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EX-VIVO STUDY AND IN-VIVO EVALUATION OF CHITOSAN-COATED OPTIMIZED LEVETIRACETAM LOADED NANOLIPOSOMES

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ABSTRACT

The study aimed to develop and evaluate chitosan-coated levetiracetam-loaded nanoliposomes (Chit-Opt-NLs) for enhanced nasal drug delivery and brain targeting. Nanoliposomes were optimized and characterized for permeation across goat nasal mucosa using ex-vivo studies in Franz diffusion cells. Confocal laser scanning microscopy (CLSM) visualized the nanoliposome's permeation profile, while a toxicity study assessed mucosal safety. In-vivo pharmacokinetic and pharmacodynamic studies were conducted in Wistar rats to evaluate the bioavailability and anticonvulsant efficacy of intranasally administered formulations. Chit-Opt-NLs demonstrated significantly enhanced permeation and brain bioavailability, with a 2.57-fold increase compared to oral levetiracetam. The pharmacodynamic study further revealed improved seizure control in a PTZ-induced seizure model. These findings suggest that Chit-Opt-NLs could be a promising intranasal formulation for epilepsy management.

Keywords *– Chitosan-coated nanoliposomes, Levetiracetam, Nasal drug delivery, Ex-vivo permeation, Pharmacokinetics, Pharmacodynamics.*

1. INTRODUCTION

Epilepsy is a neurological disorder requiring effective drug delivery to the brain for optimal seizure management. Traditional oral administration of antiepileptic drugs like levetiracetam (LEV) faces challenges such as first-pass metabolism and limited brain bioavailability [1-3]. Intranasal drug delivery has emerged as a promising alternative due to the proximity of the nasal cavity to the brain, bypassing the blood-brain barrier (BBB) [4]. Nanotechnology-based drug delivery systems, such as nanoliposomes, enhance drug absorption and brain targeting [5]. Chitosan, a mucoadhesive polymer, further improves drug permeation by prolonging retention time in the nasal cavity [6].

The objective of the present research work was to formulate chitosan coated optimized nanoliposomes of Levetiracetam through intranasal route for management of epilepsy. The drug has poor aqueous solubility and high protein binding showed less penetration through BBB after giving through oral route. Another important objective of this work was to improve the bioavailability in brain after giving through IN route. IN route provides noninvasive delivery as compared to parenteral route in addition to avoiding systemic exposure. Chitosan coating of optimized nanoliposomes also improves the therapeutic uptake in brain by improving nasal residence time.

In this study, we developed chitosan-coated optimized levetiracetam-loaded nanoliposomes (Chit-Opt-NLs) and evaluated their potential for brain-targeted drug delivery via nasal administration. Ex-vivo permeation studies, confocal imaging, toxicity assessments, and in-vivo pharmacokinetic and pharmacodynamic studies were conducted to explore the formulation's efficacy.

2. MATERIALS AND METHODS

2.1 Materials

Levetiracetam was obtained as a gift sample from the pharmaceutical industry. Other chemicals and reagents used in the study were of analytical reagent grade.

2.2 Formulation Development

In this study, optimized Levetiracetam-loaded nanoliposomes (Opt-LEV-NLs) were coated with 0.1% and 0.3% chitosan to improve nasal uptake and mucoadhesion. The formulations were evaluated for vesicle size, entrapment efficiency, in-vitro release, surface morphology, zeta potential, and pH to select the best formulation for brain targeting. Results demonstrated that 0.1% chitosan-coated nanoliposomes (Chit-NLs) retained an optimal particle size (151.72 ± 1.37 nm), suitable for nasal delivery, with a satisfactory entrapment efficiency (64.56 ± 1.03%) and effective drug release (82.51 ± 2.15%). In contrast, 0.3% Chit-NLs exhibited larger particle sizes (> 300 nm), lower release rates, and reduced suitability for intranasal delivery. TEM and zeta potential analyses confirmed the successful chitosan coating and improved mucoadhesive properties. Based on these findings, 0.1% Chit-NLs were selected as the optimized formulation for further studies.

2.3 Ex-Vivo Study

2.3.1 Preparation of Goat Nasal Mucosa

Fresh goat nasal mucosa was sourced from a local slaughterhouse post-sacrifice. The mucosa was separated from the lateral nasal wall, washed with distilled water, and preserved in 10% formalin solution [7]. For further studies, the mucosa was stored at -20ºC.

2.3.2 Ex-Vivo Nasal Mucosa Permeation Studies

Permeation studies were conducted using a Franz diffusion cell with goat nasal mucosa, using normal saline buffer (NSB, pH 6.5) containing 0.01% NaN_3 as the receptor solution [8]. Optimized levetiracetam nanoliposomes (Opt-LTG-NLs), chitosan-coated nanoliposomes (Chit-Opt-NLs), and levetiracetam suspension were applied to the donor compartment [9]. The receptor compartment was stirred at 300 rpm at 37°C. Samples were withdrawn at pre-determined time points and analyzed using HPLC [10].

