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RESEARCH ARTICLE

In vitro and *in vivo* evaluation of levodopa-loaded nanoparticles for nose to brain delivery

Sema Arisoy[a,b], Ozgun Sayiner[a], Tansel Comoglu[a], Deniz Onal[c], Ozbeyen Atalay[c] and Bilge Pehlivanoglu[c]

[a]Faculty of Pharmacy, Department of Pharmaceutical Technology, Ankara University, Ankara, Turkey; [b]Faculty of Pharmacy, Department of Pharmaceutical Biotechnology, Inonu University, Malatya, Turkey; [c]Faculty of Medicine, Department of Physiology, Hacettepe University, Ankara, Turkey

ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disease which is characterized by the loss of dopaminergic neurons in the brain. Levodopa is the drug of choice in the treatment of PD but it exhibits low oral bioavailability (30%) and very low brain uptake due to its extensive metabolism by aromatic amino acid decarboxylase in the peripheral circulation. Moreover, levodopa has psychic, gastrointestinal, and cardiovascular side effects, and most importantly, short and frequent stimulation of dopamine receptors lead to undesirable conditions such as dyskinesia over time. The challenges are to increase the therapeutic efficiency, the bioavailability and decreasing the unfavourable side effects of levodopa. Biocompatible nanosized drug carriers could address these challenges at molecular level. For this purpose, levodopa-loaded Poly (lactide-co-glycolide) acid nanoparticles were prepared by double emulsion-solvent evaporation method for nose to brain drug delivery. Parameters such as homogenization speed, and external and internal phase content were modified to reach the highest loading efficiency. F1-1 coded formulation showed prolonged release up to 9 h. Carbodiimide method was used for surface modification studies of nanoparticles. The efficacy of the selected nanoparticle formulation has been demonstrated by *in vivo* experiments in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced PD model in mice.

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1. Introduction

Parkinson's disease (PD) is a progressive degenerative central nervous system (CNS) disease that affects the dopaminergic neurons within the nigral striatal and surrounding pathways, characterized by severe motor symptoms such as bradykinesia, rigidity, and postural imbalance (Garbayo et al. 2013). In between the three classes of drugs widely used as initial PD therapy, levodopa provides somehow better control of motor symptoms than monoamine oxidase type B inhibitors (MAOBI) and dopamine (DA) agonists. Levodopa controls motor symptoms so motor complications are seen less frequently when compared to DA agonists and MAOBI. However, nausea, hallucinations, oedema, and sleep disturbance, namely non-motor side-effects are seen more often with DA agonists than with levodopa and could be more life threatening for patients and more cumbersome for carers than are motor complications. Safety is another issue, with higher mortality reported with the MAOBI, selegiline, than with levodopa alone in the studies (PD Med Collaborative Group et al. 2014). Among the drugs used in the treatment of PD, oral levodopa, which is the metabolic precursor of DA, offers the gold standard therapy as the most effective therapeutic agent in comparison to other drugs, especially with regard to the improvement of early motor symptoms and therapeutic efficacy (Kim et al. 2009; Kura et al. 2014; Leyva-Gomez et al. 2015).

In the brain, levodopa is converted to DA by l-aromatic amino acid decarboxylase (AAAD, also known as dopa decarboxylase), primarily within the presynaptic terminals of dopaminergic neurons in the striatum. However, levodopa is metabolized to DA in the peripheral tissues as well, so that relatively low amount of unchanged drug could reach the cerebral circulation (probably less than 1%) when levodopa is administered alone. Moreover, the DA released into the circulation by peripheral conversion of levodopa produces undesirable effects (Sharma et al. 2014). Another important problem that limits the use of levodopa is the involuntary movements that occur in long-term treatments, namely dyskinesias (Sharma et al. 2014). Dyskinesia and motor fluctuations called 'on/off phenomenon' limit the efficacy of the active substance after a 3–5 year treatment period with levodopa (Hurtig 1997; Cakmur 2011; Aricioglu and Yigit 2015). Also, the low oral bioavailability of levodopa (30%), results in requirement for increased dose. Keeping the problems of levodopa delivery to the brain in mind because of poor bioavailability from the blood due to the impervious nature of blood–brain barrier (BBB) higher drug dose to achieve the desired concentration at the target tissue needs to be prescribed, which causes higher level of side effects and lower compliance to the treatment (Karanth and Murthy 2008; Gabathuler 2010; Abbott 2013; Masserini 2013; Gao 2016).

