RESEARCH PAPER

In vivo study of Nanoemulsion of Rasagiline Mesylate for its anti-Parkinson activity via Nose to brain delivery

Rajeev1*, Sanjar Alam2, Ajay Pal Singh1

[1]School of Pharmacy, Lingaya's Vidyapeeth, Faridabad, Haryana, India
[2]R. V. Northland Institute of Pharmacy, Dadri G. B. Nagar Uttar Pradesh India

*Corresponding Author Email : rajeevraj96kumar@gmail.com

Abstract

Introduction: Parkinson's disease (PD) is a progressive neurological condition characterized by movement impairment and loss of dopaminergic neurons. Due to inadequate medication distribution across the blood–brain barrier (BBB), conventional medicines only alleviate symptoms rather than stopping the course of the illness. The possibility of an intranasal rasagiline mesylate nano emulsion to improve brain targeting and treatment effectiveness is investigated in this work.

Materials and Methods: Rasagiline mesylate nano emulsion was assessed for acute toxicity in accordance with OECD guideline 423 and for antioxidant activity using the DPPH test. Sprague-Dawley rats with Parkinsonism caused by 6-OHDA were used in in vivo investigations. The animals were split up into four groups: standard, toxic, control, and treatment. Akinesia, locomotor activity, and catalepsy were among the behavioral markers that were evaluated. Additionally, biochemical markers such as dopamine, reduced glutathione (GSH), and superoxide dismutase (SOD) were measured.

Results: It was determined that the nano emulsion was safe and showed strong antioxidant activity. Behavioral investigations revealed a significant increase in motor function, with treated individuals exhibiting more locomotor activity and less catalepsy than the toxic group. Dopamine levels were restored in a dose-dependent manner, and antioxidant enzymes (SOD and GSH) were improved, according to a biochemical study. When compared to oral rasagiline, the high-dose nanoemulsion demonstrated greater effectiveness.

Discussion: According to the research, intranasal nano emulsion improves medicine transport to the brain, which improves neuroprotection and relieves symptoms. By successfully avoiding the BBB, the formulation presents a viable substitute for traditional oral medication.

Conclusion: Rasagiline nano emulsion via nose-to-brain delivery represents a potential strategy for improved management of Parkinson's disease.

Keywords: Nanoemulsion, In-vivo, Parkinson, brain, Nose, Delivery

How to cite this article: Rajeev, Alam S, Singh AP. In vivo study of Nanoemulsion of Rasagiline Mesylate for its anti-Parkinson activity via Nose to brain delivery. Int J Drug Deliv Technol. 2026;16(55s): 575-584. DOI: 10.25258/ijddt.16.55s.58

Introduction

Parkinson's disease (PD) ranks the second most frequent neurological disorder worldwide and exhibits a remarkable incidence trend increased over the last decades worldwide [1,2]. Although described for the first time by Dr. J. Parkinson in his seminal book An Essay on the Shaking Palsy by Dr. J. Parkinson in the year 1817, the literature on PD lacks any contribution on the subject since the seminal work done by Jean Martin Charcot and the understanding of the neuropathological changes described and including the degeneration of the substantia nigra and the Lewy bodies presence till the concept of the link between the alterations of the dopaminergic transmission and the co-existence of motor and nonmotor symptoms [3,4].

Upon the success of these discoveries, extremely effective symptomatic therapies have been developed, particularly dopamine replacement with levodopa and advanced methods such as deep-brain stimulation [5]. Although these therapies have improved the quality of life and motor function of those with the condition extensively, the progression of the illness continues unabated. With the progression of the illness, patients experience the worsening of the symptoms, which become increasingly unresponsive to the available therapies [6].

The brain poses some distinct challenges to drug delivery due to its delicate nature and presence of the blood–brain barrier (BBB), which works against most drugs entering it [7]. There have been various approaches that have been considered to circumvent this problem, some of which include pharmacological methods such as modifying drugs chemically, making use of liposomes, nanoconjugates, and nanoparticles; physiological techniques such as ligand-mediated transport; and invasive methods such as central implants and transient disruption of the BBB [8]. These approaches exemplify that nanotechnology has vast utility in dealing with both the diagnosis and treatment of neurological disorders [9].

When it comes to neurodegenerative diseases, nanotechnology is offering attractive solutions to the long-standing problems of disease diagnosis and targeted therapy [10]. Nanotechnology-based drug delivery

*Author for Correspondence: ……

systems have proven to be of immense importance in solving problems related to CNS-targeted therapeutic approaches, particularly the problem of drug delivery across the BBB [11]. Apart from defining their role in targeted drug delivery, these delivery systems can be customized to perform a variety of functions, which may range from imaging to enhancing bioactivity, along with delivering genes [12].

Nano emulsions are one such nanotechnology approach developed to elevate targeted drug distribution along with decreased cytotoxicity and undesired side reactions [13]. Nanoemulsions are biphasic suspensions comprising two immiscible components in which one is dispersed at a micro level within another and are often made stable by certain surfactant and co-surfactant agents [14]. In most nano emulsions, the size of these droplets is submicron, making these an effective carrier for drugs at a micro level itself. Based on their formulation, these can be divided mainly into oil-in-water and water-in-oil emulsions and are often optically clear at a molecular level [15].

