

Intranasal Route For Brain Targeting Of Naringenin- A Potential Antioxidant Drug For The Treatment Of Parkinson's Disease Using Nano-Emulsion As The Drug Delivery Carrier

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Abstract

The neurodegenerative illness known as Parkinson's disease (PD) causes a shortage of dopamine in the brain's nigrostriatal region. One cause of PD is oxidative stress. The flavonoid naringenin (NAR) has been proven to be helpful in animal models of Parkinson's disease (PD). The purpose of this study is to develop a nasal-to-brain transport (PD) Nanoemulsion (NE) of vitamin E-loaded naringenin (NRG) for the treatment of Parkinson's disease. To evaluate the efficacy of the enhanced NE in PD, many behavioral tests were performed in a rat model. The findings from the present research suggest that NRG administered using a new noninvasive intranasal delivery technology may be effective in the treatment of PD-related symptoms. The purpose of this study is to develop a nasal-to-brain transport (PD) Nanoemulsion (NE) of vitamin E-loaded naringenin (NRG) for the treatment of Parkinson's disease. To evaluate the efficacy of the enhanced NE in PD, many behavioral tests were performed in a rat model.

Keywords: antioxidant; naringenin; Parkinson's disease; Nano-Emulsion

INTRODUCTION

Oxidative stress in the context of PD development is associated with mitochondrial dysfunction and impaired dopamine oxidative metabolism. It is possible that mitochondrial dysfunction, which results in the production of free radicals. In animal models of PD, antioxidants have shown to have a positive effect, but they have not demonstrated any therapeutic impact in controlled clinical studies. The search for antioxidant-based therapies for the treatment of PD, however, continues. The BBB may be avoided by using the nasal route, which is also noninvasive. After intranasal administration, It is possible for drugs to cross the blood-brain barrier (BBB) through the nasal membrane and other specific transport channels or via their lipophilicity. Drugs may enter the cerebrospinal fluid (CSF) or brain tissue via the olfactory region of the nasal cavity. Drugs typically have a short residence period in the nasal cavity, between 15 and 20 minutes, due to mucociliary clearance. For nasal delivery, this strategy may be expanded by encapsulating medications in biocompatible polymeric nanoparticles (NPs). Strong contact with mucosal membranes and protection from enzymatic degradation make polymeric NPs ideal medication carriers for the intranasal route. Researchers have created a plethora of nanoparticle medication delivery methods for nasal-to-cognitive transfer, each with its own set of benefits and drawbacks.

Vitamin E in combination with another antioxidant has been shown to be effective against neurodegenerative diseases as Alzheimer's. Moreover, intranasal administration to the brain has been studied and shown to be effective. Hence, in order to effectively treat PD, researchers want to find a way to intranasally administer an NRG NE that has been supercharged with vitamin E. The synergistic impact of intranasal treatment of vitamin E and NRG on behavioral impairment in a 6-OHDA-induced rat model of Parkinson's disease was also studied.

LITERATURE REVIEW

Bonferoni, M. C., Rassa, G., Gavini, E., Sorrenti, M., Catenacci, L., & Giunchedi, P. (2019), Damages in cerebral ischemia may be caused in part by oxidative stress, includes Alzheimer's, Parkinson's, and Huntington's diseases, in which it plays a pivotal role in disease progression. The term "oxidative stress" refers to the damage done by an excess of reactive oxygen species. Because they protect the target tissues from damage caused by free radicals, antioxidants are recommended as possible medications to thwart ROS's harmful effects. Unfortunately, the blood-brain barrier (BBB) severely restricts the absorption of the medicine into the brain after systemic doses, limiting the effectiveness of antioxidants. The so-called nose-to-brain route is an approach to improving antioxidant delivery to the brain that entails administering the antioxidant in a particular nasal formulation and having it go to the CNS primarily through the olfactory nerve pathway. The present literature provides several instances of promising outcomes from research in cell cultures and animal models indicating that antioxidants delivered through the nasal route may have neuroprotective benefits. This article discusses a novel approach to the treatment of neurological disorders by administering antioxidants directly into the brain via the nasal passages.

