Advances in Bioresearch
Adv. Biores., Vol 15 (5) November 2024: 58-62

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.15.6.5862

Advances in Bioresearch

ORIGINAL ARTICLE

Assessment of Cellular Toxicity using Entrectinib Nanosuspensions

S. Sreenivasa Chary[1], D. V. R. N. Bhikshapathi[2,3*]
[1]Research Scholar, Bir Tikandrajit University, Canchipur, Imphal West-795003, Manipur, India.
[2]Research Supervisor, Bir Tikandrajit University, Canchipur, Imphal West-795003, Manipur, India.
[3]Teegala Ram Reddy College of Pharmacy, Meerpet-500097, Hyderabad, India.
***Corresponding author's** E-mail : dbpathi71@gmail.com

ABSTRACT
In order to treat cancer, this study aims to create, test, and analyze nanosuspensions loaded with Entrectinib and their cytotoxic effects. Quantitative and qualitative criteria were used to evaluate the synthesized nanosuspensions containing entrectinib, which were prepared using a solvent-antisolvent precipitating method. Spectrophotometric measurement of mitochondrial activity in HLC-1 cell lines allowed the researchers to determine the nanosuspensions' cytotoxicity and their ability to limit cell proliferation in the MTT assay. The manufactured nanosuspensions containing Entrectinib were successfully completed, and all of the evaluated qualitative and quantitative parameters were within the allowed range. Significant anticancer efficacy was indicated by the IC50 values of 56.12 and 63.05 µg/ml, respectively, when the in vitro anticancer activity was tested on HLC-1 cell lines utilizing the nanosuspensions that were created. This result shows that the nanosuspensions loaded with Entrectinib were effectively characterized and show low levels of cytotoxicity towards the HLC-1 cell lines.
Keywords*: Nanosuspensions, entrectinib, cancer, toxicity, cell lines.*

Received 10.09.2024 Revised 13.10.2024 Accepted 19.11.2024

How to cite this article:
S. Sreenivasa Chary, D. V. R. N. Bhikshapathi. Assessment of Cellular Toxicity using Entrectinib Nanosuspensions.Adv. Biores. Vol 15 [6] November 2024. 58-62

INTRODUCTION

Entrectinib (ENT) is an innovative and powerful inhibitor targeting tyrosine receptor kinases (TRKA, TRKB, and TRKC), ROS1, and anaplastic lymphoma kinase (ALK) with oral administration capabilities [1]. To meet the demands of both adult & pediatric patient coping through advanced/solid growths that are Neurotrophic tropomyosin receptor kinase (NTRK) fusion-+ve. Solid tumors and non-small cell lung cancer (NSCLC) are two diseases that ENT has been approved to treat by FDA & European Union. Additionally, it is useful for the treatment of brain metastases [2]. Sarcoma, thyroid cancer, pancreatic cancer, breast cancer, cancer of the salivary glands, colon cancer, and a host of other malignancies are all part of this category [3]. The drug's dosage is 600mg for adults and 400mg for kids, respectively. Serious adverse effects can occur with higher dosages of the medicine [4]. Drug-drug interactions have also been associated with ENT [5].By enhancing the drug's solubility through the use of formulation methods, it is feasible to reduce the dose, so alleviating these side effects. Reducing the first-pass impact and pharmacokinetic variance linked to the current capsule formulation is also crucial [6].

In these cases, it is crucial to embrace and promote nanotechnology, a state-of-the-art technology. Colloidal nanocarriers enable the delivery of medications, which surpasses the constraints of conventional methods [6]. Nanosuspension, often known as nanocrystals, is the preferred method for increasing solubility in drugs administered at large doses. It has demonstrated potential in enhancing the bioavailability of anti-cancer drugs and decreasing fast-fed variability. The bioavailability and reduced fast-fed variability of anti-cancer drugs were both improved by nanosuspension [5].

When these nanotechnologies get close enough to a tumor, they assemble to form nanoparticulate drug delivery systems. In addition, powerful ultrasound distorts these nanoparticulate at the target area, leading to more effective and less hazardous pharmaceutical release and accumulation within the

targeted cells. The effectiveness of these methods in the treatment of different kinds of cancer requires further investigation. Both in vitro and in vivo environments could be utilized to administer anticancer medicines like Entrectinib by nanosuspensions pharmaceutical delivery [2].