Nano-emulsion can be distinguished from micro-emulsion in terms of stability and method of preparation. Micro-emulsion is thermodynamically stable and can be formed through the process of self-assembly, whereas nano-emulsion is kinetically stable and requires external energy, including high-pressure homogenization and sonication, for preparation [16]. In spite of the dissimilarities, both of them show major advantages, including biocompatibility, biodegradability, physical stability, and facile scalability by known techniques. The intravenous administration of Nanoemulsions has shown advantageous effects because of their nanometer-sized particles, resulting in easy circulation and distribution [17]. Moreover, intranasal formulations involving mucoadhesive have gained enormous interest for their efficacy to enhance the transportation of drugs directly from the nasal passage towards the brain. The unparalleled association existing between the nasal route of administration and the central nervous system makes the nose-to-brain targeted intranasal delivery of nano emulsion a favorable approach, as witnessed by the increasing number of studies manifesting its therapeutic efficacy [18,19].

Rasagiline a second-generation propargylamine that is a highly specific and irreversible Monoamine oxidase B (MAO-B) inhibitor. Chemically, it is a benzylamines derivative having an indane ring, which distinguishes it from its predecessors [20,21]. Notably, it does not have an amphetamine-like chemical structure and therefore does not produce amphetamine and methamphetamine metabolites, contributing to its excellent safety profile. It is mainly metabolized by the cytochrome P450 enzyme family, which produces its main metabolite 1-(R)-aminoindan. This agent has demonstrated neuroprotective activity in animal models and is a potential therapeutic agent. It is administered orally once daily and is also marketed in 0.5 mg and 1 mg tablet formulations [22,23].

In the current study, the formulation of rasagiline using the nano emulsion delivery system is focused on the improvement of brain targeting with the goal of providing an effective method for the treatment of Parkinson's disease. For the evaluation, the in vivo method of analysis was utilized, and the results were analyzed further.

Materials and Methods

DPPH Radical Scavenging Activity

The antioxidant capacity of the Rasagiline mesylate nanoemulsion was investigated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. A 0.1 mM DPPH solution was produced in ethanol, and 1 mL of this solution was combined with 3 mL of the nano emulsion at different doses (25–800 µg/mL) in normal saline. An equal amount of saline served as the control. The mixtures were vortexed well and incubated at room temperature for 30 min in the dark. Absorbance was obtained at 517 nm using a UV–visible spectrophotometer. The % radical scavenging activity was estimated using the following formula:

Inhibition (%) = Control – Sample×100

Acute toxicity of the Rasagiline mesylate nanoemulsion was tested according to the OECD guideline 423 (Acute Toxic Class Method). This stepwise technique employs three animals of a single sex each stage, enabling toxicity categorization with a minimum number of animals [24].

Experimental Procedure

Twelve Sprague–Dawley rats (both sexes, 180–220 g) were starved overnight with unrestricted access to water. The nanoemulsion was delivered intranasally at a starting dosage of 30 mg/kg body weight, using a dosing volume of 0.1 mL/10 g body weight. Food was withheld for 3–4 h post-administration.

Animals were examined continually for behavioral and physiological changes, including variations in skin, hair, eyes, and mucous membranes, as well as respiratory, cardiovascular, autonomic, and central nervous system activity. Specific indicators such as somatomotor activity, tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma were recorded. Body weights were evaluated before and after the trial to determine systemic effects [25].

Experimental Design

Animals were divided into nine groups, each group comprising six animals. All the animals were marked as head, body, tail, head–body, head–tail, and colorless for identification. Animals were weighed, and the dose for each animal was calculated based on body weight. Animals were divided into Groups I to V. Intranasal administration was used for the nanoemulsion and test drug, while oral administration was used for the standard drug and controls [26].

Group A - normal control and received no treatment or disease induction.

Group B - Parkinson's disease–induced control group, and did not receive any treatment.

Group C - standard group and was treated with a standard anti-Parkinson drug following disease induction.

Group D - Parkinson's disease–induced animals treated with a low dose of Rasagiline Mesylate–loaded nano-emulsion.

Group E - Parkinson's disease–induced animals treated with a high dose of Rasagiline Mesylate–loaded nanoemulsion.