Cheng, G., Liu, Y., Ma, R., et al., (2018) An increasing number of elderly people are diagnosed with Parkinson's disease (PD), a neurological disorder. Due to the lack of a therapeutic treatment, innovative anti-PD medicines are desperately needed. A major obstacle to the creation of such medicines is the selective permeability of the blood-brain barrier (BBB). Thankfully, nanotechnology may help solve this issue and improve medication transport over the BBB by using tactics based on the BBB's physiological features and making other alterations, such as increasing BBB permeability. The biological action of nanoparticles is largely disregarded, despite their widespread usage as carriers in PD therapy. Numerous studies over the last few years have shown that nanoparticles, via their own nano-bio impacts, may alleviate PD symptoms. In this study, we first explore the creation of suitable brain-targeted delivery nanoplatfoms for PD therapy, and then briefly explain the physiological properties of the BBB. We then focus on the unique approaches to overcoming the BBB and creating nanomaterials with anti-PD nano-biological effects. Lastly, we look to the future of nanomaterial-based PD therapy and explore the present hurdles that exist in this area. Based on recent patents, our evaluation highlights the therapeutic significance of nanotechnology in PD therapy and may serve as a roadmap for future studies.

Misra, Shashi Kiran, and Kamla Pathak. (2019), The highly vascularized nasal cavity provides a pathway for therapeutics to enter and spread throughout the brain through the olfactory and trigeminal neurons. Several neurological conditions, may be treated using nano-emulsions because of their ability to transport actives from the nose directly to the brain. This article provides a summary of current studies on the subject. The data and information come from over a hundred papers indexed in Scopus and PubMed. The olfactory and trigeminal routes, the authors conclude, provide an alternate route for the administration of hydrophobic, poor-absorption, and enzyme-degradative therapies since they promote improved biodistribution and circumvent BBB difficulties. By investigating these benefits, researchers have shown that intranasal nanoemulsions are effective, non-invasive, and safe brain-targeting cargos for the treatment of neurological diseases.

Materials and methods

Sigma Aldrich was used to get NRG and 6-OHDA (St. Louis, MO, USA). Loba Chemical was where we got our oils like peanut and olive (Mumbai, India). The Evion Capsules brand of vitamin E was bought. Fischer Scientific Co. supplied the water and methanol used in the experiment (Mumbai, India). The research only employed high-quality, analytical-grade chemicals and solvents.

We used a column and a mobile phase of 7 parts methanol to 3 parts water containing 0.1% glacial acetic acid to chromatographically separate substances at room temperature (25 0.5 C) at a flow rate of 1 ml/min. Samples (20

l) were injected with a Rheodyne injector and filtered through microfilters with a 0.45 m pore size before being analyzed at 289 nm.

Before taking any readings, we diluted all of the formulations with around 200 times as much deionized distilled water. Thereafter, Tese were vigorously shaken to reduce the effects of repeated scattering. To determine the average globule diameter, 1 cc of this mixture was pipetted out and placed in the sample cell. At 25 C and a 90 degree angle, light scattering was observed. One milliliter of each sample was poured into a clear polystyrene cuvette, and the globule diameters were measured. Then, we made three sets of measurements to ensure accuracy.

The dimensions, composition, and shape of the dried grid were further analyzed. The dialysis bag was set up according to protocol, and the machine was operated with a magnetic stirrer at 100 rpm and 37.2 C. Two milliliters of each formulation was placed in individual bags and dipped into a hundred milliliters of phosphate buffer to mimic the pH of nasal fluid, which is 6.4. To maintain the same volume in the sink, 1 ml of the sample was removed at 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 360, and 420 minutes and replaced with fresh media of the same volume. Analysis of NRG content was performed on the samples. After proper dilution, samples were analyzed using a UV spectrophotometer at 322 nm. There were three sets of measurements taken.

