Evaluation of Eslicarbazepine-loaded reduced Graphene oxide Polymeric nanoparticles by Pentylenetetrazole (PTZ) induced convulsion in mice

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Abstract: Millions of people worldwide suffer greatly from epilepsy, a chronic neurological illness that severely lowers quality of life. Typical antiepileptic medications frequently have negative side effects, low absorption, and inadequate patient compliance. The objective of this research is to devise and evaluate polymeric nanoparticles loaded with escicarbazepine to improve the treatment of epilepsy. A new antiepileptic medication called eslicarbazepine acetate has encouraging therapeutic potential, however its physicochemical characteristics limit its clinical usefulness.

In this paper, we investigated polymeric nanoparticles loaded with Eslicarbazepine using biocompatible and biodegradable polymers. They are put through in-vivo release experiments to look at the kinetics of eslicarbazepine's release from the polymeric nanoparticles. To ascertain the drug release mechanisms and guarantee a prolonged and regulated release, the release profiles were examined. The outcomes showed that the polymeric nanoparticles offered Eslicarbazepine a stable and effective delivery mechanism, increasing its bioavailability and therapeutic efficiency.

According to our research, polymeric nanoparticles loaded with Eslicarbazepine may be able to minimize adverse effects and lower the frequency of doses, which would increase patient compliance and treatment results overall. This study demonstrates how medication delivery systems based on nanotechnology may transform the way epilepsy is treated and lays the groundwork for future in vivo research and clinical use.

Keywords: Eslicarbazepine, Polymeric Nanoparticles, Epilepsy, Drug Delivery, In-vivo Release, Nanotechnology.

INTRODUCTION

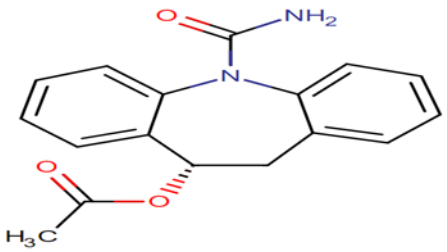
About 50 million people worldwide suffer from epilepsy, a common neurological disorder marked by recurrent, unprovoked seizures. Despite advancements in medical research, managing epilepsy is still difficult because traditional antiepileptic drugs (AEDs) frequently have suboptimal bioavailability, substantial side effects, and variable patient response. Eslicarbazepine acetate, a third-generation AED, has shown promise in treating partial-onset seizures; however, its physicochemical properties hinder its therapeutic potential, resulting in uneven absorption and bioavailability.

Nanotechnology has become a game-changer in medication delivery in recent years, providing ways to improve the effectiveness and safety of medicinal medicines. In particular, polymeric nanoparticles have drawn interest because of their capacity to increase drug solubility, shield the molecule from deterioration, and offer controlled and prolonged release. These benefits may be able to get beyond the drawbacks of conventional medication compositions and enhance patient results while treating epilepsy.

The formulation techniques and in-vitro release dynamics of polymeric nanoparticles loaded with Eslicarbazepine for the treatment of epilepsy are the main topics of this work. Utilizing the advantages of nanotechnology, our goal is to create a unique delivery method that will increase eslicarbazepine's bioavailability, decrease the frequency of doses, and limit side effects, all of which will improve patient compliance and quality of life.

MATERIALS AND METHOD

Drug Profile: Eslicarbazapine
Chemical Name: Eslicarbazepine acetate
Molecular Formula: C17H16N2O3
Molecular Weight: 296.32 g/mol
Chemical Structure:



Sodium Alginate:
Sodium alginate, sourced from a reputable supplier (UV Scientifics, Hyderabad), is a biocompatible natural hydrophilic polysaccharide derived from brown seaweed. Its high molecular weight and structural integrity render it an excellent candidate for various drug delivery applications.

METHODOLOGY

Synthesis of Reduced Graphene Oxide (rGO)
Preparation of Graphene Oxide (GO)
Graphene oxide (GO) was synthesized using a modified Hummers' method. Initially, natural graphite flakes underwent oxidation with a mixture of potassium permanganate (KMnO4) and concentrated sulphuric acid (H2SO4). The resulting GO was then subjected to multiple washing and filtration steps to eliminate residual reactants.

