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Original Article

OPTIMIZING LEVETIRACETAM SURFACTANT-BASED NANOVESICLES (LEV-NVS) GEL FOR TREATING EPILEPSY USING EXPERIMENTAL DESIGN

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ABSTRACT

Objective: To develop and estimate the intranasal delivery of Levetiracetam surfactant-based nanovesicles (Lev-Nvs) as a brain-targeted antiepileptic delivery system prepared via solvent evaporation technique.

Methods: Optimized formulation F (OPT) chosen by the Design-Expert® program gave the highest entrapment efficiency (EE%) was incorporated into the gel. An experimental design was adopted utilizing various (span 65) surfactants and different cholesterol ratios. The (Lev-Nvs) nanovesicles were formulated by solvent evaporation technique and evaluated for *in vitro* characterization parameters such as zeta sizer, Transmission Electron Microscopy (TEM), zeta potential. The nasal gel was evaluated for drug-excipient interactions utilizing Fourier Transform Infrared Spectroscopy (FTIR) and subjected to *in vitro* and *in vivo* release studies.

Results: The results indicated that the entrapment efficiency (EE%) of Levetiracetam surfactant-based nano-vesicles (Lev-Nvs) could be modulated by the alterations in surfactant and cholesterol concentrations. Optimized formulation F (OPT) showed an entrapment efficiency of (87.9±1.06 %), (206.7±20.43 nm) particle size, (-34.1) zeta potential and (0.979) PDI. The nanovesicle nasal gels of the F(OPT) were prepared using Carbopol 940 at different concentrations. G 0.375 formulation showed the best *in vitro* drug release (87.36%) after 12 h. Finally, the comparative *in vivo* pharmaco-kinetics release studies on rats revealed considerable, sustained release of the nanovesicle nasal gel and higher relative bioavailability than an equivalent dose of oral solution (293.85%).

Conclusion: Our study proves the improved efficacy of Levetiracetam as a surfactant-based nanovesicle intranasal gel in the brain targeting antiepileptic medication.

Keywords: Intranasal**,** Levetiracetam, Nanovesicles, Brain-targeted, Antiepileptic, Solvent evaporation technique

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INTRODUCTION

Epilepsy was noted by the world health organization (WHO) in April 2018 as the fourth most common neurological disorder. Treatment of epilepsy is based on the type of seizures the patient suffers from, other factors such as age, and co-administration of other drugs [1, 2]. Epilepsy affects people of all ages; however, pediatric epilepsy may impair brain development. As a result, epilepsy in children is markedly different from epilepsy in adults, and they must be treated differently in the majority of cases [3].

Levetiracetam is an Anti-epileptic drug commonly used as a first-line choice for seizures in palliative care [4]. It is used therapeutically in adults and children to man-age three unique types of seizures. It is typically used as an adjuvant therapy in adults and children≥ 4 y of age with partial or focal onset seizures, in individuals 12 y of age or older with myoclonic seizures, and in adults and children≥ 6 y of age with primary tonic-clonic seizures [5]. While the oral route is the most frequently utilized for chronic medication, it is not recommended throughout an epileptic attack due to the risk of nausea or vomiting. Other buccal, rectal, and parental routes are also viable alternatives to the oral route, but each has a number of limitations.

The intranasal (IN) route is a promising therapy option for chronic or acute conditions. Because it allows medications to reach the brain directly, it is regarded an alternative to parenteral administration, and hence the effective dosage predicted via other administration routes is scheduled to be reduced using the IN route [6]. Different pathways have been suggested for IN route: the olfactory and trigeminal nerve and/or the systemic path [7]. Various researchers have compared the effect of administrating AEDs by the conventional and the IN route. Preliminary findings are promising and encouraging [8, 9].

Nano-Loaded devices may facilitate the extended/controlled release profiles of medications used in the therapy of chronically ill patients [10-12]. Numerous CNS active medicines have been regarded suitable carriers for increasing their brain-targeting potential using nanoparticles [8, 13]. Due to the fact that nanovesicles are composed of Span® and cholesterol, they can be employed to transport both hydrophilic and hydrophobic medicines via the inside hydrophilic compartment and the outer lipid layer, respectively [14, 15].