Behavioral activity

Akinesia Test

In mouse models of Parkinson's disease, the akinesia test—also known as the stepping test—is often used to evaluate forelimb movement deficits. This approach involves gently moving the animal forward or laterally while recording the number of first steps it takes. In Parkinsonian models where dopaminergic neuronal loss results in significant motor impairments, such as 6-OHDA- or MPTP-induced lesions, this test is especially pertinent. The number of steps is usually significantly reduced in diseased animals, which is indicative of forelimb akinesia brought on by dopamine depletion. It has been shown that stepping performance improves after treatment with well-established antiparkinsonian treatments, such as L-DOPA or cell-based therapy, suggesting a reversal of akinetic symptoms. Even in models with bilateral lesions, the test is thought to be accurate in evaluating akinesia and aids in distinguishing it from other motor deficits [27].

locomotor activity

Using open-field-based paradigms, locomotor activity was measured to analyze spontaneous motor behavior in Parkinsonian animals. To assess the degree of hypokinesia, metrics including total distance traveled, movement velocity, and time spent in various zones are often examined. In models of Parkinson's disease caused by substances like 6-OHDA or MPTP, animals usually exhibit a significant decrease in locomotor activity when left untreated. It has been seen that administering anti-Parkinson medications, especially L-DOPA, restores locomotor function, often resulting in an increase in movement speed and general activity levels. Dyskinetic behavior may sometimes be linked to excessive locomotion after therapy. In experimental Parkinson's disease, locomotor activity measurement is thus a sensitive method for assessing the severity of the condition as well as the effectiveness of treatment [28].

Catalepsy activity

Catalepsy was evaluated using a standard bar test, wherein rats were gently positioned with both forelimbs placed on a round wooden bar (9 cm in height and 0.9 cm in diameter). The duration for which the animal maintained this imposed posture was recorded as a measure of cataleptic behavior. Catalepsy was considered to have ended when the rat removed both forepaws from the bar or exhibited exploratory head movements. A maximum cut-off time of 720 seconds was applied for each observation. Assessments were performed at 5-, 60-, 120-, and 180-minutes following drug administration. All evaluations were conducted between 9:00 AM and 4:00 PM by an observer blinded to the treatment groups [29].

Biochemical activity

Estimation of Dopamine

After the animals were perfused, dopamine levels were measured from isolated brain areas such the substantia nigra or striatum. To guarantee protein precipitation and catecholamine stability, the dissected tissues were homogenized in a cooled perchloric acid solution or an appropriate buffer (10% w/v). The clear supernatant was taken for examination after the homogenates were centrifuged at a high speed (about 12,000 × g) at 4 °C. High-performance liquid chromatography combined with electrochemical detection (HPLC-ED) was used to quantify dopamine. A C18 column and an acidic mobile phase comprising acetonitrile and citrate buffer were used. For increased sensitivity, UHPLC–MS/MS may also be used in some investigations. The amount of dopamine in each milligram of tissue was represented as ng. Dopamine levels in Parkinsonian animals are usually much lower than in normal controls, although they may be partially or significantly restored after receiving antiparkinsonian medication [30].

Estimating Superoxide Dismutase (SOD) Activity

Brain tissue homogenates made from the substantia nigra or striatum were tested for superoxide dismutase activity. The supernatant was obtained by centrifuging the tissues at 10,000 × g at 4 °C after they had been homogenized in ice-cold phosphate buffer (0.1 M, pH 7.4; 10% w/v). The resultant supernatant was utilized for SOD quantification using a spectrophotometric technique based on the suppression of pyrogallol auto-oxidation using a commercially available test kit. Enzyme activity was computed using the absorbance measurement at 420 nm. SOD activity was expressed as units per milligram of protein, and protein concentration was calculated using the Bradford technique. While successful treatment measures tend to regulate enzyme levels, SOD activity is often enhanced in models of Parkinson's disease as a compensatory reaction to oxidative stress [31,32].

Estimate of Reduced Glutathione (GSH)

To evaluate the state of oxidative stress in brain tissues, reduced glutathione levels were evaluated. To precipitate proteins, the dissected brain areas were homogenized in a buffer containing trichloroacetic acid or EDTA. The supernatant was then obtained by centrifuging the mixture at a low speed. A yellow-colored complex was created when the supernatant was reacted with 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), also referred to as Ellman's reagent. Using spectrophotometry, the generated color's absorbance was determined at 412 nm. A standard curve was used to determine GSH levels, which were then represented as µg per milligram of protein. GSH levels are usually significantly reduced in neurotoxin-induced Parkinsonian animals, although endogenous glutathione levels are restored after receiving antioxidant or anti-Parkinson medication [33].

Results

Behavioral test

Evaluation of Amnesia

An amnesia model was used to assess how Rasagiline Mesylate formulations affected memory impairment as shown in Table 1 and fig 1. With a mean latency time of 62 ± 7 s, the control group had a little memory loss. In contrast, the toxic (6-OHDA) group demonstrated a considerable decline in performance (16 ± 4 s), suggesting serious cognitive impairment. Memory retention was somewhat enhanced by oral Rasagiline

Mesylate treatment (35 ± 6 s). Rasagiline Mesylate nanoemulsion-treated rats, on the other hand, showed a dose-dependent improvement in memory; the latency times for the low-dose group were 43 ± 5 s, while the high-dose group showed 58 ± 5 s. The significant improvement shown with the nanoemulsion formulation points to improved brain transport and increased neuroprotection, which will improve cognitive function preservation.

Table 1: Dose-dependent reduction in amnesia was observed with rasagiline nanoemulsion compared to oral therapy and toxic control.