Reduction of Graphene Oxide to Reduced Graphene Oxide (rGO)
The reduction of graphene oxide to reduced graphene oxide (rGO) was accomplished by introducing a reducing agent, specifically ascorbic acid. This reduction process was meticulously monitored to regulate the degree of reduction, ensuring the formation of rGO with enhanced electrical conductivity and diminished oxygen-containing functional groups[13].

Preparation of Eslicarbazepine-Loaded Sodium Alginate Microbeads
Sodium Alginate Solution

A 2% (w/v) sodium alginate solution was prepared by dispersing sodium alginate powder in deionized water. Continuous stirring using a magnetic stirrer at room temperature facilitated complete dissolution. The solution was then allowed to stand briefly to eliminate any air bubbles.

Incorporation of rGO and Eslicarbazepine
The sodium alginate solution was combined with the previously prepared reduced graphene oxide (rGO) and Eslicarbazepine to achieve a homogeneous mixture. The rGO-Eslicarbazepine suspension was gradually added to the sodium alginate solution under constant stirring to prevent agglomeration. Additional stirring ensured uniform distribution of rGO and Eslicarbazepine within the sodium alginate matrix.

Microbeads Formation
Microbeads were formed using the droplet method. The sodium alginate-rGO-Eslicarbazepine mixture was loaded into a syringe pump connected to a syringe needle. Controlled droplets of the mixture were dispensed by the syringe pump into a calcium chloride solution (2% w/v) under constant stirring. The crosslinking reaction between calcium ions and sodium alginate resulted in the formation of spherical microbeads[15].

Table: 1. Formulation of Eslicarbazepine microbeads

S.NO	Batch code	Sodium alginate (% w/v)	Eslicarbazepine(mg)	Curing agent	Curing agent %	Curing time (h)	RPM
1	A	2	400	Cacl2	2.5	0.25	100
2	B	2	400	Cacl2	2.5	0.25	100
3	C	3	400	Cacl2	2.5	0.25	100
4	D	3	400	Cacl2	5	0.25	100
5	E	4	400	Cacl2	5	0.5	200
6	F	4	400	Cacl2	5	0.5	200
7	G	5	400	Cacl2	5	1	300
8	H	6	400	Cacl2	5	1	300

In-Vivo Studies:
In-Vivo studies of selected formulations of Eslicarbazepine loaded Reduced Graphen oxide nanoparticles were performed and reported.

Animals
Albino Mice of either sex weighing 18-22g were used for in-vivo studies. The animals were kept in the animal house and maintained under standard rates and diet ad Libitum. Experimentation on

animals was conducted after proper approval from IEAC

Evaluation of Pharmacokinetic Parameters

Every animal study was conducted in accordance with CPSCEA criteria and following methods authorized by IAEC. The open labeled parallel research design was used to carry out the pharmacokinetic investigation. KINETICA software was used to determine the various pharmacokinetic parameters, including Cmax, Tmax, Half-life, MRT, Clearance and Volume of distribution, AUC0-t, and AUC0-∞.

The studies were performed in albino rats weighing about 200–250 gm. The rats were fasted overnight before experimentation and were accessed to water *ad libitum*. The rats were randomly separated into four groups and administered as follows. Eslicarbazepine pure in first group, drug loaded reduced graphene oxide NPs in second group and third group served as control The drug concentration was measured in the blood plasma at several time intervals after administration. Blood samples were withdrawn via cardiac puncture at 0, 0.25, 0.5, 1, 2, 4, 6, 8, 20 and 24 h in micro centrifuge tubes and EDTA was added as an anticoagulant. The collected blood was mixed properlywith the anticoagulant and centrifuged at 3000 rpm for 20 min. The plasma was separated and stored at -21°c until drug was analyzed. At the same interval of blood collection, the rats were sacrificed to collect brain and tissues. The separated tissues were rinsed with saline and homogenized with different volumes phosphate buffer with pH 7.4

RESULTS

Bioavailability Studies:

Parameters	Pure drug	Marketed Formulation	Optimized Nanoparticles
Cmax (µg/mL)	1.23±0.01	1.37±0.01	1.63±0.01*
Tmax (h)	1.00±0.00	1.00±0.00	1.00±0.00
t1/2 (h)	2.60±0.06	2.37±0.37	1.05±0.06*
$AUC_{0\rightarrow 8}$(µg-h/mL)	5.77±0.66	6.89±0.37	7.11±0.15*
Ke (h^{-1})	0.267±0.005	0.294±0.04	0.652±0.03*
Fr (%)	-	119.41	123.22