2.3.3 Ex-Vivo Confocal Laser Scanning Microscopy (CLSM) Visualization

Rhodamine B dye-loaded Opt-LTG-NLs and Chit-Opt-NLs were prepared to visualize their permeation into nasal mucosa. After 12 hours of incubation, sections of the treated nasal mucosa were imaged using CLSM [11].

2.4 Toxicity Study of Nasal Mucosa

The toxicity of the formulations was evaluated by treating the mucosa with Opt-LTG-NLs, Chit-Opt-NLs, and control solutions (LEV suspension, NSB, and isopropyl alcohol). After 2 hours of exposure, mucosal samples were histologically examined for cellular damage using hematoxylin and eosin staining [12].

2.5 In-Vivo Animal Study

2.5.1 Animal Care and Study Approval

The in-vivo study was conducted on Wistar rats (150-200 g) with approval from the Institutional Animal Ethical Committee. Animals were maintained under standard conditions with a 12-hour light/dark cycle. The study was carried out as per standard procedure mentioned in the approved protocol [13].

2.5.2 Pharmacokinetic Study

Thirty-six rats were divided into three groups: intranasal administration of Opt-LTG-NLs, Chit-Opt-NLs, and oral levetiracetam.

Blood and brain samples were collected at various intervals (0.5, 2, 6, and 24 hours), processed, and analyzed by HPLC for drug concentration [14]. Pharmacokinetic parameters such as Cmax, Tmax, and AUC were determined [15].

2.5.3 Pharmacodynamic Study

The anticonvulsant efficacy of the formulations was evaluated using a PTZ-induced seizure model [16]. Seizure latency and incidence were recorded for control and treated groups.

3. RESULTS AND DISCUSSION

3.1 Ex-Vivo Nasal Mucosa Permeation Studies

Chit-Opt-NLs and Opt-LTG-NLs exhibited significantly higher permeation (85.25% and 95.15%, respectively) compared to the pure drug suspension (14.73%) [17]. The improved permeation is attributed to the nanoliposomes' phospholipid composition, which enhances membrane permeability [18].

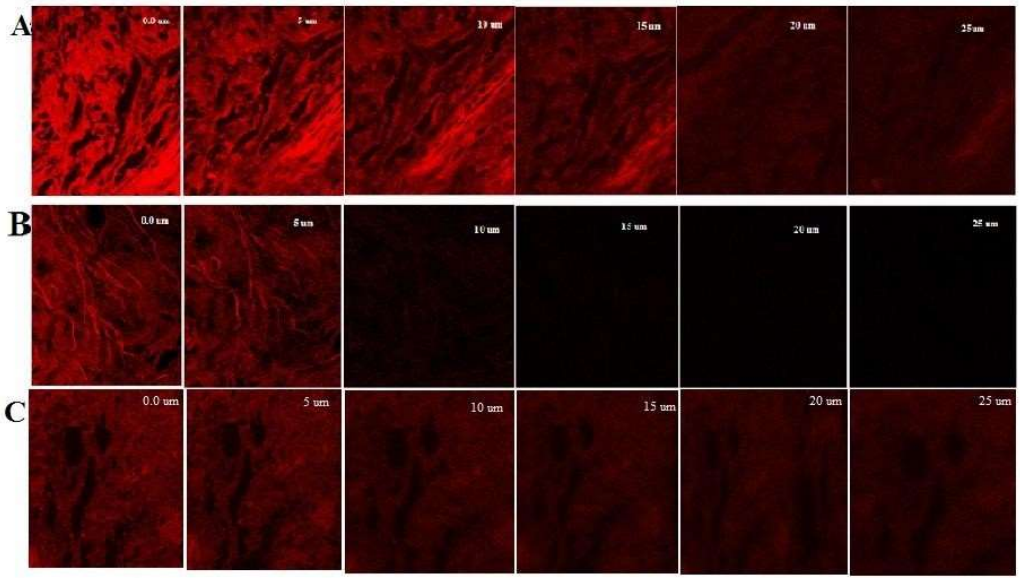

Fig. 1: CLSM image of goat nasal mucosa treated with (A) Rhodamine loaded nanoliposomes (B) Rhodamine pure suspension (C) Rhodamine loaded chitosan coatednanoliposomes showing depth of penetration.

3.2 Ex-Vivo CLSM Visualization

CLSM imaging revealed that both Chit-Opt-NLs and Opt-LTG-NLs penetrated deeper into the mucosal layers than the control formulation [11]. The chitosan coating further enhanced retention in the nasal mucosa, allowing for prolonged drug absorption.

3.3 Toxicity Study

Histological analysis showed no significant epithelial damage from Chit-Opt-NLs and Opt-LTG-NLs compared to isopropyl alcohol, confirming the safety of these formulations for nasal administration [12].