Ex Vivo Nasal Mucosa Permeation Study. The Franz diffusion cell was used for permeation tests outside of living tissue (15.2 ml receiver volume). Using recently extracted goat nasal mucosa obtained from a slaughterhouse, NRG NE was compared to NRG suspension. Mucosa from a goat was frozen at -80 degrees Celsius after being stored in a 10% formalin solution. After gently peeling it away, the nasal membrane was washed down with isopropyl alcohol to remove any remaining debris. The Franz diffusion cell was used to harvest tissue, which was then put between the donor and receiver chambers at a thickness of 0.2 mm. The mucosal side of the tissue will be facing the donor, while the dermal side will be facing the receiver. In order to maintain mucosal integrity, we mixed running media with dissolving medium. At 37.1 C, it was done 10 times, with new media each time. Each donor compartment received 1 cc of the specified NRG solution and NRG NE. Fresh media was added at the same volume and samples were taken at 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 360, and 420 minutes. The amount of NRG present in the samples was then determined. After proper dilution, materials were analyzed at 322 nm using a UV spectrophotometer. There were three sets of measurements taken.

Results and Discussion

Thermodynamically stable systems, NEs are made by combining a certain amount of Smix, oil separation, and water. Creaming, cracking, and phasing shouldn't be present in the final formulation. We used a Franz diffusion cell with a 0.2 mm tissue gap between the donor and recipient chambers. Ostwald ripening may be to blame for the cloudiness of certain formulations.

Table 1: Studies on the physiochemical composition of NE formulations

Formulation code	Surfactant : co-surfactant ratio	Oil %	S _{mix} %		Water %	Physical Stability test			Inference
			Surfactant	Co-surfactant		Heating	Freeze thaw cycle	Centrifugation	
F1	4:1	10	32	8	50	✓	✓	✓	Passed
F2		20	25	5	50	-	-	-	Failed
F3		18.18	21.82	5.45	54.55	-	-	-	Failed
F4		14.04	16.84	4.21	64.91	-	-	-	Failed
F5		11.94	14.33	3.58	70.15	✓	✓	✓	Passed
F6		14.81	23.71	5.92	55.56	✓	-	-	Failed
F7		11.63	18.61	4.65	65.12	✓	✓	✓	Passed
F8		11.11	19.445	19.445	50	✓	✓	✓	Passed
F9		10	17.5	17.5	55	✓	-	-	Failed
F10		8.89	15.55	15.55	60	✓	-	-	Failed
F11		7.75	13.56	13.56	65.12	✓	-	-	Failed
F12		6.67	11.665	11.665	70	✓	✓	✓	Passed
F13		7.49	18.725	18.725	55	✓	-	-	Failed
F14		6.67	16.665	16.665	60	✓	✓	✓	Passed
F15		5.8	14.495	14.495	65.22	✓	✓	✓	Passed
F16		5	12.5	12.5	70	✓	-	-	Failed

The Making of Drug-Infused NE. NRG was dissolved in the oil phase to make the drug-loaded formulation. After that, the necessary amount of S_{mix} was added, and the mixture was gradually diluted with distilled water. (Table 2).

Table 2: Studies on the structure and physical properties of drug-loaded NE formulations

Formulation code	Surfactant : co-surfactant ratio	Oil %	S _{mix} %		Water %	Physical Stability test			Inference
			Surfactant	Co-surfactant		Heating	Freeze thaw cycle	Centrifugation	
F1	4:1	10	32	8	50	✓	✓	✓	Passed
F5		11.94	14.33	3.58	70.15	-	-	-	Failed
F7		11.63	18.61	4.65	65.12	✓	-	-	Failed
F8		11.11	19.445	19.445	50	✓	-	-	Failed
F12		6.67	11.665	11.665	70	✓	-	-	Failed
F14		6.67	16.665	16.665	60	✓	-	-	Failed
F15		5.8	14.495	14.495	65.22	-	-	-	Failed

That might be because of the S_{mix}'s bigger emulsification area. Since surfactants form a thin, dense film at the oil-water contact, increasing their concentration improves stability. This data points to the surfactant as the primary player in the oily mixture's nanoemulsification, rather than the cosurfactant. The stability of a formulation and the uniformity of its globule sizes are both enhanced by a low PDI.