Levetiracetam can thus be encapsulated in surfactant-based nanovesicles (Lev-Nvs). The nanovesicle's ability to cross the BBB and reach the brain is determined by its properties, not those of the therapeutic substance; additionally, they can paracellularly transport.

The objective of this study was to establish and validate the efficiency of intranasally delivered Levetiracetam surfactant-based nanovesicles (Lev-Nvs) in comparison to the commercial oral solution (Tiratam®) in order to produce a more promising and convenient dosage form for the patients.

MATERIALS AND METHODS

Materials

Levetiracetam and Commercial Levetiracetam (Tiratam®) were gifted by El Andalous Medical company (Cairo, Egypt). Sorbitan tristearate (Span® 65) and Triethanolamine 98% were gathered from Sigma-Aldrich Company (St. Louis, MO, USA). Ethanol 95%, acetonitrile, chloroform, sodium hydroxide, and potassium dihydrogen phosphate were obtained from El-Nasr Pharmaceutical

chemicals Co. (Cairo, Egypt). Cholesterol was gathered from Loba Chemie (Mumbai, India). Carbopol 940 was obtained from Athos chemicals (Gujarat, India). VISKING ® Dialysis Tubing molecular weight cutoff 12,000–14,000 pore diameter 25 A ° received from SERVA Electrophoresis (Uetersen, Germany).

Animals (Ethics approval)

Male Wistar rats with normal eyes and no sickness were acquired from the Animal House of Cairo University's Faculty of Pharmacy in Cairo, Egypt. Animal housing and handling were undertaken in accordance with the requirements of the Faculty of Pharmacy, Cairo University's Research Ethics Committee (REC). The study followed protocols issued by the National Research Center's Animal Care Committee (Cairo, Egypt) and was authorized by the Faculty of Pharmacy's Research Ethics Committee for clinical and experimental studies (Approval date: 28/10/2019, Permit number: PI 2535).

Methods

Preparation of Levetiracetam surfactant-based nanovesicles (Lev-Nvs)

Applying a 3^2 Factorial design in table 1, nine formulations were prepared using different cholesterol concentrations and span 65. Table 2 showed the composition of the prepared formulations. The surfactant-based nanovesicles formulations were prepared using the solvent evaporation technique [16-18]. Accurately weighed amounts of span 65 and cholesterol were dissolved in chloroform, forming the organic phase. The aqueous phase (drug in ethanol) was added to the previously prepared organic phase under continuous stirring with a magnetic stirrer at 900 rpm using a stirrer (model 275 T, Crest Ultrasonic Corp, NY, USA). After the complete evaporation of the organic phase, the formed Levetiracetam surfactant-based nanovesicles (Lev-Nvs) were cooled.

Table 1: Independent variables and their impact on dependent variables utilizing 3^2 *factorial designs*

Independent variables	Levels		
	-1	0	+1
X1: Span 65 (mg)	25	50	75
X2: Cholesterol (mg)	25	50	75
Dependent variables	Constraints		
Y1: Particle size (nm)	Minimum		
Y2: Entrapment efficiency (%)	Maximum		
Y3: Zeta potential (mV)	Maximum		

Table 2: Composition of levetiracetam surfactant-based nanovesicles (Lev-Nvs)

Formula No.	Drug (mg)	Span65 (mg)	Cholesterol (mg)
F1	50	25	25
F2	50	50	25
F3	50	75	25
F4	50	25	50
F5	50	50	50
F6	50	75	50
F7	50	25	75
F8	50	50	75
F9	50	75	75
F (OPT)	50	42.954	62.419

Characterization of levetiracetam surfactant-based nanovesicles (Lev-Nvs) formulation

Determination of percentage drug entrapment efficiency (%EE)

Determination of Entrapment efficiency can be done either directly or indirectly [14]. Direct determination of EE%: one milliliter of the nano vesicular dispersion subjected for cooling ultracentrifuged at 20,000 rpm for 30 min at 4 ºC utilizing a centrifuge (Sigma 3K 30, Osterode am Harz, Germany). The precipitated nanovesicles were washed with 1 ml phosphate buffer (pH 6.8) and re-centrifuged for an additional 10 min to ensure the extraction of the unentrapped drug [19].

$$EE\% = \left(\frac{(\text{Total amount of drug} - \text{amount of free drug})}{\text{Total amount of drug}}\right) * 100$$

In direct determination of EE%: The gathered supernatant was measured after dilution at 205 nm employing a UV/V is spectrophotometer (Shimadzu UV 1650 Spectrophotometer, Kyoto, Japan) [5]. The unentrapped drug concentration was determined and contrasted to the original amount administrated.