All values are expressed as mean ±S.D., n=6

The pharmacokinetic parameters were calculated from plasma concentration-time curve. The results are shown in Table Absorption of Eslicarbazepine from oral administration was rapid with three groups as indicated by the low Tmax value about 1.00 h. However, the Cmax value was high with nanoparticles of Eslicarbazepine showing maximum drug absorption. The elimination half-life (t1/2) of Eslicarbazepine with nanoparticles was less, representing the drug is rapidly eliminated from the body. It was further supported evidence by high elimination rate constant value (Ke) of nanoparticles when comparing with pure drug and marketed formulation. The prepared nanoparticles showed a high area under the curve (AUC) value specifying the greater bioavailability of drug than pure drug and marketed formulation.

This is one more supports to the higher values of Cmax which were observed with Eslicarbazepine nanoparticles. Hence forth the pharmacokinetic study shows rapid, higher absorption followed to higher bioavailability of the drug from nanoparticles when comparing with pure drug and marketed formulation. Bioavailability study showed significantly greater extent of absorption of Eslicarbazepine nanoparticles than pure drug and marketed formulation ($p < 0.05$). Eslicarbazepine absorption from nanoparticles and marketed formulation resulted in 1.23 and 1.19 fold increases in bioavailability when compared to pure drug. The result of the study shows that nanoparticles technique can be effectively used for improvement of oral bioavailability of Eslicarbazepine

PTZ stimulation can be administered using Trans corneal or Tran's auricular (ear-clip) electrodes from an electroshock device at a power that causes 100% of the control animals to exhibit tonic hind limb extension (HLE). Generally speaking, a seizure is deemed maximal when changes in current intensity do not affect the pattern or duration of its different components.

Standardized parameters like a 50mA (for mice) or 150mA (for rats) fixed current, a 50-60-Hz pulse frequency, a 0.6ms pulse width, and a 0.2s stimulus

duration are all part of the traditional MES test. Corneal electrodes are primarily used briefly. After applying a stimulus, an immediate severe tonic seizure occurs, causing the anterior and posterior legs to extend to their maximum extent and the body to stiffen. Clonic seizures begin at the end of the tonic phase, which typically lasts for 10–15 s, and are characterized by the hind limbs flailing and the body shaking. Twenty to thirty seconds later, the animal is usually able to return to an upright position and begin moving around, seemingly regaining its normal behavior.

If, within 10 seconds of stimulation, the animal displays a tonic extensor seizure with rearward HLE greater than 90 degrees from the body and lasts longer than 3 seconds, the test is deemed successful. At the commencement of widespread clonus, the tonic HLE ends. Thus, the goal of the current investigation was to assess the anticonvulsant profile.

The standard medicine Valproic acid (SD fine chemicals) 200 mg/kg and the test substance derivative 50 and 100 mg/kg in 10% DMSO were administered orally. We bought Albino Wistar mice from Mahaveer Enterprises in Hyderabad. Before administering the dose, the animals were kept in the experimental settings for roughly seven days in order to acclimate them to the surroundings. Animal identification is based on the cage number and individual markings on the tail. The animals were kept in polypropylene cages with rice husk bedding, six of each sex. The animals were fed a conventional diet of pellet chews under appropriate management practices, and they were given access to unlimited water. The temperature was kept between 20 and 25 oC, and the light and dark cycles lasted for 12 hours each.