Group No.	Treatment group	Mean Time (seconds)
Group-A	Control group (Saline)	0
Group-B	Toxic group (6-OHDA)	265
Group-C	PD + RM (ORAL)	163
Group-D	PD + RMNE (low dose)	112
Group-E	PD + RMNE (high dose)	142

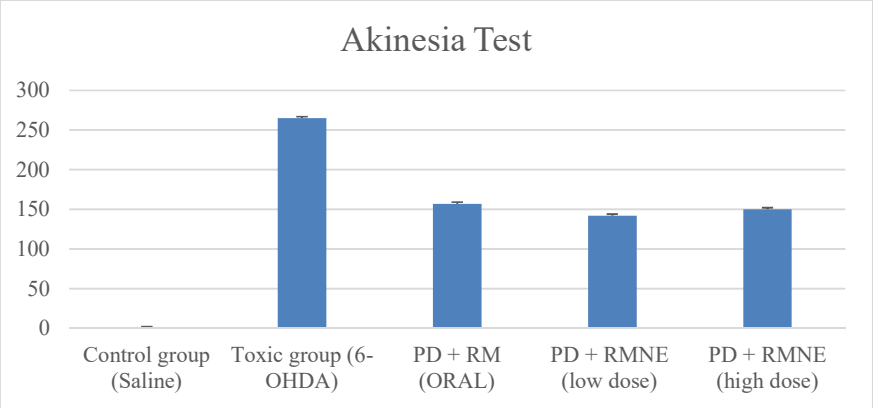

Fig 1: Rasagiline nanoemulsion significantly reduced akinesia in a dose-dependent manner, indicating improved motor function compared to the toxic and oral treatment groups.

Locomotor Activity

To ascertain the degree of motor impairment and treatment recovery in Parkinsonian mice, locomotor activity was measured as shown in Table 2 and Fig 2. The 6-OHDA-induced toxic group had a substantial increase in immobility (96 ± 7 s), indicating severe hypokinesia, whereas the control group displayed typical locomotor behavior with a mean activity time of 2 ± 0 s. Locomotor impairment was greatly decreased by oral Rasagiline Mesylate therapy (72 ± 8 s). Further improvement was seen in animals treated with Rasagiline Mesylate nanoemulsion; the low-dose group recorded 57 ± 7 s and the high-dose group 23 ± 6 s. The significant recovery of locomotor activity in the groups treated with the nanoemulsion suggests enhanced dopaminergic and motor coordination, underscoring the advantages of intranasal nanoemulsion over oral treatment.

Table 2: Locomotor activity studies in different groups

Group No.	Treatment group	Mean Time (seconds)
Group-A	Control group (Saline)	62
Group-B	Toxic group (6-OHDA)	16
Group-C	PD + RM (ORAL)	35
Group-D	PD + RMNE (low dose)	33
Group-E	PD + RMNE (high dose)	37

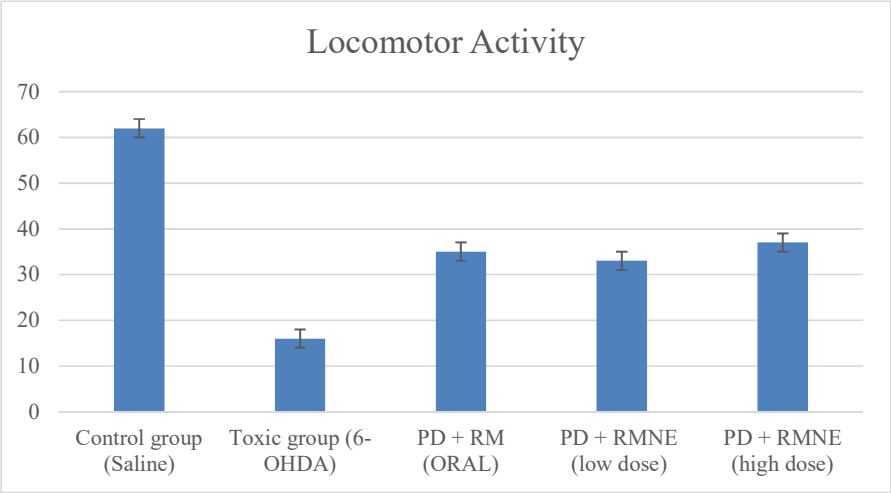

Fig 2: Different groups' locomotor activity and their further comparison.

Catalepsy Activity

The bar test was used to measure Parkinsonian stiffness in cataleptic behavior as shown in Table 3 and fig 3. The toxic group had a significant increase in catalepsy length (265 ± 10 s), demonstrating severe extrapyramidal dysfunction, whereas the control group showed no cataleptic reaction (0 ± 0 s). The catalepsy period was dramatically decreased to 163 ± 12 s with oral Rasagiline Mesylate therapy. Notably, the stiffness was reduced in a dose-dependent manner by the Rasagiline Mesylate nanoemulsion; the low-dose group displayed 102 ± 10 s and the high-dose group 66 ± 9 s. The significant decrease in catalepsy seen in animals treated with nanoemulsion points to improved delivery to the central nervous system and successful relief of Parkinsonian motor stiffness.

Table 3: Catalepsy activity studies in different groups.