Particle size (PS) analysis, polydispersity index (PDI), and zeta (ζ)-potential

The Levetiracetam surfactant-based nanovesicles (Lev-Nvs) dispersion was established using dynamic light scattering with Malvern Zeta sizer (Malvern Instruments Ltd., UK), at 25 °C at a scattering angle of 90 [20, 21], to measure The Particle Size (PS) analysis, Polydispersity index (PDI), and Zeta (ζ)-Potential. The PDI was established for the determination of particle size distribution of the niosomal vesicles.

Optimization of levetiracetam surfactant-based nanovesicles (Lev-Nvs) formulation

With the aid of Design-Expert® program version 10.0, an optimized formula F(OPT) was conducted. This formula was further used to prepare and evaluate levetiracetam nanovesicle gels after studying the Compatibility of Levetiracetam with Carbopol 940, cholesterol, Span 65, and their physical mixture was performed utilizing Fourier transform Infrared spectroscopy (FTIR).

Transmission electron microscope (TEM) of the optimized formula of levetiracetam surfactant-based nanovesicles (Lev-Nvs)

To evaluate the morphology of Levetiracetam surfactant-based nanovesicles (Lev-Nvs), the optimized formula was studied using a transmission electron microscope (TEM) (JEM-1230, Joel, Tokyo, Japan). Specimens were placed on a car-bon-coated grid surface and stained negatively with a 1 percent phosphotungstic acid aqueous solution. They were then allowed to air dry completely before visualization at room temperature [21].

Preparation of Levetiracetam surfactant-based nanovesicles (Lev-Nvs) gel

The composition of prepared Levetiracetam surfactant-based nanovesicles (Lev-Nvs) gel is shown in table 3. The specified

amounts of Carbopol 940 were dispersed gradually in deionized water using a magnetic stirrer model 275 T (Crest Ultrasonic Corp.; New York, NY, USA) at a medium speed of 100 rpm. In order to allow for full expansion, the stirring was maintained after the gel base had formed. Disperse in each formula 45.5% W/V of the selected optimized Levetiracetam surfactant-based nano-vesicles. This was followed by adjusting the pH to approximately 6.0 using triethanolamine [21, 22].

Table 3: Gel formulation codes for different carbopol 940 concentrations

Gel code	Carbopol 940 concentration (%W/V)	Nanovesicle content (%W/V)
G1	1%	45.5 %
G 0.75	0.75%	45.5 %
G 0.375	0.375%	45.5 %

***In vitro* release of levetiracetam surfactant-based nanovesicles (Lev-Nvs) gel**

Drug release assessment was performed utilizing a modified dissolution apparatus I. A cylindrical tube of 2.5 cm diameter and 6 cm length (donor compartment) was hung on the dissolution apparatus shaft. The gel formulation was kept in the donor compartment over a dialysis membrane (cut off = 12-14 kDa) and immersed beforehand for 24 h in a 7.4 phosphate buffer. The receptor compartment contained 100 ml of 7.4 phosphate buffer and was kept at 37±0.5 °C with constant stirring at 100 rpm [23]. Three milliliters of the aliquot were withdrawn from the diffusion medium at (0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, and 12 h.) was replaced with three milliliters of fresh phosphate buffer. The drug concentration was determined spectrophotometrically at 205 nm.

***In vivo* pharmacokinetic study of levetiracetam surfactant-based nanovesicles (Lev-Nvs) gel**

Animal handling and drug injection

In this investigation, nine healthy male Wistar rats weighing between 250 and 300 g were employed. Three groups (I, II, and negative control) of rats were randomly allocated. All rats were fasted for 12 h and given access to water on an ad libitum basis [24]. The study followed animal ethical criteria for laboratory animal research, and the methodology was authorized by the Faculty of Pharmacy at Cairo University's Animal Ethics Committee (serial no. PI 2535, valid from 28 October 2019).