In the recent years, transport of exogenous materials directly from nose-to-brain as a potential route has been reported to bypass the BBB and provides an effective route for central administration of drugs. Intranasal administration of levodopa will prevent peripheral degradation and eliminate the disadvantages of oral administration (Brime et al. 2000; Illum 2003; Özsoy 2007; Wang et al. 2008; Mistry et al. 2009; Seju et al. 2011; Kondrasheva

CONTACT Tansel Comoglu comoglu@pharmacy.ankara.edu.tr Faculty of Pharmacy, Department of Pharmaceutical Technology, Ankara University, Ankara 06100, Turkey

et al. 2012; Sharma et al. 2014; Khan et al. 2017; Pires and Santos 2018). In addition, in contrast to conventional preparations that have a short duration of action, controlled release systems that provide continuous stimulation of receptors can be considered as an effective method for reducing levodopa-induced dyskinesias (Garbayo et al. 2013; Kura et al. 2013). In our study, in order to transport the drug from nose to brain, levodopa was formulated with Poly (lactide-co-glycolide) acid (PLGA) in nanoparticle form with wheat germ aglutunin (WGA) graftion.

Yang et al. (2012) examined the efficacy of levodopa methyl ester benserazide-loaded PLGA nanoparticles in rats in order to prevent levodopa-induced dyskinesias and reported that nanoparticle formulations are effective in reducing dyskinesia. Sharma et al. (2014) developed a thermo-reversible pluronic gel formulation for the intranasal administration of levodopa-containing chitosan nanoparticles and observed a significant increase in the amount of active substance in the brain as a result of *in vivo* studies. However, the viscosity of the gel was an obstacle to the mucosal uptake, although the residence time of the nanoparticles in the nasal mucosa increased.

The main limitation of intranasal drug application is the removal of nanoparticles before nasal drainage is sufficiently absorbed. In order to prevent this, Wen et al. (2011) prepared PEG-PLGA nanoparticles for PD treatment and modified the outer surface with lectin-derived odorrana lectin (OL) to improve nasal absorption. *In vivo* studies in rats with PD showed that the transitions to the brain and therapeutic efficacy of nanoparticles have increased with OL modification. In another study, in order to increase the nasal absorption of PEG-PLGA nanoparticles, a lectin derivative of WGA was conjugated to their surfaces, and a 2-fold increase was observed in the transition of nanoparticles to the brain (Gao et al. 2006).

Taking all the previous data into consideration, we aimed to investigate the use of WGA grafted PLGA nanoparticles as an alternative biocompatible delivery system for intranasal levodopa administration in this study. To serve this aim specifically, PLGA nanoparticles containing levodopa were prepared, the selected formulations by the analysis were morphology, dimensions, structural properties and drug distribution was tested for therapeutic efficiency to attenuate motor deficits in *in vivo* 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP) induced PD model in mice.

2. Material and methods

2.1. Material

Resomer® RG 502 H (Poly (D,L-lactide-co-glycolide) acid, acid terminated, Mw 7.000-17.000), Poly (vinyl alcohol) (PVA), D-Mannitol (≥98%), N-Hydroxysuccinimide (NHS), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDAC), HEPES buffer, Lectin from *Triticum vulgaris*, Sodium dodecyl sulfate, Lectin from *Triticum vulgaris* FITC conjugate, Glycerol and Poloxamer 188 and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) were purchased from Sigma-Aldrich. Trifluoro acetic acid (TFA) was from Merck. Levodopa was a product of Divis' Laboratories and it was a gift from ILKO (Turkey). Sodium chloride, hydrochloric acid solution (1 M) and dichloromethane were obtained from Merck. Mouse dopamine ELISA Kit was provided by Bioassay Technology Laboratory.

