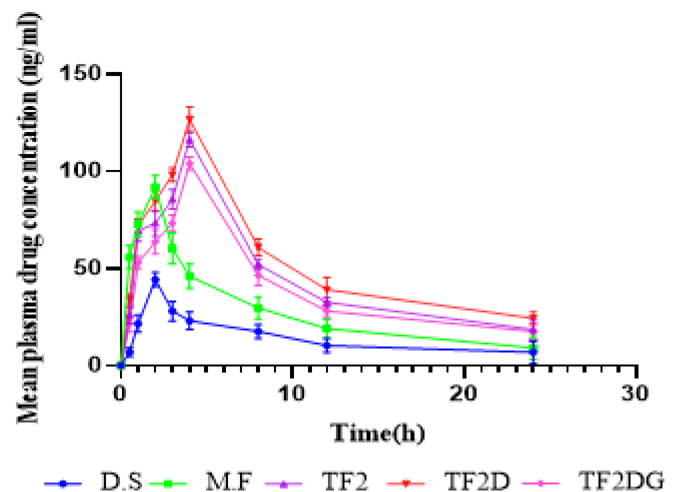

Figure 4: Plasma concentration vs. time profiles of transferosomal formulations and Drug suspension (DS) (mean±SD, *n*=3).

Table 4: Pharmacokinetic parameters of Nebivolol formulations after oral and Transdermal route (mean±SD, *n*=6).

Groups	Treatments	C_{max} (ng/mL)	AUCtotal (ng*hr/mL)	T_{max} (hr)	$t_{1/2}$ (hr)	MRT (hr)	F
I	DS (oral)	44.3±3.67	425.3±4.61	2	7.1±0.8	9.73±0.7	-
II	MF (oral)	91.64±5.23	752.4±2.75	2	9.6±0.9	10.23±1.4	1.77
III	TF2	116.23±3.76**	1334.8±7.32***	4	12.28±1.0**	15.46±1.1**	3.12
IV	TF2D	126.62±6.43***	1744.1±3.53****	4	15.53±1.08***	19.11±1.4***	4.13
V	TF2DG	103.83±3.44	1102.3±2.51**	4	10.32±0.9	13.26±0.9	2.59

**** Significant at $p<0.0001$,*** at $p<0.001$,** at $p<0.01$ compared with drug suspension.F (R elative bioavailability)=AUC of respective formulation /AUC of DS (oral).

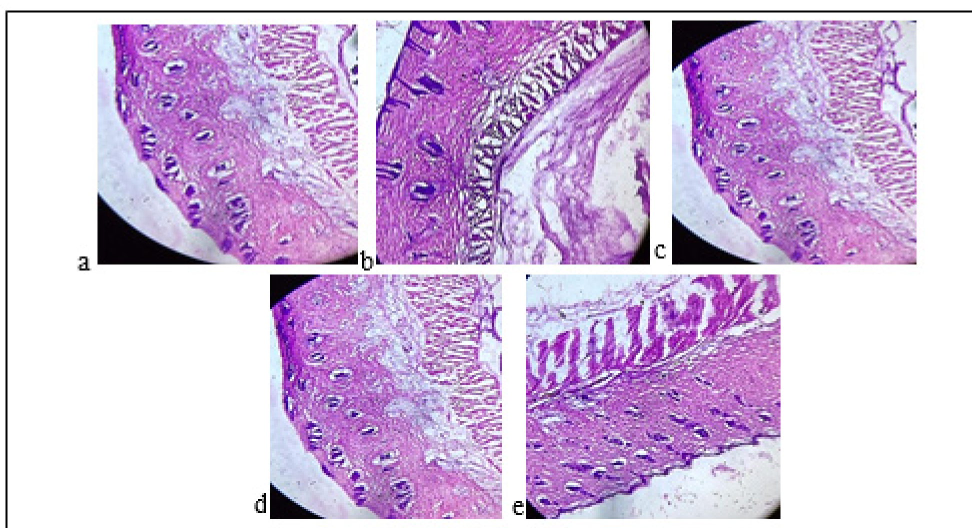

Figure 5: Histopathology photomicrographs of rat skin treated with a) untreated b) SLS c)TF2 d) TF2D e) TF2DG.

Visual observation of skin after treatment showed no redness and oedema. Histopathological images of rat skin are shown in Figure 5. The skin of rats treated with transferosomal formulations (TF2, TF2D) was intact with clear dermal and epidermal layers without any significant tissue damage. The skin of the SLS treated rats exhibited significant damage due to lipid extraction.