Changes in shape, growth rate, cell mortality, and cell disintegration occur in cells exposed to nanosuspensions. Consequently, it is imperative that all compounds with experimental pharmacology potential undergo cell viability testing. The usage of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) allows for the detection of changes in mitochondrial activity. Research on the cellular toxicity of Entrectinib nanosuspensions is being conducted in vitro [7]. Nanotechnology methods are advanced drug carriers with a gas-filled core and a protein, polymeric, or phospholipid coating. When exposed to high-pressure ultrasound, NBs undergo inertia cavitation, causing shock waves and microjets. This process has significant bioeffects, exerting mechanical impacts on nearby tissues or cells (8). Formulating, evaluating, and assessing the cellular toxicity of nanosuspensions loaded with Entrectinib during cancer treatment is the goal of the current study.

MATERIAL AND METHODS

Materials

Entrectinib was gifted by Hetero Laboratories Pvt. Ltd. Hyderabad, India. We used DMEM [#AL007A], EMEM [#AL047S], Fetal Bovine Serum [#RM10432], and D-PBS [#TL1006] from Hi Media. DMSO [#PHR1309] and MTT Reagent [#M5655] were also purchased from Sigma. We used a 96-well plate from Corning, USA, to culture the cells. Using Eagle's minimal necessary medium, cell lines procured from India's National Center for Cell Sciences in Pune.

Methods

Preparation, Characterization, & evaluation of drug loaded nanosuspensions

Method of solvent-antisolvent precipitation for nanosuspension of Entrectinib. Dynamic light scattering was used to measure particle size, polydispersity index, and zeta potential. Scanning electron microscopy gotutilized to observesound structure of both plain nanosuspensions and those loaded with Entrectinib. The drug and excipients were also examined. The dialysis bag method determined the in vitro release kinetics, while fourier transformed infrared spectroscopy & differential scanning calorimetry were utilized for compatibility tests. Using a UV-visible spectrophotometer set at 254 nm, the saturation solubility was investigated. Three months of stability testing followed drug release trials in four different media: simulated gastric fluid (SSGF), simulated intestinal fluid (SIF), with fed state (FeS), and Fasted state (FaSSGF).

In-vitro cytotoxic study

In order to determine if the nanosuspensions containing Entrectinib were cytotoxic or could spectrophotometrically reduce cell proliferation in response to mitochondrial activity in live HLC-1 cell lines, the MTT test was employed.

Preparing cell line

We quickly brought one vial of every cell line obtainable of liquid nitrogen & brought it to room heat. Following addition of the material from vials to 9ml of whole medium, the mixture was centrifuged at 125g (5min). Next, pellet got combined by 10ml of whole media & suspended in T-25 flask. The flask was then nurtured at 37°C with 5% CO_2 after centrifugation, with the supernatant being discarded. Upon reaching 80% cell confluence, the cells were spun at 125g (5min). The resulting pellet got combined with 15mL of whole media & moved T-75 flasks. Experiment was conducted using cells in the flask once their confluence had reached around 80-90%.

MTT assay

Without the test agent, 20,000 cells gotsowed into 96-well plate using 200µl of cell suspension in complete culture media with 10% FBS. The cells were then left to develop for 24 hours. The 96-well plates incubated at 37°C in 5% CO_2 environment aimed at 48 hours after first 24 hours. The wasted medium in each well was replaced with nanosuspensions loaded with Entrectinib at the appropriate dosages. Following incubation time, plates taken out of incubator, the used media got drained, and then MTT reagent was added until it reached a closing concentration of 0.5mg/mL, using a 0.2 µm filter that had been sterilized. After three hours in the incubator, plates got covered by aluminum foil to keep them from touching any light. Following the incubation period, 100µl of DMSO added after MTT substance had been withdrawn. At 570 nm, the Tecan™ Infinite 200Pro spectrophotometer was used to measure the absorbance [9].