2.2. Methods

2.2.1. Preparation of levodopa-loaded PLGA nanoparticles

Levodopa-loaded PLGA nanoparticles were prepared with emulsion-solvent evaporation method using PVA as a stabilizer. 100 mg of PLGA and was dissolved in 2 ml dichloromethane, then 5 mg levodopa containing 1 ml % 0.1 TFA solution was added and mixed in an ultrasonic homogenizer (Bandelin SONOPULS UW 2200 Ultrasonic Homogenizer, Germany) for 1 min at 30 W; ×3 cycle. This emulsion was added to 75 ml of PVA solution at different concentrations (1−5% w/v) and mixed in the homogenizer at 9400 rpm for 5 min (IKA T18 digital ULTRA-TURRAX Disperser, Germany). All operations were carried out in ice-water bath. Resultant emulsion was stirred for 5 h at 1500 rpm on a magnetic stir plate to allow the evaporation of dichloromethane. Then, nanoparticles were recovered by centrifugation at 2000 rpm for 15 min at 4 °C (Sigma 3-30 KS CENTRIFUGE, Germany), and supernatant was centrifuged again at 26 000 rpm for 30 min at 4 °C to collect the remaining nanoparticles. The nanoparticles were washed with water in order to remove the adsorbed PVA and levodopa. Nanoparticles lyophilized at −80 °C under vacuum (Christ LYOPHILIZATOR, Germany). Then, dried nanoparticles were collected and stored at 4–8 °C (Zeini et al. 2012; Shin et al. 2014). This was used as initial formulation method, further studies have been presented in Section 3. In this study, the most suitable formulation was designed by changing the variables step by step.

2.2.2. Preparation of WGA-conjugated nanoparticles

About 500 µL 7% EDAC solution and 500 µL 0.3% NHS solution in 20 mM HEPES/NaOH pH 7.4 was added to nanosphere suspension in the same buffer and incubated end-over-end for 2/0.5 h. To remove the excessive amount of cross-linking agent, the suspensions were centrifuged (Sigma 3-30 KS CENTRIFUGE, Germany). The supernatant was discarded, and the pellet was resuspended in 20 mM HEPES/NaOH pH 7.4. After the addition of 0.1% F-WGA solution, the nanospheres were incubated end-over-end for 18/24 h. In order to saturate free coupling sites, 20% glycine solution in 20 mM HEPES/NaOH pH 7.4 was added and incubated end-over-end for 0.5 h. Finally, the particles were washed with 20 mM HEPES/NaOH pH 7.4, resuspended in isotonic HEPES/NaOH pH 7.4, and stored at 4–8 °C for further use (Weissenbock et al. 2004).

2.2.3. In vitro *drug release*

The nanoparticles which were suspended in buffer solution were put in a dialysis bag, and both ends were tied. The dialysis bag was suspended in 49.5 ml (total 50 ml) buffer solution at pH 4.5 and pH 7.4, and maintained at 37 ± 0.5 °C. At predetermined time intervals, 1 ml aliquots were sampled and replaced with 1 ml fresh pH 4.5 and pH 7.4 phosphate buffer solutions. pH 4.5 phosphate buffer solution was prepared with dissolving 6.8 g potassium dihydrogen phosphate, 2% (w/v) Tween 80, 0.244% (w/v) sodium metabisulphite in 1000 ml distilled water. Also, pH 7.4 phosphate buffer solution was prepared with dissolving 2.38 g disodium hydrogen phosphate, 0.19 g potassium dihydrogen phosphate, 8 g sodium chloride, 2% Tween 80, 0.244% (w/v) sodium metabisulphite in 1000 ml distilled water. The pH 4.5 phosphate buffer was selected to represent the pH of the endo-lysosomal compartment while the pH 7.4 phosphate buffer was selected to represent the pH of the brain since the nanoparticles are thought to pass to the brain through the endocytosis pathway. 2 % Tween 80 was added to the buffer solutions to overcome the low solubility of levodopa that would result in unrealistically low release rates *in vitro* assays relative to *in vivo*. 0.244% (w/v) sodium metabisulphite was added to protect L-Dopa from oxidation during study. Drug concentrations were quantified using high-performance liquid chromatography (HPLC) (Agilent 1100 HPLC, USA) as stated in literature (Zhou et al. 2013). Mobile phase was comprised of acetonitrile-0.05% (v/v):trifluoroacetic acid (TFA) solution (5:95, v/v) (pH 3)