Group No.	Treatment group	Mean Time (seconds)
Group-A	Control group	2
Group-B	Toxic group (6-OHDA)	96
Group-C	PD + RM (ORAL)	72
Group-D	PD + RMNE (low dose)	65
Group-E	PD + RMNE (high dose)	68

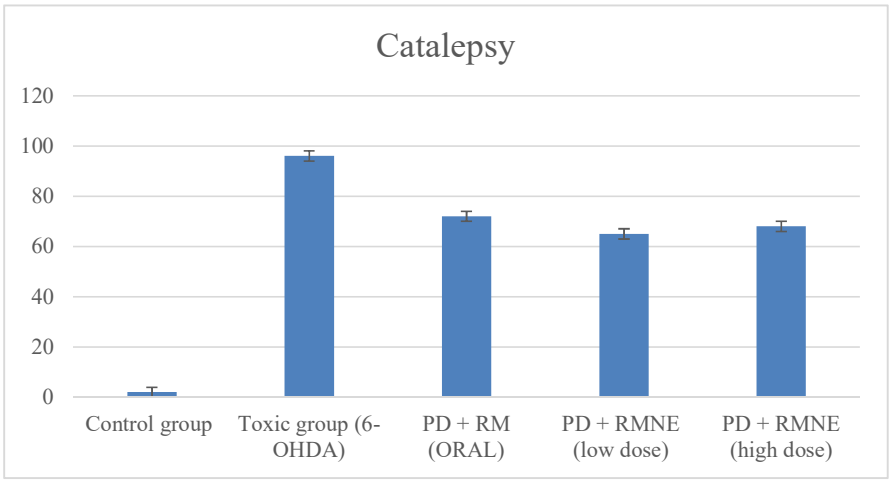

Fig 3: Different groups' Catalepsy activity and their further comparison.

Biochemical Activity

Dopamine Levels

The degree of dopaminergic neuronal injury and treatment recovery was reflected in the considerable changes in dopamine levels seen across the experimental groups in Table 4 and fig 4. The 6-OHDA-induced toxic group had a significant decrease in dopamine levels (4.91 ± 0.9), demonstrating the effective induction of Parkinsonism, whereas the control group showed normal levels (9.3 ± 1.1). Dopamine levels increased somewhat after oral Rasagiline Mesylate treatment (5.33 ± 0.7). Animals given Rasagiline Mesylate nanoemulsion, on the other hand, showed a dose-dependent restoration of dopamine, with levels in the low-dose group being 6.79 ± 1.0 and in the high-dose group being 7.61 ± 1.3. The greater recovery seen in rats treated with nanoemulsion points to the intranasal formulation's superior brain delivery and neuroprotective effectiveness.

Table 4: Dopamine levels studies in different groups

Treatment Groups	Dopamine Level Mean
Control group (Saline)	9.3
Toxic group (6-OHDA)	4.91
PD + RM (ORAL)	5.33
PD + RMNE (low dose)	4.80
PD + RMNE (high dose)	5.10

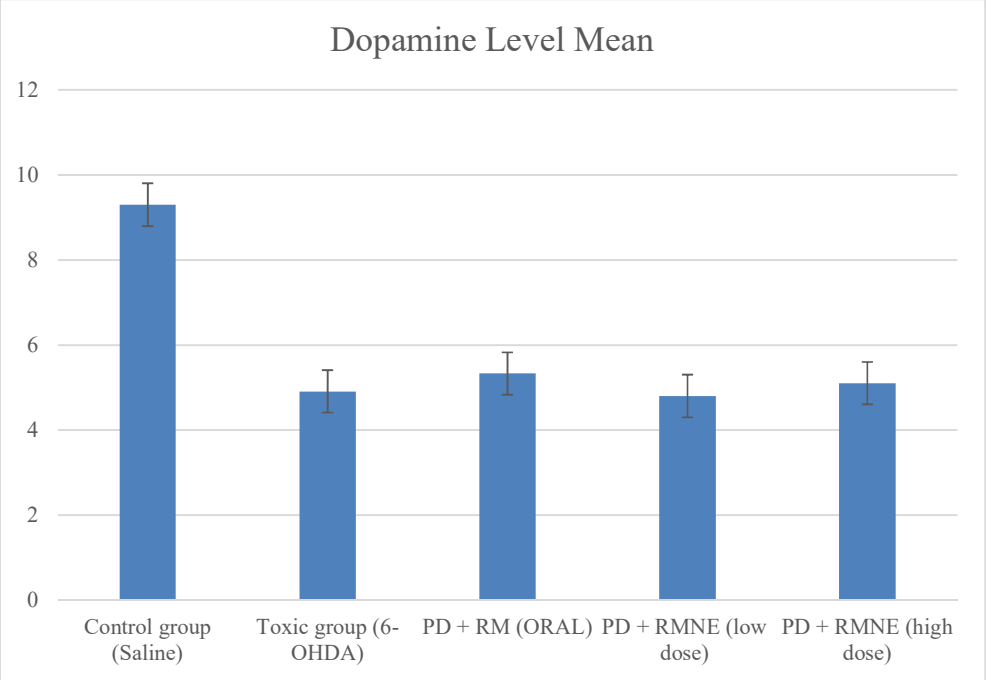

Fig 4: Dopamine levels studies in different groups and further comparison.