The rats were split into three groups. Group I underwent the commercial Tiratam® solution equivalent to 0.015 gm Levetiracetam, the dose was administered using a syringe. Group II underwent the developed nasal gel, 50-µl nasal gel equivalent to 0.0075 gm Levetiracetam into each nostril (total of 100-µl equivalent to 0.015 gm Levetiracetam). The negative control group was used to provide blank plasma.

Sample collection and analysis

At time intervals of 0, 0.25, 0.5, 0.75, 1, 2, 4, and 8 h, one milliliter of blood was taken from the rats' retro-orbital vein into accessible commercially plasma tubes pre-pared with EDTA. After centrifuging the blood samples for 15 min at 4000 rpm to separate plasma, they were promptly kept at-20 °C until HPLC analysis. A liquid-liquid extraction technique was used to assess levetiracetam concentrations in plasma acquired during *in vivo* pharmacokinetic research. HPLC analysis was then carried out [25, 26].

Chromatographic conditions

Levetiracetam was measured utilizing an assessed HPLC approach for determination in plasma [27]. Agilent 1200 series HPLC instrumentation was utilized. Data analysis and processing were performed using the Agilent Chemstation software program, re-vision A.10.02. Chromatography was performed on a Phenomenex Luna C18 column (250 4.6 mm, 5 µm particle size) and quantification was performed using UV detection at 205 nm. The mobile phase was composed of a 50 mmol KH2PO4 buffer (6.8045 g/l) and acetonitrile combination (90:10, v/v). Sodium hydroxide (NaOH) was used to ad-just the pH of the mobile phase. The NaOH was added to the mobile phase at the rate of 1 ml/min and the injection volume was 10 µl.

The pharmacokinetic parameters

PKSolver, a Microsoft Excel add-in for pharmacodynamic and pharmacokinetic and data analysis, were used to calculate the pharmacokinetic features. The following parameters were modified using data. Maximum plasma concentration (C_{max}), the time necessary to attain Cmax (Tmax), the area under the plasma concentration-time curve from zero to the last perceptible concentration (AUC0–t), the area under the plasma concentration-time curve from zero to infinity (AUC0-∞), the abolition half-life (T1/2), and the elimination rate constant (K_e).

Statistical analysis

The findings are noted as means±SD. The pharmacokinetic features, AUC0–t, AUC0-∞, C_{max}, and T_{max}, were examined statistically via analysis of variance (ANOVA). The P-value was measured from the gathered F-value utilizing IBM SPSS statistics version 20 Microsoft software. A statistically significant difference was regarded when $p<0.05$ [28]. The following equation calculated the relative bioavailability of nasal gel [29].

$$F\% = \left(\frac{(\text{AUC } 0-\infty \text{ nasal X oral dose})}{(\text{AUC } 0-\infty \text{ oral X nasal dose})}\right) * 100$$

Table 4: EE%, polydispersity index, particle size, and zeta potential of levetiracetam surfactant-based nanovesicles (Lev-Nvs) formulation

Formula No.	E. E.%	Particle size (nm)	PDI	Zeta potential (mV)
F1	81.31±2.3	219.13±30.03	1	-32.1
F2	83.19±2.03	266.03±22.56	1	-40.5
F3	65.19±2.3	137.26±15.50	1	-28
F4	66.46±0.5	362.16±10.68	0.695	-19.9
F5	88.4±0.53	372.46±93.41	0.73	-24.9
F6	67.35±0.56	381.46±20.16	0.818	-24.3
F7	74.42±0.52	373.83±143.2	0.759	-28.6
F8	76.02±0.97	329±39.5	0.789	-28.5
F9	77.35±0.56	516.2±65.03	0.532	-25.1
F (OPT)	87.9±1.06	206.7±20.43	0.979	-34.1

E. E% and diameter are represented as mean±SD (n=3)

RESULTS

Characterization of Levetiracetam surfactant-based nanovesicles (Lev-Nvs) formulation

Determination of entrapment efficiency (E. E.%), Particle size (PS) analysis, Polydispersity index (PDI), and Zeta (ζ)-potential of Levetiracetam surfactant-based nanovesicles (Lev-Nvs) formulation

The proportion of Levetiracetam entrapped in nanovesicles varied between 65.19 % and 88.4 %. The entrapment efficiencies differed by the amount used of surfactant and cholesterol. F5, F2, and F1 showed the highest drug entrapment, whereas F3, F4, and F6 showed the lowest entrapment efficiency. PS, PDI, Zeta potential of all formulas showed in (table 4). As illustrated in fig. (1), the zeta potential analysis noted that the surface charge of all nanovesicles was negative.