Data examination

Percentage of viable cells in control set, which did not receive any treatment, got set at 100%, while percentage of viable cells in treatment groups got calculated comparative to this control. A dose response

analysis was performed by plotting the percentage of viability against the concentration. A suitable model fitted to evaluate I_{max}& IC_{50} based on the dosage response relationships.
Percentage viability formula:

$$\% \, Viability = \frac{100 \, X \, OD570e}{OD570b}$$

Where,
OD570e: mean value of Optical Density test item dilutions;
OD570b: mean value of Optical Density of -ve control

RESULTS AND DISCUSSION

Preparation, Characterization, and evaluation of drug loaded nanosuspensions

The current investigation used the solvent-antisolvent precipitation method to create nanosuspensions. A portion of the medication gotmelted in an organic solvent, and a share of additive in an antisolvent. The fast desolvation process results in the rapid formation of nanosuspensions and the precipitation of the medication.

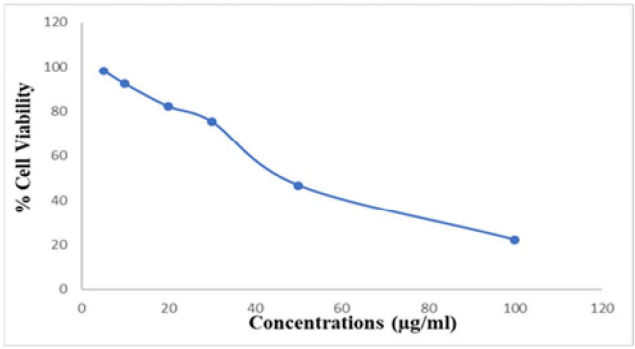
Characterization and evaluation of nanosuspensions loaded with Entrectinib were carried out independently. The nanosuspensions loaded with Entrectinib had particle sizes ranging from 83.22 to 258.2 nm, zeta potentials of 0.189± 0.013 mV, & polydispersity indices of 0.055 to 0.258, respectively. The assessment of Entrectinib loaded nanosuspensions using transmission electron microscopy and the dynamic light scattering technique are well linked. Possible drug entrapment in the suspended particles is suggested by the lack of drug-specific peaks in fourier transformed infrared spectra & thermogram of nanosuspensions containing Entrectinib. The solubility of the nanosuspension was 19.33 times higher than that of the pure medicine. Nanosuspensions exhibited drug release rates of 85.03.18 ± 6.66%, 94.61 ± 12.91%, 98.56 ± 14.66%, and 98.12 ± 9.66% in the two-hour FaSSGF, two-hour FeSSGF, and four-hour FeSSIF experiments, respectively. Even after a long time in storage, the formula remained unchanged.

***In-vitro* cytotoxic study**

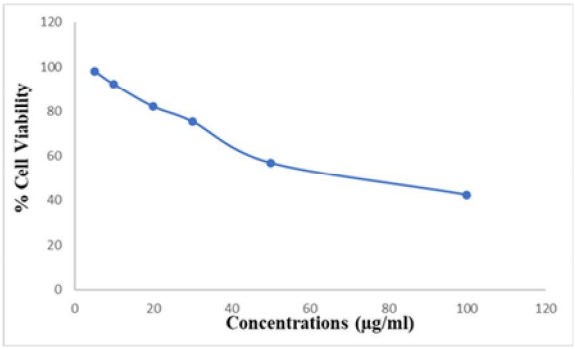
The benefits and limitations of model for screening & mechanistic evaluation of possibly hazardous substances are shed light on by the in vitro cytotoxicity experiments. In order to detect chemical-induced disruption, a variety of morphological & biochemical marker isobtainable for use in cellular and molecular level data acquisition [9]. Under controlled laboratory conditions, in vitro cell line representations are better to implement. Human tumor cell lines are the mainstay of cellular screening in cancer research [10]. When it comes to management and reproducibility, this system is top-notch [11]. Cell viability tests were conducted on the HLC-1 (Table 1; Figure 1) cell lines using the MTT method to calculate IC50 values in order to measurenoxiousness of different amounts of nanosuspensions loaded with Entrectinib.