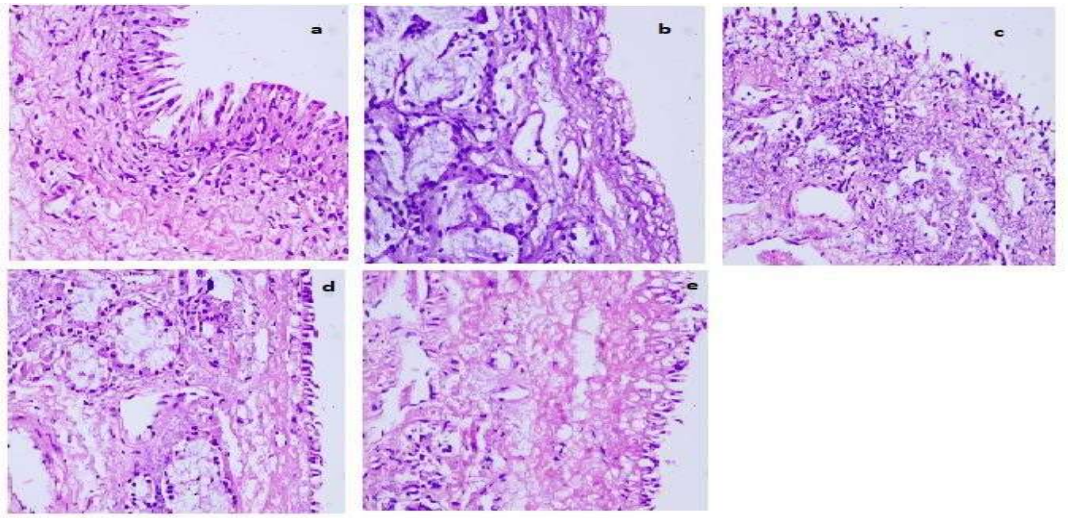

Fig. 2: Optical micrographic image of histopathological structure of goat nasal mucosa treated with (a) Nasal saline buffer pH 6.5 (b) Isopropyl alcohol (c) Drug suspension (d) Opt-LEV-NLs (e) Chit-Opt-NLs.

3.4 Pharmacokinetic Study

The intranasal formulations showed a higher brain-to-plasma drug ratio compared to oral LEV, with Chit-Opt-NLs achieving a 2.57-fold increase in brain bioavailability [13]. This highlights the potential of chitosan-coated nanoliposomes for targeted brain delivery.

Table 1: Pharmacokinetic drug distribution of Opt-LEV-NLs (i.n.), Chit-Opt-NLs (i.n.)and LEV-marketed formulation (oral) in Rats.

Formulations	Brain/ Plasma	C_{max} (ng/ml)	T_{max} (hr)	AUC (0-24h)	AUC (0-inf)	AUMC (0-24)	K_e	$t_{1/2}$
Marketed formulation (Oral)	Brain	1345.23	6	19460.08	28291.94	150022.5	0.1010	23.205
	Plasma	2167.89	2	27051.66	37267.77	212893.7	0.134	14.64252
Opt-LEV-NLs (IN)	Brain	2818.56	2	41297.91	94394.75	404804.1	0.027	30.706
	Plasma	1063.57	2	12154.09	17497.41	95441.68	0.049	16.9181
Chit-Opt-NLs(IN)	Brain	3468.69	2	47403.73	123441.2	442597.4	0.0124	42.634
	Plasma	839.64	2	8665.803	13651.58	71384.97	0.0423	18.92492

3.5 Pharmacodynamic Study

In the PTZ seizure model, Chit-Opt-NLs demonstrated superior anticonvulsant activity, significantly delaying seizure onset and reducing seizure frequency compared to oral LEV [16].

Table 2: Scoring of seizure of different treated groups of Wistar rats.

Groups	Treatment	Route of delivery	Doses (mg/Kg)	Myoclonic jerk (Sec. ± SEM)	Generalized tonic clonic seizure (Sec.± SEM)
Group 1	PTZ	Intraperitoneal	70 mg/Kg	148.81±10.39	865.2± 18.15
Group 2	PTZ + Opt- LEV-NLs	Intraperitoneal + Intranasal	70 mg/Kg +2 mg/Kg	45.74 ± 6.39	8.19 ± 2.10
Group 3	PTZ + Chit- Opt-NLs	Intraperitoneal + Intranasal	70 mg/Kg +2 mg/Kg	5.75 ± 3.45	1.20 ± 1.75
Group 4	PTZ+ Marketed formulation	Intraperitoneal + Oral	70 mg/Kg +2 mg/Kg	85.5 ± 3.71	310 ± 15.45

4. CONCLUSION

Chitosan-coated levetiracetam-loaded nanoliposomes significantly enhance nasal permeation and brain bioavailability, offering a promising alternative for epilepsy management. The formulation exhibited favorable safety profiles and superior therapeutic efficacy in in-vivo studies, positioning it as a viable candidate for intranasal delivery in epilepsy treatment.

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