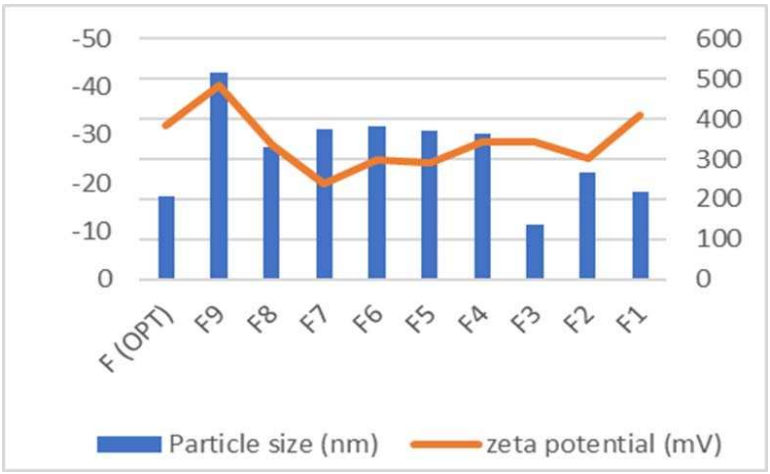

Fig. 1: Illustration of size and zeta potential of levetiracetam surfactant-based nanovesicles (Lev-Nvs) formulation

Compatibility of levetiracetam with polymer and excipient using fourier transform infrared spectroscopy

Drug-excipient interactions were analyzed utilizing Fourier Transform Infrared Spectroscopy (FTIR). According to fig. 2, the Levetiracetam FT-IR spectra have shown carbonyl peak at 1680 cm-1, NH stretching absorption 3500–3200 cm-1, 2989 cm-1 CH stretching, CN stretching at 1083 cm-1. The same peaks were found in the FT-IR spectra of physical mixtures, ensuring selected excipients' suitability. There is no specific interaction between drugs and excipients employed in the formulations.

Transmission electron microscopy (TEM)

TEM images were carried out for the optimized Levetiracetam surfactant-based nano-vesicles (Lev-Nvs) formulation. Fig. 3 indicated that the surfactant-based nano-vesicles prepared by solvent evaporation technique are well identified, spherical in shape, have a smooth surface, and segregated from each other. Additionally, the images revealed that the size of these surfactant-based nanovesicles is very consistent, which is a distinctive morphological dimension of nanovesicles.

In vitro release study of Levetiraceam surfactant-based nanovesicles dispersion and nano-vesicle gel

In vitro drug release of drug solution, Levetiraceam surfactant-based nanovesicles (Lev-Nvs) dispersion, and Levetiraceam surfactant-based nanovesicles gels of Carbo-pol 940 (1, 0.75, 0.375) % are shown in fig. 4. The initial drug release in 3 h from all the formulations was (G1: 28.34%), (G 0.75: 35.27%) and (G 0.375: 39.25%). The release of levetiracetam from the produced gels demonstrated a decrease in the amount of medication released after 4 h by (48%, 39.3% and 33.64%) contrasted to free drug solution and (29.55%, 20.85% and 15.19%) reduction compared to nanovesicle formulation for G 1, G 0.75 and G 0.375 respectively. Drug uptake after 12 h from G1, G 0.75 and G 0.375 gels was shown to be (63.25%, 65.42% and 87.36%) respectively.