DISCUSSION

Nebivolol is a selective β blocker which is commonly used to treat hypertension and heart failure. Oral bioavailability of nebivolol is low due to first pass metabolism. Delivery of nebivolol via transdermal route improves bioavailability and minimizes the GIT side effects and prolongs the activity.[1,2]

In the present study, optimized transferosomal formulation showed significantly high antihypertensive activity in dynamic studies and high bioavailability in Pharmacokinetic studies.

Transferosomes were developed and characterized.[3-5] Based on vesicle size, entrapment efficiency formulation TF2 was selected and further modified with permeation enhancer DMSO to form TF2D. The TF2D was finally incorporated into Carbopol gel 934 to produce transferosomal gel TF2DG. Based on steady state flux the formulation TF2D was optimized.[6,7] The vesicle size of transferosomes ranged between 141.9±3.8 to 222.5±5.9 nm. At a 90:10 ratio lecithin and tween80 (TF2) showed small vesicle size (148.6±1.2 nm) and high entrapment efficiency. When the concentration of edge activator (Tween80) increased from 5 to 15 mg, size of vesicle decreased due to lowering the interfacial tension. Further increase in Tween 80 concentration from 15 to 25 mg increased the vesicle size. This could be due to fluidization of lipids in stratum corneum.[11-13]

Permeability coefficient of transferosomal formulations was significantly high when compared with drug suspension. This is due to nano size and ultra deformable nature of transferosomes which makes them easy to pass intercellularly. We found that vesicular transport through the skin is aided by surfactant (tween 80) optimal concentrations. The reduced drug delivery at high and low surfactant concentrations could be due to increased size and reduced entrapment efficiency.[16,17]

In vivo, the pharmacodynamic activity of transferosomes was studied by the NIBP method. The optimized formulation TF2D decreased Systolic Blood Pressure (SBP) gradually showing peak activity at 12 hr and the effect was continued up to 24 hr. A significantly high percent reduction in SBP (R) 31.6% was

observed with TF2D formulation at 12 hr time point. Whereas, in oral drug suspension and marketed formulation, R was found to be 9.2% and 12.6% respectively. Based on the outcomes it is noticed that the TF2 and TF2D released the drug gradually over a prolonged period, which resulted in sustained anti-hypertensive effects up to 48 hr.[23-25] The transferosomal gel showed less activity compared to formulation TF2 and TF2D. This is due to reduced thermodynamic mobility of transferosomes in gel. The transferosomal formulations TF2 and TF2D successfully reduced rat blood pressure to normal. The results proved the efficiency of transdermal formulations of nebivolol hydrochloride in the management of hypertension.

In pharmacokinetic study, The AUC_{total} and C_{max} of transdermal formulations were significantly high compared to oral drug suspension and marketed formulation. Transferosomal formulation bioavailability ratio (F) was greater than 3. This clearly proves the potential advantage of transferosomes via transdermal route. This is due to the avoidance of hepatic first-pass metabolism and enhanced permeability of nanosized vesicles carrying lipophilic drugs intercellular route.[27,28]

DMSO as permeation enhancer improved bioavailability by 30%. The observations were tallying with earlier reports.[12,17] Converting into gel form significantly reduced the bioavailability and also anti-hypertensive effect due to reduction in thermodynamic mobility of transferosomes in gel. Peak time for the activity of transdermal formulations was significantly high (4 hr) compared to oral route (2 hr). This could be due to the barrier properties of skin. The $t_{1/2}$ and MRT of TF2D were significantly high ($p<0.0001$) compared to oral drug suspension and marketed formulation. This could be due to the avoidness of first pass metabolism and slow absorption of drug via transdermal route.[30] The bioavailability of optimized transferosomal formulation (transdermal route) was 4.13folds compared to oral drug suspension and 2.31 folds compared to oral marketed formulation.

CONCLUSION

In the present investigation, transferosomal formulations of nebivolol were developed and characterized. Steady state flux of optimized transferosomal formulation TF2D was 4.23 folds compared to flux of drug suspension. The formulation TF2D showed significantly high antihypertensive activity and bioavailability (4.13 folds) in comparison to oral drug suspension, 2.31 folds to oral marketed formulation. Hence transferosomes via the transdermal route could significantly enhance the bioavailability of nebivolol hydrochloride.

ACKNOWLEDGEMENT

The authors acknowledge the UCPSc, Kakatiya University, Warangal, Telangana, India, for providing all the facilities to perform the experimental work.

CONFLICT OF INTEREST

The authors declare that there is no conflicts of interest.