Table 1: Cytotoxicity of Entrectinib pure drug andEntrectinib loaded nanosuspensions on HLC-1 cell line

Concentrations (µg/ml)	**Entrectinib Pure Drug**		**Entrectinib Loaded Nanosuspensions**	
	% Mean Viability	**IC50 (µg per ml)**	**% Mean Viability**	**IC50 (µg per ml)**
Blank	-	70.03	-	56.12
Vehicle Control	100.00±0.00		100.00±0.00	
5.00	98.37±2.56		98.37±2.65	
10.00	92.36±3.24		92.36±2.84	
20.00	82.26±2.14		82.26±2.78	
30.00	75.54±2.75		75.54±2.96	
50.00	46.87±1.27		56.87±1.47	
100.00	22.36±1.65		42.36±1.65	



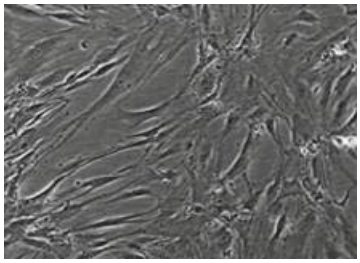
Cytotoxicity of Entrectinib Pure Drug on HLC-1 cell line



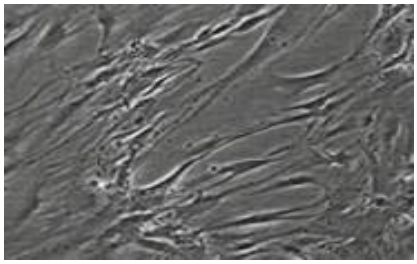
Cytotoxicity of Entrectinib nanosuspensions on HLC-1 cell line

Figure 1: Cytotoxicity of Entrectinib Pure Drug and Entrectinib nanosuspensions on HLC-1 cell line

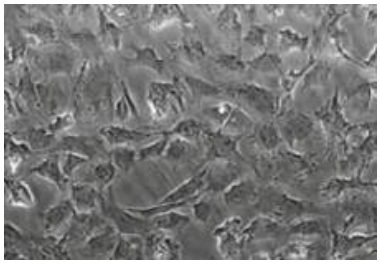
Figure 2 shows results of morphology of HLC-1 cell lines after treatment with different concentrations of Entrectinib and nanosuspensions containing Entrectinib. Substantial surface deformations and alterations were seen on the cells, which were most likely caused by the investigated nanosuspensions [11]. The effect on cell morphology was less pronounced with pure medications [12, 13]. Cell shape and apoptotic modifications were shown to be linked with the rise in concentration of Entrectinib-loaded nanosuspensions. The anticancer activity of nanosuspensions loaded with Entrectinib is associated with the processes via which they induce cell death and break cell membranes [14].



Vehicle control



Entrectinib test formulation against HLC-1 cellline



Entrectinib Pure Drug on HLC-1 cellline

Figure 2:Morphologic variations of HLC-1 cancer cells once treated with Entrectinib nanosuspensions

One colorimetric approach to measuring cellular metabolism is the MTT test. For preliminary screening of apoptosis or necrosis, the cytotoxic potential of a test drug was evaluated using the HLC-1 cell lines [14]. The yellow tetrazolium MTT [3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] is converted into insoluble (E, Z)-5-(4,5-dimethylthiazol-2-yl)-1,3-diphenylformazan (formazan) by NAD(P)H-dependent cellular oxidoreductase enzyme, which is involved in MTT assay's biological mechanism. The MTT experiment showed minimal cytotoxicity in the formulation containing the aforementioned safe substances. We confirmed that, at all concentrations, the modified formulation had a cell viability percentage higher than 97% (<3% cell death). This finding proves that the created microsuspensions have very little cytotoxic effect on the HLC-1 cell lines.

CONCLUSION

In order to administer various anticancer drugs, including synthetic and natural compounds, nanoparticulated drug delivery methods have lately attracted a lot of attention. Particle size for tumor accumulation was shown to be optimal in the revised formulations. The uniform monodispersity of the formulation was confirmed by the narrow size distribution seen in nanosuspensions. A notable cytotoxic effect of the formulation was shown in the MTT experiment. These results proved that the nanosuspensions loaded with Entrectinib were important in clinical settings. Research shows that the nanosuspensions loaded with Entrectinib showed very little cytotoxicity towards the HLC-1 cell lines.

CONFLICTS OF INTEREST
No conflict of interest.

AUTHOR CONTRIBUTION
S.S.C. and D.V.R.N.B. wrote up the strategy, reviewed it, and made any necessary edits after finishing the research and execution. The submission and publication have been approved by both authors. The published form of work reviewed &accepted by both writers.

FUNDING
This study acknowledged no outside funding.

ACKNOWLEDGMENTS
Authors express their gratefulness to Bir Tikandrajit University in Canchipur, Manipur, India, for their unwavering assistance during the entire research process.