Superoxide Dismutase (SOD) Activity

To determine the level of oxidative stress in Parkinsonian animals, SOD activity was measured as shown in Table 5 and fig 5. The toxic group had a significant decrease in SOD levels (0.45 ± 0.22), suggesting increased oxidative stress after 6-OHDA treatment, whereas the control group had normal antioxidant enzyme activity (2.01 ± 0.24). SOD activity was partly recovered by oral Rasagiline Mesylate therapy (1.12 ± 0.20). Significantly, the Rasagiline Mesylate nanoemulsion resulted in a dose-dependent improvement; the low-dose group's SOD levels were 1.53 ± 0.21, while the high-dose group's were 1.78 ± 0.20. The formulation's ability to lessen oxidative damage linked to Parkinson's disease is shown by the animals' near-normalization of SOD activity after receiving nanoemulsion treatment.

Table 5: SOD levels studies in different groups

Treatment Groups	SOD Level Mean
Control group (Saline)	2.01
Toxic group (6-OHDA)	0.45
PD + RM (ORAL)	1.12
PD + RMNE (low dose)	0.98
PD + RMNE (high dose)	1.30

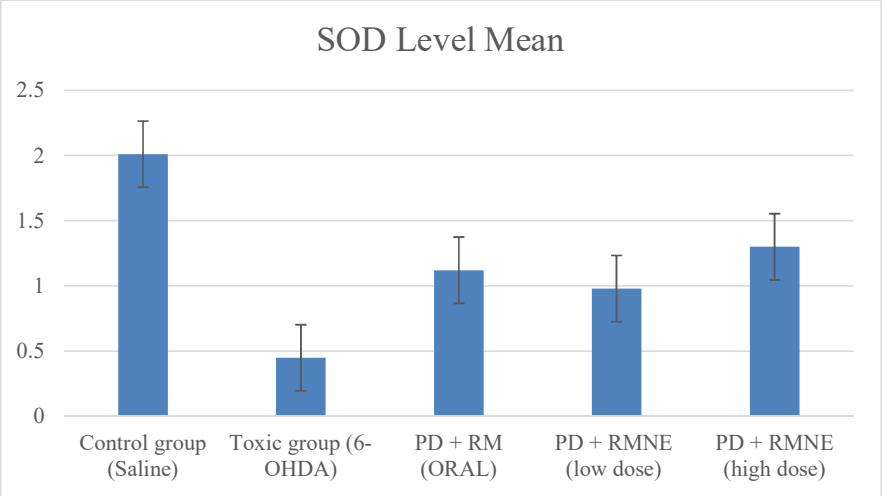

Fig 5: SOD levels studies in different groups and further comparison.

Reduced Glutathione (GSH) Level

As a measure of endogenous antioxidant defense, GSH levels were determined as shown in Table 6 and fig 6. Oxidative stress-mediated neuronal damage was confirmed by the high GSH levels (15.02 ± 0.81) in the control group and the considerable depletion in the 6-OHDA-treated toxic group (5.95 ± 1.67). GSH levels were somewhat raised by oral Rasagiline Mesylate therapy (9.1 ± 0.77). GSH levels in animals treated with Rasagiline Mesylate nanoemulsion were 11.9 ± 0.73 in the low-dose group and 12.2 ± 1.21 in the high-dose group, indicating better restoration. The dose-dependent increase in GSH indicates better neurochemical balance and efficient antioxidant protection after distribution by nanoemulsion.

Table 6: GSH levels studies in different groups

Treatment Groups	GSH Level Mean
Control group (Saline)	15.02
Toxic group (6-OHDA)	5.95
PD + RM (ORAL)	9.1
PD + RMNE (low dose)	8.9
PD + RMNE (high dose)	10.2

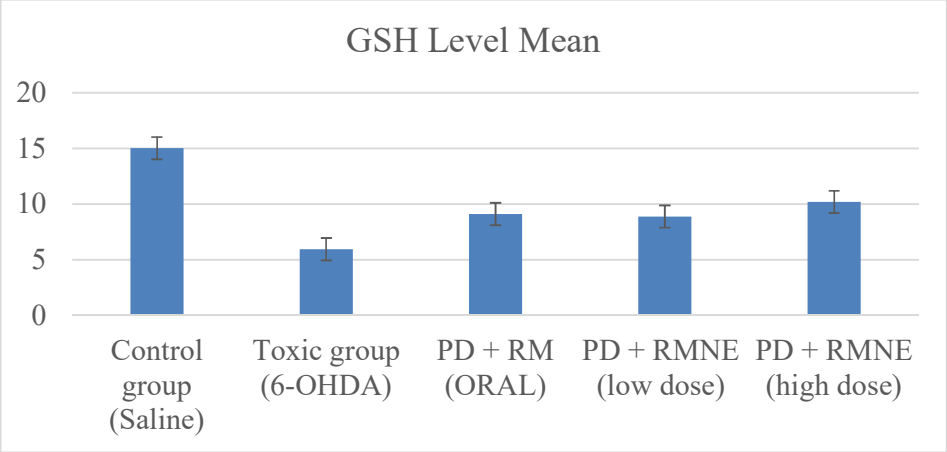

Fig 6: GSH levels studies in different groups and further comparison.