ABBREVIATIONS

NEB: Nebivolol hydrochloride; **TF:** Transferosomes; **PDI:** Polydispersity Index; **DMSO** Dimethyl sulfoxide; **FTIR:** Fourier transform infrared; **HPLC:** High-Performance Liquid Chromatography; **%EE:** Entrapment efficiency; **SEM:** Scanning electron microscopy; **PBS** Phosphate buffer saline; **SBP:** Systolic blood pressure; **HR:** Heart rate; **SLS:** Sodium lauryl sulphate.

REFERENCES

1. Opatha SA, Titapiwatanakun V, Chutoprapat R. Transfersomes: A promising nanoencapsulation technique for transdermal drug delivery. Pharmaceutics. 2020;12(9):855. doi: 10.3390/pharmaceutics12090855, PMID 32916782.
2. Reddy YK, Reddy DM, Kumar MA. Transdermal drug delivery system: a review. Indian J Res Pharm Biotechnol. 2014;2(2):1094.
3. Rastogi V, Yadav P. Transdermal drug delivery system: an overview. Asian J Pharm. 2012;6(3):161-70. doi: 10.4103/0973-8398.104828.
4. Dhopavkar S, Kadu P. Transfersomes-a boon for transdermal delivery. Indo Am J Pharm Sci. 2017;4(9):2908-19.
5. Pahwa R, Pal S, Saroha K, Waliyan P, Kumar M. Transferosomes: unique vesicular carriers for effective transdermal delivery. J Appl Pharm Sci. 2021;11(5):001-8.
6. Varia U, Joshi D, Jadeja M, Katariya H, Detholia K, Soni V. Development and evaluation of ultra deformable vesicles loaded transdermal film of boswellic acid. Future J Pharm Sci. 2022;8(1):39. doi: 10.1186/s43094-022-00428-2.
7. Sultana SS, Krishna Sailaja A. Formulation and evaluation of diclofenac sodium transferosomes using different surfactants by thin film hydration method. Pharm Lett. 2015;7(11):43-53.
8. Manohar SD, Sridhar DA, Mallikarjuna SC. UV spectrophotometric method for the estimation of nebivolol HCL in bulk and pharmaceutical formulations. J Adv Pharm Educ Res. 2013; 3;3-2013: 244-7.
9. Yilmaz B. Reverse phase HPLC method for determination of nebivolol in pharmaceutical preparations. Int J Pharm Sci Rev Res. 2010;1(2):14-7.
10. SrilekhaVelupla RKV, Janapareddi K. Formulation and characterization of glibenclamide loaded transferosomal gel for transdermal delivery. Eur Chem Bull. 2023; 12;Special Issue 4: 5536-51.
11. Ahad A, Al-Saleh AA, Al-Mohizea AM, Al-Jenoobi FI, Raish M, Yassin AE, *et al.* Formulation and characterization of Phospholipon 90 G and Tween 80 based transfersomes for transdermal delivery of eprosartan mesylate. Pharm Dev Technol. 2018;23(8):787-93. doi: 10.1080/10837450.2017.1330345, PMID 28504046.
12. Lee PJ, Ahmad N, Langer R, Mitragotri S, Prasad Shastri VP. Evaluation of chemical enhancers in the transdermal delivery of lidocaine. Int J Pharm. 2006;308(1-2):33-9. doi: 10.1016/j.ijpharm.2005.10.027, PMID 16321488.
13. Ahad A, Aqil M, Kohli K, Sultana Y, Mujeeb M, Ali A. Formulation and optimization of nanotransfersomes using experimental design technique for accentuated transdermal delivery of valsartan. Nanomedicine. 2012;8(2):237-49. doi: 10.1016/j.nano.2011.06.004, PMID 21704600.
14. Jangdey MS, Gupta A, Saraf S, Saraf S. Development and optimization of apigenin-loaded transfersomal system for skin cancer delivery: *in vitro* evaluation. Artif Cells Nanomed Biotechnol. 2017;45(7):1452-62. doi: 10.1080/21691401.2016.1247850, PMID 28050929.
15. Pasha I, Khan AB, Sudheer P. Formulation and evaluation of transdermal ultradeformable vesicles of aspirin. Acta Pharm Sci. 2022;60(4):389-412. doi: 10.23893/1307-2080.APS6025.
16. Iizhar SA, Syed IA, Satar R, Ansari SA. *In vitro* assessment of pharmaceutical potential of ethosomes entrapped with terbinafine hydrochloride. J Adv Res. 2016;7(3):453-61. doi: 10.1016/j.jare.2016.03.003, PMID 27222750.
17. Nippani AD, Janapareddi K. Development and *ex vivo* evaluation of Atorvastatin microemulsions for transdermal delivery using box-Behnken design. IJPBS.2018;8(3): 81-93.
18. Kotha Y, Kandhula AG, Janapareddi K. Development and characterization of levodopa loaded pharmacosomes for brain targeting via intranasal route: pharmacodynamic evaluation in rats. J Young Pharm. 2020; 12(2s):s56-62. doi: 10.5530/jyp.2020.12s.47.
19. Albash R, Abdelbary AA, Refai H, El-Nabarawi MA. Use of transethosomes for enhancing the transdermal delivery of olmesartan medoxomil: *in vitro, ex vivo* and *in vivo* evaluation. Int J Nanomedicine;1. 2019;14:1953-68. doi: 10.2147/IJN.S196771, PMID 30936696.
20. Panchaxari Gadad A, Patil AS, Singh Y, Mallappa Dandagi P, Bolmal UB, Basu A. Development and evaluation of flurbiprofen loaded transethosomes to improve