REFERENCES
1. Konda Sri Chaya Reddy, Darna Bhikshapathi (2024), Design and optimization of DPC-crosslinked HPβCD nanosponges for entrectinib oral delivery: formulation, characterization, and pharmacokinetic studies, Future Journal of Pharmaceutical Sciences (2024) 10:101: 1-15, https://doi.org/10.1186/s43094-024-00680-8.
2. Rybarczyk-Kasiuchnicz, A., Ramlau, R., Stencel, K. (2021). Treatment of brain metastases of non-small cell lung carcinoma. Int. J. Mol. Sci. Multidisciplinary Digital Publishing Institute (MDPI)., 1–21.
3. Frampton, J.E.(2021). Entrectinib: A Review in NTRK+ Solid Tumours and ROS1+ NSCLC. Drugs. Springer, 81:697.
4. Menichincheri, M., Ardini, E., Magnaghi, P., Avanzi, N., Banfi, P., Bossi, R., et al. (2016). Discovery of Entrectinib: A New 3-Aminoindazole As a Potent Anaplastic Lymphoma Kinase (ALK), c-ros Oncogene 1 Kinase (ROS1), and Pan-Tropomyosin Receptor Kinases (Pan-TRKs) inhibitor. J Med Chem.,59:3392–408.
5. Sigal, D., Tartar, M., Xavier, M., Bao, F., Foley, P., Luo, D., et al. (2017). Activity of Entrectinib in a Patient With the First Reported NTRK Fusion in Neuroendocrine Cancer. J Natl Compr Canc Netw. J Natl Compr Canc Netw., 15:1317–22.
6. Delgado, J., Pean, E., Melchiorri, D., Migali, C., Josephson, F., Enzmann. H., et al. (2021). The European Medicines Agency review of entrectinib for the treatment of adult or paediatric patients with solid tumours who have a neurotrophic tyrosine receptor kinase gene fusions and adult patients with non-small-cell lung cancer harbouring ROS1 rearrangements. ESMO open. ESMO Open., 2021;6.
7. Kumar, P., Nagarajan, A., Uchil, P.D. (2018). Analysis of Cell Viability by the MTT Assay. Cold Spring Harb Protoc., 2018(6).
8. Bijili Vijaya Laxmi, Darna Bhikshapathi, Penakalapati Sailaja Rao (2025), Optimization and Enhancement of Oral Bioavailability of Dabrafenib as Nanobubbles Using Quality by Design Approach, Pharmaceutical Sciences, 31(1), 1-15. doi:10.34172/PS.2024.31 https://ps.tbzmed.ac.ir.
9. Buranaamnuay, K. (2021). The MTT assay application to measure the viability of spermatozoa: A variety of the assay protocols. Open Vet J., 11(2):251-269.
10. Khorsandi. L., Orazizadeh, M., Niazvand, F., Abbaspour, M.R., Mansouri, E., Khodadadi, A. (2017). Quercetin induces apoptosis and necroptosis in MCF-7 breast cancer cells. Bratisl Lek Listy., 118(2):123-128.
11. Arafath, A.A.M.Y., Jayakar, B. (2019). Enhancement of Oral Bioavailability via Solid Lipid Nanoparticles of Anticancer Drug Dasatinib - An in Vitro Cytotoxicity and Pharmacokinetic Study. Asian J Pharm Clin Res.,12(6):143-5.
12. Cheng, X., Tan, S., Duan, F., Yuan, Q., Li, Q., Deng, G. (2019). Icariin induces apoptosis by suppressing autophagy in tamoxifen-resistant breast cancer cell line MCF-7/TAM. Breast Cancer., 26(6):766-775.
13. Xu, B., Peng YJ, Zhu WJ. (2022). Curcumin Inhibits Viability of Clear Cell Renal Cell Carcinoma by Down-Regulating ADAMTS18 Gene Methylation though NF-κ B and AKT Signaling Pathway. Chin J Integr Med., 28(5):419-424.
14. Tang X, Zhao, Q., Liu, J, Wang, S., Zhang, N., Yang, Y. (2021). The compound AST-003 could effectively promote apoptosis of renal cell carcinoma cells *in vitro*. Transl Cancer Res., 10(5):2120-2133.