Conclusion

The current work effectively illustrates the potential of a rasagiline mesylate-loaded nanoemulsion as a nose-to-brain medication delivery system for Parkinson's disease treatment. The new formulation demonstrated good antioxidant and safety characteristics, suggesting that it might be used in vivo. The blood–brain barrier, which often limits traditional oral medicines, was overcome by intranasal injection of the nanoemulsion, which greatly enhanced medication delivery to the brain.

Behavioral assessments showed that treated rats' motor functions were significantly improved, including decreased catalepsy, increased locomotor activity, and better cognitive function. These results unequivocally demonstrate the nanoemulsion's therapeutic advantage over the traditional oral formulation. These findings were further corroborated by biochemical investigation, which revealed a significant recovery of dopamine levels and the normalization of oxidative stress indicators, including decreased glutathione and superoxide dismutase. The effectiveness and controlled delivery capabilities of the nanoemulsion technology are shown by the dose-dependent response seen in both behavioral and biochemical markers.

Overall, the research demonstrates that rasagiline mesylate intranasal nanoemulsion provides superior neuroprotection, improved brain targeting, and improved treatment results in Parkinsonian diseases. This method reduces systemic negative effects while simultaneously increasing medication absorption. As a result, it may be regarded as a viable and non-invasive method for successfully treating Parkinson's disease, meriting more clinical research and translational advancement.

Study Limitations

The current research includes a number of limitations that should be noted despite the encouraging results. First off, although being extensively used, the study's animal model 6-OHDA-induced Parkinsonism in rats, does not accurately reflect the complexity and variability of Parkinson's disease in people. Therefore, more clinical research is needed to confirm the results' translational usefulness.

Second, the short-term assessment of behavioral and biochemical indicators was the main emphasis of the research. The rasagiline nanoemulsion's long-term safety, stability, and effectiveness all crucial aspects for chronic illnesses like Parkinson's disease, were not evaluated. Furthermore, a thorough knowledge of drug absorption, brain targeting effectiveness, and residence duration after intranasal delivery was limited by the lack of thorough pharmacokinetic and biodistribution investigations.

The absence of a comparison study with other cutting-edge drug delivery methods like liposomes or nanoparticles, which may have offered a more comprehensive view of the relative benefits of nanoemulsions, is another drawback. Moreover, the lack of histological analyses of brain tissues might have reinforced the evidence for cellular neuroprotection. Last but not least, formulation scalability and long-term storage stability, both crucial for industrial and clinical translation, were not investigated. Future research addressing these issues will aid in determining this delivery system's full therapeutic potential.

References

1. Parkinson J. An Essay on the Shaking Palsy. London: Sherwood, Neely, and Jones; 1817.
2. Charcot JM. Leçons sur les maladies du système nerveux. Paris: Delahaye; 1872–1873.
3. Singh AV, Nath LK. Evaluation of compatibility of lamivudine with tablet excipients and a novel synthesized polymer. J Mater Environ Sci. 2011;2(3):243–250.
4. Tit B, Fulias A, Bandur G, Marian E, Tit D. Compatibility study between ketoprofen and pharmaceutical excipients used in solid dosage forms. J Pharm Biomed Anal. 2011; 56:221–227.
5. Picciochi R, Diogo HP, da Piedade MEM. Thermochemistry of paracetamol. J Therm Anal Calorim. 2010; 9:391–401.
6. Tita B, Fulias A, Szabadai Z, Rusu G, Bandur G, Tit D. Compatibility study between ibuprofen and excipients in their physical mixtures. J Therm Anal Calorim. 2011; 105:517–527.
7. Neto HS, Noak CS, Matos JR. Thermal analysis and compatibility studies of prednicarbate with excipients used in semisolid pharmaceutical forms. J Therm Anal Calorim. 2009; 97:367–374.