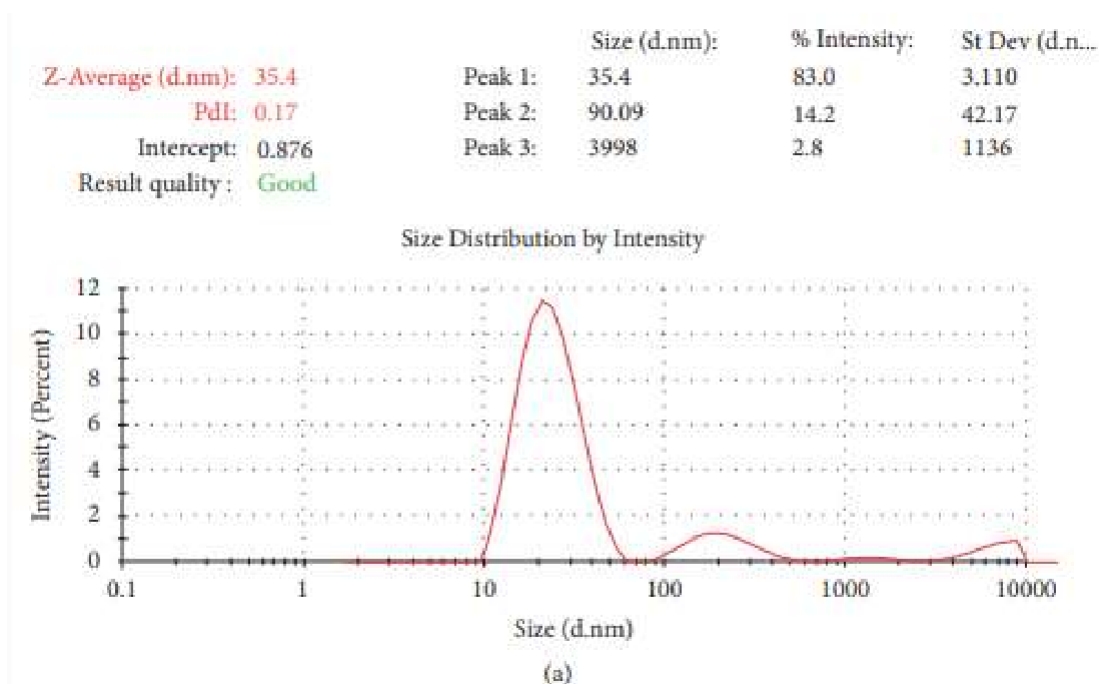
The RI value demonstrates the formulation's isotropy. The chemistry of the medication and its excipients is also described by RI. Statistically insignificant differences in RI between drug-loaded NE and a placebo formulation were not observed due to NE's chemical stability and isotropic nature. So, it is preferable to have a formulation with a viscosity that is just right so that it may be administered without difficulty, penetrate effectively, and overcome mucociliary clearance. With the use of an Abbe's refractometer, we were able to determine that the

NE had a viscosity of 19.67 0.25 Pa s. The wide range of linearity between shear rate and shear stress, from 0 to 100 s⁻¹ and from 0 to 20 Pa, was indicative of a Newtonian flow in NE.

Percent Transmittance. The created formulation has a transmittance of 98.120.07%, demonstrating the NE's stability. The fact that the NEs transmittance was so close to that of the aqueous phase confirmed that the oil droplets were spread continuously throughout the medium. The formulation is see-through because the largest globules of the dispersed phase were determined to be less than a quarter of the wavelength of visible light.

Electron microscopy transmission. The TEM experiments investigated the NE's morphology after it was produced. Optimized formulation was shown to include non-aggregated spherical globules with a size of 35.43 4.10 nm (Figure 1(d)). The TEM examination of globule size agreed with that obtained by zetasizer.

Measurement of pH. The optimal NE had a pH of 6.200.7. The nasal medication delivery device was compatible with NE since its pH was within a nonirritating range.