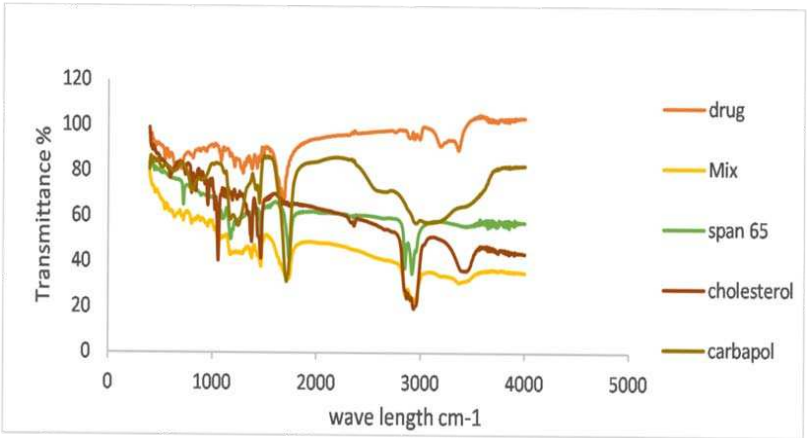

Fig. 2: FTIR of the drug, crabapol, span 65, cholesterol, and physical mixture

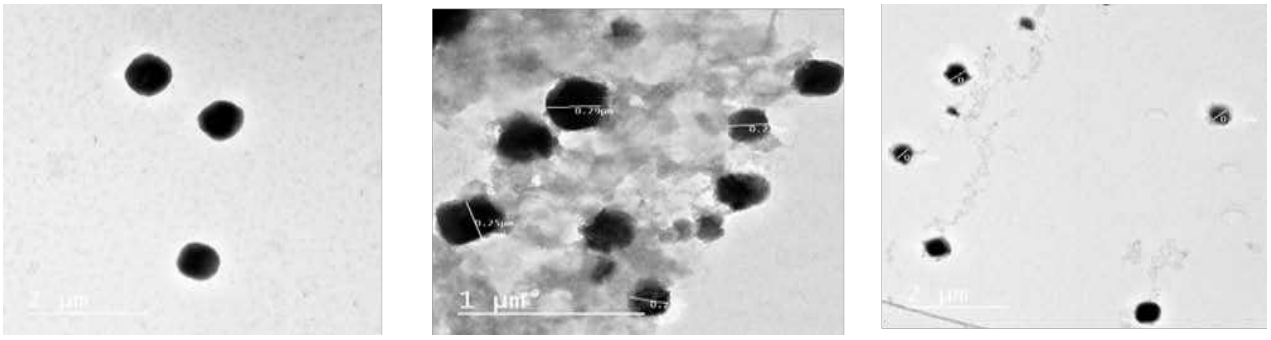

Fig. 3: **Transmission electron microscope (TEM) images of optimized levetirace-tam surfactant-based nanovesicles (Lev-Nvs) formulation**

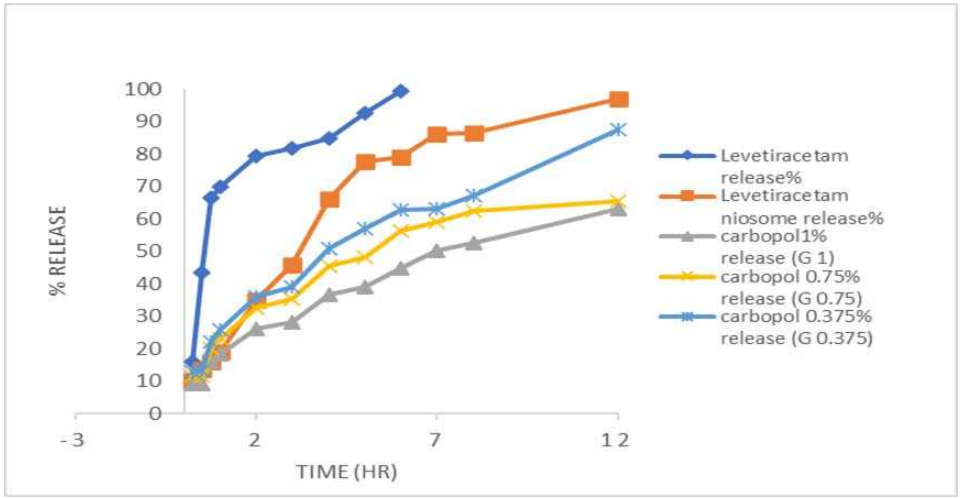

Fig. 4: ***In vitro*** **release of levetiracetam drug from solution, levetiracetam nanovesicle formulation and (1, 0.75 and 0.375%) Carbopol nanovesicle gels. Data is expressed as mean**

In vivo **pharmacokinetic study of Levetiracetam surfactant-based nanovesicles (Lev-Nvs) gel**

An established HPLC approach was used to quantify the concentration of Levetiracetam in rat plasma [15]. Under HPLC settings, where interferences were found in blank plasma samples, levetiracetam was well isolated. The bioavailability of Levetiracetam gel was tested using the optimized formulation (Carbopol 0.37 percent), which demonstrated the best drug release findings, and contrasted to a commercial oral solution containing the same dose (15 mg) of Levetiracetam.