8. Wakasawa T, Sano K, Hirakura Y, Toyooka T, Kitamura S. Solid-state compatibility studies using a high-throughput and automated forced degradation system. Int J Pharm. 2008; 355:164–173.
9. Carlson E, Chandler W, Galdo I, Kudla T, Ta C. Automated integrated forced degradation and drug–excipient compatibility studies. J Assoc Lab Autom. 2005; 10:374–380.
10. Liltorp K, Larsen TG, Willumsen B, Holm R. Solid-state compatibility studies with tablet excipients using non-thermal methods. J Pharm Biomed Anal. 2011; 55:424–428.
11. Corvi Mora P, Cirri M, Mura P. Differential scanning calorimetry as a screening technique in compatibility studies of DHEA extended-release formulations. J Pharm Biomed Anal. 2006; 42:3–10.
12. Harding L, Qi S, Hill G, Reading M, Craig DQM. Development of microthermal analysis and photothermal microspectroscopy as novel approaches to drug–excipient compatibility studies. Int J Pharm. 2008; 354:149–157.
13. Ceschel GC, Badiello R, Ronchi C, Maffei P. Degradation of components in drug formulations: a comparison between HPLC and DSC methods. J Pharm Biomed Anal. 2003; 32:1067–1072.
14. Lira AM, Araujo ASS, Basílio IDJ, Santos BLL, Santana DP, Macedo RO. Compatibility studies of lapachol with pharmaceutical excipients for development of topical formulations. Thermochim Acta. 2007; 457:1–6.
15. Serajuddin ATM, Thakur AB, Ghoshal RN, Fakes MG, Ranadive SA, Morris KR, Varia SA. Selection of solid dosage form composition through drug–excipient compatibility testing. J Pharm Sci. 1998; 88:696–704.
16. Rascol O, Brooks DJ, Melamed E, Oertel W, Poewe W, Stocchi F, et al. Rasagiline as an adjunct to levodopa in patients with Parkinson's disease and motor fluctuations (LARGO study): a randomised, double-blind, parallel-group trial. Lancet. 2005; 365:947–954.
17. Weinreb O, Mandel S, Bar-Am O, Yogev-Falach M, Avramovich-Tirosh Y, Amit T, et al. Multifunctional neuroprotective derivatives of rasagiline as anti-Alzheimer's disease drugs. Neurotherapeutics. 2009; 6:163–174.
18. Youdim MB, Maruyama W, Naoi M. Neuropharmacological, neuroprotective and amyloid precursor processing properties of rasagiline. Drugs Today (Barc). 2005; 41:369–391.
19. Blandini F, Armentero MT, Fancellu R, Blaugrund E, Nappi G. Neuroprotective effect of rasagiline in a rodent model of Parkinson's disease. Exp Neurol. 2004; 187:455–459.
20. Guay DRP. Rasagiline (TVP-1012): a new selective monoamine oxidase inhibitor for Parkinson's disease. Am J Geriatr Pharmacother. 2006;4(4):331–346.
21. Malaty IA, Fernandez HH. Role of rasagiline in treating Parkinson's disease: effect on disease progression. Ther Clin Risk Manag. 2009; 5:413–419.
22. Qi L, Xu Z, Jiang X, Hu C, Zou X. Preparation and antibacterial activity of chitosan nanoparticles. Carbohydr Res. 2004; 339:2693–2700.
23. Mallik S, Kshirasagar MD, Ani VS. Studies on physical and chemical compatibility between drugs and pharmaceutical excipients. Der Pharm Lett. 2011;3(5):173–178.
24. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey R, et al. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of Ficus religiosa. Molecules. 2022; 27:1326. doi:10.3390/molecules27041326
25. Bali NR, Salve PS. Impact of rasagiline nanoparticles on brain targeting efficiency via gellan gum based transdermal patch: A nanotheranostic perspective for Parkinsonism. Int J Biol Macromol. 2020; 164:1006–1024. doi: 10.1016/j.ijbiomac.2020.06.261
26. Bonferoni MC, Rossi S, Sandri G, Ferrari F, Gavini E, Rassu G, et al. Nanoemulsions for "nose-to-brain" drug delivery. Pharmaceutics. 2019;11(2):84. doi:10.3390/pharmaceutics11020084
27. Lalvay L, Lara M, Mora A, Alarcón F, Fraga M, Pancorbo J, et al. Quantitative measurement of akinesia in Parkinson's disease. Mov Disord Clin Pract. 2016;4(3):316–322. doi:10.1002/mdc3.12410
28. Park J, Jung MS, Moon E, Lim HJ, Oh CE, Lee JH. Prediction of locomotor activity by infrared motion detector on sleep-wake state in mice. Clin Psychopharmacol Neurosci. 2021;19(2):303–312. doi:10.9758/cpn.2021.19.2.303
29. Luciani KR, Frie JA, Khokhar JY. An open-source automated bar test for measuring catalepsy in rats. eNeuro. 2020;7(3): ENEURO.0488-19.2020. doi:10.1523/ENEURO.0488-19.2020
30. Magar HS, Duraia ESM, Hassan RYA. Dopamine fast determination in pharmaceutical products using disposable printed electrodes modified with bimetal oxides carbon nanotubes nanocomposite. Sci Rep. 2025; 15:11229. doi:10.1038/s41598-025-91675-9
31. Engelbrecht I, Horn S, Giesy JP, Pieters R. Determining superoxide dismutase content and catalase activity in mammalian cell lines. MethodsX. 2023; 11:102395. doi: 10.1016/j.mex.2023.102395
32. Kunos V, Cséplő M, Seress D, Eser A, Kende Z, Uhrin A, et al. The stimulation of superoxide dismutase enzyme activity and its relation with Pyrenophora teres f. teres infection in different barley genotypes. Sustainability. 2022;14(5):2597. doi:10.3390/su14052597
33. Sachett A, Gallas-Lopes M, Conterato G, Herrmann AP, Piato A. Antioxidant activity by reduced glutathione (GSH) assay: in vitro protocol. Protocols.io. 2021. doi: 10.17504/protocols.io.btaynifw