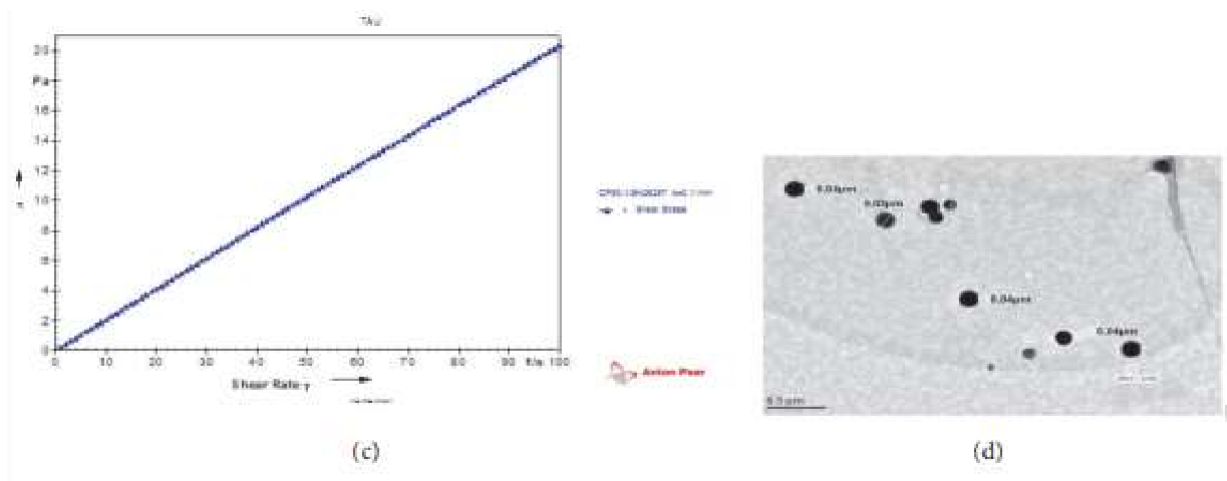
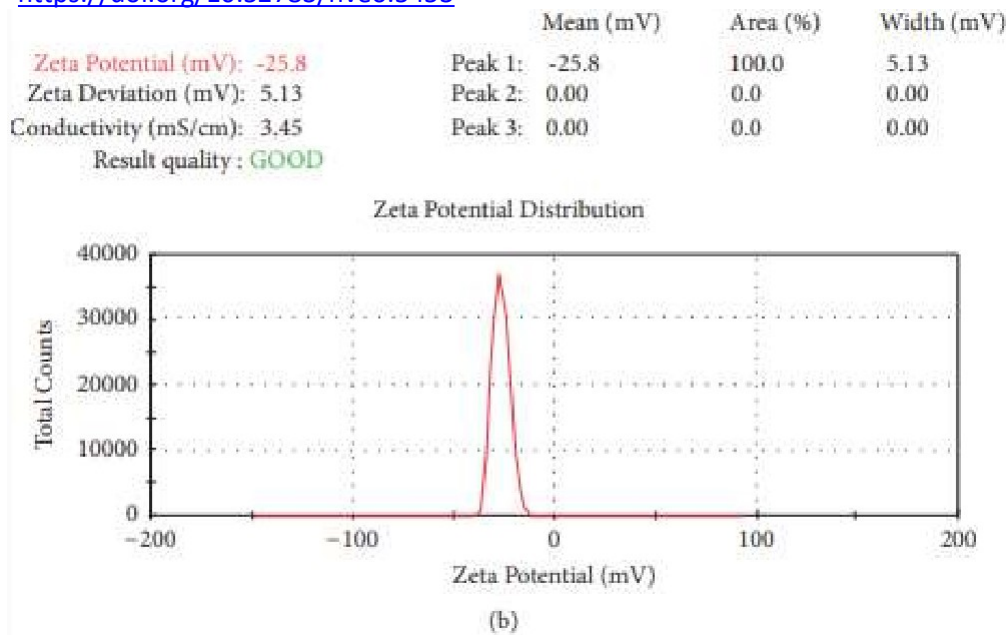


Figure 1: optimized formulation

In vitro release experiments compared NRG release from NRG NE to NRG release from NRG suspension. The amount of drug released from NRG NE was found to be substantially higher than from NRG suspension ($p < 0.05$). After 8 hours, the cumulative percentage of NRG released from NE was discovered to be larger than the release from NRG suspension (88.875% 2.875%). The NRG suspension dialysis bag also showed medication precipitation (Figure 2).

To determine the mechanism of drug release in NE, we collected data and then fit it to the zero order, first order, Higuchi, and Hixson-Crowell release kinetic models. With an R^2 value of 0.937, which is near to 1, the data supported a zero-order model for drug release from NE. (Table 3).