(Agilent 1100 HPLC, USA). An HPLC column of 250 mm × 4.6 mm, 5 μ C18 was used for chromatographic separation with a rate 1 ml/min flow, 7-min run time and 10 μL injection volume.

2.2.4. Characterization of nanoparticles

2.2.4.1. Size distribution and zeta potential. Particle size (z-average diameter, d/nm), size distribution (PDI) and zeta potential (ZP) of nanoparticles were determined in distilled water at 25 °C by Zetasizer (Malvern ZetaSizer Nano ZS, Germany). Formulations were tested before and after lyophilization ($n = 6$).

2.2.4.2. Determination of entrapment efficiency. The amount of levodopa entrapped in the nanoparticles was determined in the supernatant. In order to determine the entrapment efficiency (EE) of levodopa, a validated HPLC assay was used with a mobile phase of acetonitrile-0.05% (v/v):trifluoroacetic acid (TFA) solution (5:95, v/v) (pH 3) (Agilent 1100 HPLC, USA). An HPLC column of 250 × 4.6 mm, 5 μ C18 was used for chromatographic separation with a rate 1 ml/min flow, 7-min run time and 10 μL injection volume. Equation (1) was used to calculate EE (Kumar et al. 2020).

$$\mathrm{EE}(\%) = \left(\frac{\text{Amount of drug in nanoparticles}}{\text{Total amount of drug added}}\right) \times 100 \qquad (1)$$

2.2.4.3. Determination of WGA binding constant. PLGA nanoparticles were incubated with 2 ml of 0.05 N NaOH/0.5% SDS at room temperature for 2 h. The amount of F-WGA was evaluated by using UV-VIS spectrophotometry at 493 nm (Thermo Scientific Evolution 201 UV/VIS spectrophotometer, USA). Equation (2) was used to calculate the binding constant.

$$\text{Binding constant}(\%) = \left(\frac{\text{Amount of binded WGA to nanoparticles}}{\text{Total amount of WGA added}}\right) \times 100 \qquad (2)$$

2.2.4.4. DSC analysis. Levodopa, polymer or nanoparticle formulations that were contained in the aluminum pan was placed in the device to examine their thermal behavior. Thermograms were taken at a temperature range of 25–400 °C at a rate of 5 °C/min (Shimadzu DSC 60, USA).

2.2.4.5. TEM analysis. A FEI brand Tecnai G2 Spirit Biotwin Model High Contrast Transmission Electron Microscope (CTEM), which operates under accelerator voltage in the range of 20–120 kV with electron gun, is used for high contrast imaging of biological and polymeric materials. The capacity of the CTEM to operate with a fast transition with an accelerating voltage range from 20 to 120 kV is an ideal feature to reduce electron beam damage in biological and polymeric specimens. The surface properties and structures of the nanoparticles thus obtained have been investigated.

2.2.4.6. FT-IR analysis. Cary 630 FT-IR with Diamond ATR (Agilent Technologies FT-IR, USA) was used to record FT-IR spectra. Spectral studies were performed in specular-reflection mode by putting thin layers of sample on a metallic IR-reflective surface. The background spectrum was collected from the metallic IR-reflective surface in the absence of the sample. A sampling spot on the surface was selected through an objective (10× optical or 15× infrared). The FT-IR spectra of the samples were obtained ranging from 4000 to 400 cm^{-1}. (Portaccio et al. 2015).