Acute Toxicity Studies

Organization for Economic Cooperation and Development (OECD) guidelines 423 (Acute Toxic Class Method) were used in the process. The acute toxic class approach involves three animals of a single sex in a step-by-step process. A minimum of two to four steps may be required to assess the acute toxicity of the test drug, depending on the animals' mortality and/or morbidity status. This process uses the fewest amount of animals necessary to reach a valid scientific conclusion based on the available data. The process (acute oral toxicity OECD guideline 420 fixed dosage procedure) and the outcomes enable a material to be categorized and graded in accordance with the Globally Harmonized System (GHS) for the categorization of the chemical that causes acute toxicity. The fixed doses (5, 50, 300, and 2000 mg/kg b.wt). To test for toxicity, six mice weighing between 18 and 22 grams were employed The mice were given an oral starting dose of 50 mg/kg b.wt of eslicarabazipine because the majority of the crude extracts had LD50 values greater than 4000 mg/kg b.wt per oral dose. The mice were then allowed to fast for the entire night with water at their discretion, denied food for an additional 3–4 hours following the drug administration, and monitored for an additional 14 days. The mice's body weight was recorded both before and after the treatments, along with any alterations to their eyes, mucous membranes, skin, and fur. Additionally changes were noted to their autonomic and central nervous systems, somatomotor activity, behavior patterns, and signs of tremors, convulsions, salivation, diarrhoea, lethargy, sleep, and coma. It was also important to observe when poisoning started and when it showed symptoms (OECD 423). Since there was no animal mortality when the dosage of Eslicarbazapine was 200 mg/kg, >200 mg/kg was considered the LD 50 cut off value.

Methods for Antiepileptic activity

Experimental design, Albino wistar mice of either sex (18-22g) were used in the present study. Animals were provided with standard diet and water ad libitum. The mice were divided in to four groups containing 6 each.

Group I- Control, administered vehicle orally 10% DMSO).

Group II- Administered standard drug at a dose of 200mg/kg b.wtorally.

Group III- Administered test drug (Eslicarbazapine loaded, reduced Graphene nanoparticles) a dose of 50mg/kg b.wt per orally.

Group IV- Administered test drug (Eslicarbazapine loaded, reduced Graphene nanoparticles) at a dose of 100mg/kg b.wt per orally.

Albino mice (18-22 g) of either sex were partitioned into four gatherings of six mice and abstained for the time being before the test however water was provided not obligatory. Bunch I was kept up with as control which was given with

sodium alginate (10 ml/kg p.o.) when every day for seven days. Group II was managed with diazepam (5 mg/kg i.p.) alone on first day solely after 30 min treatment anticonvulsant movement was recorded. Bunches III and IV were treated with various Eslicarbazepine definitions once every day for seven days. On seventh day, 30 min after diazepam and 60 min after drug organization Pentylenetetrazol (80 mg/kg i.p.) was regulated. The boundaries like idleness (beginning of clonus), beginning of tonic seizure, and status of creature following 30 minutes and 24 hours and level of insurance were recorded during test meeting

Table no. Pentylenetetrazole (PTZ) induced convulsion in mice

Group	Design of Treatment	Flexion (Seconds)	Extension (Seconds)	Clonus (Seconds)	Stupor (Seconds)	Recovery (Seconds)	% Protection
I	Control(DMSO) 50mg/kg, p.o	5.06± 0.66	14±1.46	4.66± 0.33	46.06± 1.35	148.08± 4.03	40
II	Valproic Acid 200mg/kg,.p.o	1.06 ± 0.44*	0.54± 0.14*	2.8± 1.20	10.11± 0.29**	89.76± 2.59	100
III	50mg/kg, p.o	4.02± 0.32	608± 0.37	2.21± 0.55**	38± 1.29	154.33± 7.57	81.47
IV	100mg/kg, p.o	1.71± 0.17**	1.05± 0.19**	0.72± 0.27**	19.11± 3.17**	97.5± 2.69	100

Values are expressed as Mean ± S.E.M; n=6, *p<0.05, **p<0.01, ns – non significant (One-way ANOVA way ANOVA followed by Dunnet'stest). Experimental groups values are compared with control group and standard. P.o.: per oral route of administration.

DISCUSSION

Pentylenetetrazole is a selective blocker of the chloride ionophore complex to the GABA-A receptor, and after repeated or single dose administration leads to a decrease in GABAergic function and to the stimulation and modification of density or sensitivity of different glutamate receptor subtype in many brain regions. Pentylenetetrazole may also trigger a variety of biochemical processes including the activation of the membrane phospholipase, proteases and nucleases. Alteration in membrane phospholipids metabolism cause liberation of free fatty acids, diacylglycerols, eicosanoids, lipid peroxidase and free radicals. The tonic extensor phase is selectively abolished by the drugs effective in generalized tonic clonic seizure. Eslicarbazapine is the active principles responsible for the anticonvulsant activity

CONCLUSION

The present study indicates that the drug Eslicarbazapine has potential anticonvulsant activity against chemically induced convulsion in experimental animals.

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