Table 3: Optimized NRG NE medication release kinetics in vitro

Various release models	Coefficient of correlation (R ²)
Zero Order	0.937
First Order	0.861
Higuchi	0.9269
Hixon-crowell	0.899

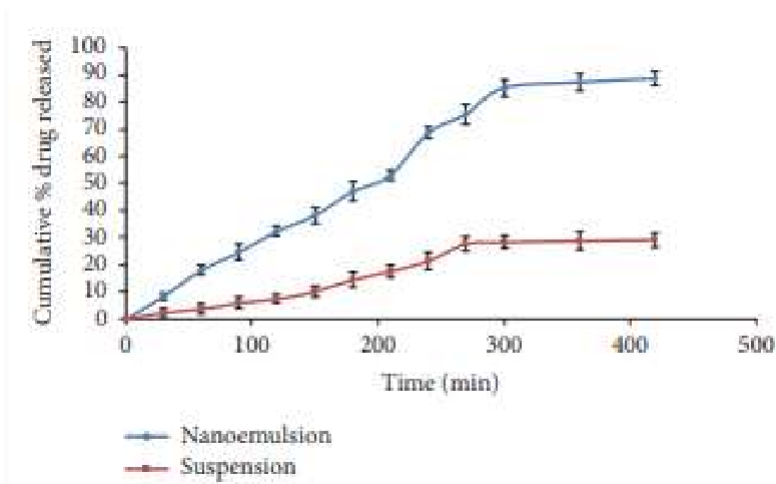


Figure 2: Release profiles of NRG suspension and NRG-loaded NE were compared in vitro.

After 8 hours, permeation was greatest for NRG-loaded NE (85.45 ± 3.404%), compared to the NRG suspension (24.644 ± 3.107%). (Figure 3). High permeability across the nasal mucosa is favorable in vivo because drug was swiftly eliminated from nasal mucosa due to mucociliary clearance. As compared to NRG's suspension, NE's flux and permeability coefficient were almost three times higher. Similarities were found between the drug release patterns seen in in vitro and ex vivo permeation tests. The results of the foregoing investigation suggest, however, that a smaller globule size results in more drug absorption through the nasal mucosa.

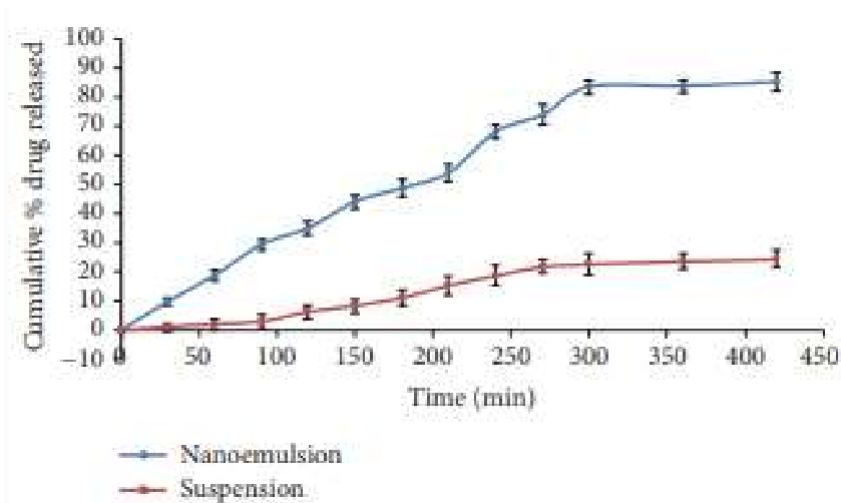


Figure 3: NRG NE and suspension: a comparison of ex vivo release profiles.

CONCLUSION

In the current investigation, a vitamin E-loaded NRG NE formulation was created that is physically stable. Trans-nasal mucosal flux was shown to be very high *ex vivo* and at its highest *in vitro*. The drug's concentration was also shown to be greater in the brain, demonstrating the targeting efficiency of NE when administered intravenously. Adding NE to standard treatment for 6-OHDA-induced rats improved motor coordination, grip strength, and swimming activity, as indicated in the study. Therefore, NRG NE administered through intranasal injection avoids first pass metabolism by being protected from the action of nasal metabolizing enzymes and allowing for its enhanced brain absorption independent of its systemic circulation.

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