transdermal delivery. Indian J Pharm Educ Res. 2020;54(4):954-62. doi: 10.5530/ijper.54.4.189.

21. Godse S, Mohan M, Kasture V, Kasture S. Effect of myricetin on blood pressure and metabolic alterations in fructose hypertensive rats. Pharm Biol. 2010;48(5):494-8. doi: 10.3109/13880200903188526, PMID 20645789.
22. Chen HH, Chu CH, Wen SW, Lai CC, Cheng PW, Tseng CJ. Excessive fructose intake impairs baroreflex sensitivity and led to elevated blood pressure in rats. Nutrients. 2019;11(11):2581. doi: 10.3390/nu11112581, PMID 31731536.
23. Hwang IS, Ho H, Hoffman BB, Reaven GM. Fructose-induced insulin resistance and hypertension in rats. Hypertension. 1987;10(5):512-6. doi: 10.1161/01.hyp.10.5.512, PMID 3311990.
24. Qadri GR, Ahad A, Aqil M, Imam SS, Ali A. Invasomes of isradipine for enhanced transdermal delivery against hypertension: formulation, characterization and *in vivo* pharmacodynamic study. Artif Cells Nanomed Biotechnol. 2017;45(1):139-45. doi: 10.3109/21691401.2016.1138486, PMID 26829018.
25. Dudhipala N, Veerabrahma K. Candesartan cilexetil loaded solid lipid nanoparticles for oral delivery: characterization, pharmacokinetic and pharmacodynamic evaluation. Drug Deliv. 2016;23(2):395-404. doi: 10.3109/10717544.2014.914986, PMID 24865287.
26. Salem HF, El-Menshawe SF, Khallaf RA, Rabea YK. A novel transdermal nanoethosomal gel of lercanidipine HCl for treatment of hypertension: optimization using Box-Benkhen design, *in vitro* and *in vivo* characterization. Drug Deliv and Transl Res. 2020;10(1):227-40. doi: 10.1007/s13346-019-00676-5.
27. Dudhipala N, Veerabrahma K. Pharmacokinetic and pharmacodynamic studies of nisoldipine-loaded solid lipid nanoparticles developed by central composite design. Drug Dev Ind Pharm. 2015;41(12):1968-77. doi: 10.3109/03639045.2015.1024685, PMID 25830370.
28. Jadhav PB, Parwe CD. RP-HPLC method development and Validation for simultaneous estimation of nebivolol and valsartan in bulk and dosage form. Int J Pharm Sci Res;2: 756-62:2019.10.
29. Pratap SN, Ratna JV. Development and validation of a sensitive and rapid bioanalytical RP-HPLC method for the quantification of nebivolol hydrochloride in rat plasma. Curr Trends Biotechnol Pharm. 2022; 16(3s):87-95.
30. Brito Raj S, Chandrasekhar KB, Reddy KB. Formulation, *in vitro* and *in vivo* pharmacokinetic evaluation of simvastatin nanostructured lipid carrier loaded transdermal drug delivery system. Future J Pharm Sci. 2019;5(1):1-4. doi: 10.1186/s43094-019-0008-7.

Cite this article: Dunaka S, Janapareddi K. Bioavailability Enhancement of Nebivolol Hydrochloride Loaded Transferosomes via Transdermal Route: Pharmacokinetic and Pharmacodynamic Evaluation in Rats. J Young Pharm. 2024;16(4):772-80.