The mean plasma drug concentration-time profiles following oral as well as nasal gel administration of Levetiracetam are depicted in fig. 5.

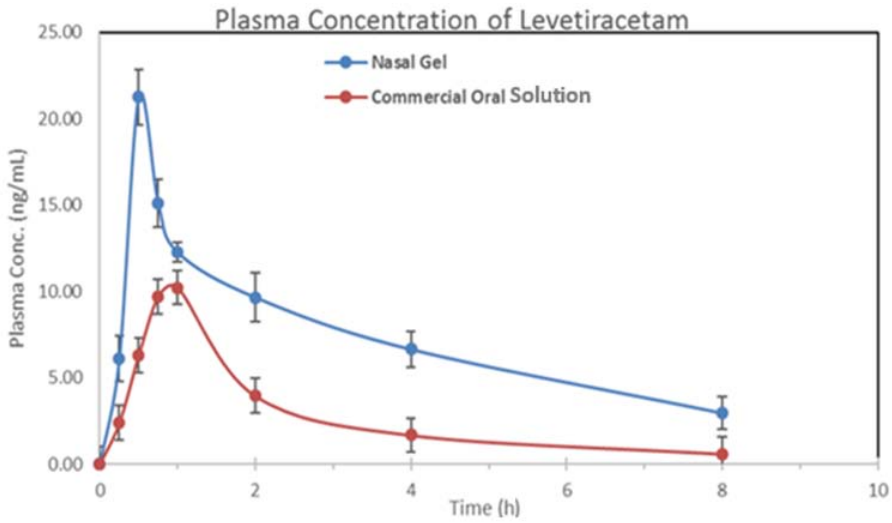

Fig. 5: **Levetiracetam plasma conc.-time profile for oral solution and nasal gel. All** ***data showed as mean±SD (n=3)***

Table 5 showed plasma pharmacokinetic parameters from various dosage form. The Cmax values were 21.26±1.61521.26 ng/ml and 10.421±0.295 ng/ml for nasal gel and oral solution, respectively while AUC0–t results were 58.715±7.74 h*ng/ml and 23.135±0.43 hr*ng/ml for nasal gel and oral solution respectively. Tmax values for nasal gel and oral solution were 0.5±0 and 0.917±0.144 h, respectively, in terms of absorption rate. The plasma means residence time (MRT) of levetiracetam obtained from nasal in situ gel was slightly longer (2.88±0.17 h) than that obtained from oral solution (2.186±0.025 h), but both values are extremely short, indicating a rapid drug release.

Statistical analysis of the previous data revealed a significantly greater Cmax in case of the nasal gel ($p < 0.05$). Although the time required to perform Cmax was greater in case of oral solution contrasted to nasal gel, the statistical analysis showed no significance regarding Tmax ($p>0.05$). Moreover, statistical analysis demonstrated a significant difference between plasma (AUC0–∞) of nasal gel (74.11±14.954 ng h/ml) and oral tablets (25.224±2.095 ng h/ml) ($p < 0.05$), demonstrating that nasal route performs optimal relative bioavailability of (293.85%) contrasted to oral route.

Table 5: Pharmacokinetic parameters of drug released from oral solution and nasal gel in rat plasma

PK parameters	Treatment	
	Commercial oral solution	Nasal gel
Cmax (ng/ml)	10.421±0.295	21.26±1.615
AUC_{0-t}(h*ng/ml)	23.135±0.43	58.715±7.74
$AUC_{0-\infty}$(h*ng/ml)	25.224±2.095	74.11±14.954
Tmax (h)	0.917±0.144	0.5±0
AUM Clast (h*h*ng/ml)	50.577±1.521	169.999±32.175
AUMCINF_obs (h*h*ng/ml)	74.986±22.312	374.308±139.309
$T_{1/2}$ (h)	2.18±0.714	3.467±0.601
MRT last (h)	2.186±0.025	2.88±0.17
Ke (1/h)	0.345±0.124	0.204±0.037

All data showed as mean±SD (n=3).

DISCUSSION

The entrapment efficiency of Levetiracetam is directly proportional to cholesterol concentration [24]. The 1:1 ratio of cholesterol to nonionic surfactant showed an optimum ratio for obtaining the highest entrapment of the drug [24]. Cholesterol is known to be included in the formulation to stabilize the prepared nanovesicles as it can diminish the gel to the lipid phase transition of nanovesicle systems, which could effectively avoid leakage of drug from nanovesicles [25, 26]. However, increasing the cholesterol concentration to 75 mg decreased the entrapment efficiency in (F8, EE percent 76.02) compared to (F5, EE percent 88.4), possibly because cholesterol molecules compete with the drug for space within the bilayer, culminating with disruption of the vesicular membrane structure and withdrawal of the drug. Similar results were observed by Rahul Kumar Singh and Anirudh Singh Deora; when cholesterol exceeded its optimum concentration level, drug entrapment decreased [30].

Surfactant effect on entrapment efficiency: surfactant is crucial in forming nanovesicle vesicles. The variation in its concentration may impact the entrapment efficiency and particle size. The observation concluded that with the increase of Span 65 concentration from 25 mg to 50 mg, a significant rise in entrapment efficiency was (F4, EE% 66.46) to (F5, EE% 88.4). However, further, increase in span 65 concentration from 50 mg to 75 mg, the entrapment efficiency reduced (F6, EE% 67.35). The number of nanovesicles formed increased by an initial increase in surfactant concentration; thus, the volume of the hydrophobic domain rises and hence elevates the amount of drug entrapped. Subsequent increases in surfactant concentration resulted in a reduction in entrapment efficiency, probably due to the generation of combined micelles in addition to the nanovesicle with a great concentration of surfactant, leading to lowering of entrapment efficiency [27, 31]. This leaded to the conclusion of the suitability of 50 mg surfactant as an optimum quantity for nanovesicle formulation.

The particle sizes of various formulations (table 4) revealed that the size of the nanovesicle grows as the quantity of cholesterol in the formulation rises, as per the surfactant concentration utilized in fig. (1). This could be owing to the hard cholesterol molecule's inverted cone structure. Thus, when hydrated above the gel/liquid transition temperature, it can intercalate between the fluid hydrocarbon chains of the surfactant, hence expanding the vesicle size [28]. As illustrated in the fig. (1), the zeta potential analysis noted that the surface charge of all nanovesicles was negative, suggesting that they were stable systems [29]. The high surface charge provides sufficient electrostatic repulsion between the vesicles, which makes them stable by preventing aggregation [32]. The increase in cholesterol concentration reduces zeta potential values [33]. This is unfavorable as the zeta potential increases; the charged particles repel one another and become more stable against aggregation [34].

The reduction in *in vitro* drug release was accompanied with elevating polymer content due to the trapping of the active substance inside the polymer. Also, the movement area of the active substance is limited at high polymer content as a result of the chain structure augmentation density [35]. The release of Levetiracetam from gel formulations was shown to be markedly less than the release of drug solution. This can be justified by the inverse relation between drug release and viscosity of gel formulations.

CONCLUSION

In this study, Levetiracetam was successfully incorporated into (cholesterol/span 65) nanovesicle intended for intra-nasal delivery. According to Design-Expert®, (F OPT) was selected as the best nanovesicle formulation with an entrapment efficiency of (87.9±1.06%), (206.7±20.43 nm) particle size, (-34.1) zeta potential and (0.979) PDI. This formula was further used for the preparation of nanovesicle nasal gels. G (0.375) nasal gel formulation indicated a favorable (87.36 %) drug release in a span of 12 h.

In vivo release study showed a prolonged release of the nanovesicle nasal gel and a higher relative bioavailability (293.85%) when contrasted to a similar dose of oral solution, indicating that nanovesicle nasal gels are superior to oral solutions for antiepileptic drug administration and patient compliance.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

AUTHORS CONTRIBUTIONS

The article comprises original, unpublished material that is not published elsewhere, in any language and is not under consideration for publication elsewhere. All authors have read and approved the text and consent to its publication.

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