2.2.4.7. Stability of nanoparticles. Lyophilized nanoparticles (F1-1 and WGA-F1-1) in amber-colored glass bottle containers stored at the refrigerator with a temperature set at 4–8 °C based on the stability conditions of PLGA. After 6 months of stability testing, ZP, particle size and PDI values were evaluated to determine the stability of nanoparticles.

2.2.5. Cell viability in PC-12 neural-like cells

PC-12 neural-like cells have been used for cell viability analysis. RPMI 1640 media, supplemented with 10% fetal bovine serum, 100 units/mL penicillin (international unit of penicillin is the specific penicillin activity of 0.6 micrograms of sodium salt), and 100 mg/mL streptomycin was used throughout the experiment, and cells were cultured under a humidified atmosphere (5% CO_2 + 95% air) at 37 °C. The cells were seeded in culture flasks and treated with 100-50-25-12.5-6.25 μg/mL levodopa, F1-1 and WGA-F1-1 (corresponding to 100-50-25-12.5-6.25 μg/mL levodopa concentration) and levodopa-free F1-1 and WGA-F1-1 (in the same amount of polymer and WGA with F1-1 and WGA-F1-1), separately. To obtain the best results, cells were exposed for 24 h to assess the impact of our treatment on their viability against toxicity effects. Well contained cells and media were only used as positive control, and dimethyl sulfoxide (DMSO) as negative control for comparison. Experiment was performed for eight times, and results were presented as mean ± SD. After 24 h of incubation, cell viability was determined by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay (Sadigh-Eteghad et al. 2013). All experiment was done at HUNITEK.

2.2.6. In vivo studies

2.2.6.1. Experimental animal model for Parkinson's disease. The experimental protocol was approved by the Local Ethics Committee for animal experiments. The male CD57/BL6 mice ($n = 30$) were housed in groups with *ad libitum* access to standard rat chow and tap water under 12 h light-dark cycle. They were tested for motor activity (see below) after a week of adaptation in the laboratory. Then the acute PD model in mice with MPTP was employed to all of the animals. For the PD induction, four doses of MPTP (20 mg/kg/application; total dose 80 mg/kg/day; ip) injections at 2 h intervals on the same day were performed. A week after the MPTP injections, the animals were evaluated for motor performance to approve the development of PD and allocated to the following groups:

Group 1: Oral levodopa treatment group (16 mg/kg levodopa/day; po) ($n = 7$)
Group 2: Intranasal levodopa treatment group (16 mg/kg levodopa/day; intranasal) ($n = 7$)
Group 3: Levodopa-loaded nanoparticle treatment group (16 mg/kg levodopa containing nanoparticle (F1-1)/day; intranasal) ($n = 7$)
Group 4: WGA-grafted levodopa-loaded nanoparticle treatment group (16 mg/kg levodopa containing WGA-grafted nanoparticle (WGA-F1-1)/day; intranasal) ($n = 9$)

For oral and intranasal levodopa administration, levodopa was dissolved in distilled water (1 mg/1 ml w:v). The nanoparticles were suspended in distilled water to obtain the same w:v ratio. The intranasal application was performed by administering an equal volume of solution/suspension to the right and left nose openings (rhinarium) in a maximum volume of 20 μL. Oral levodopa was given via oral gavage. The treatments were continued for 7 days. After the last day of the 7-day treatment period, motor activity tests were repeated at 30, 60, and 120 min, and the

animals were sacrificed by cervical dislocation. Blood and brain tissue samples were obtained to measure DA level and stored at −80 °C until the day of analysis.

2.2.6.2. Motor tests. The groups were determined according to spontaneous and locomotor activity of the animals before and after PD model was employed and after treatment with levodopa.

2.2.6.3. Locomotor activity. Wired rod test is used to score locomotor activity. A total of four gradually narrowing rod pieces from 3.5 apparatus to 2 cm width, placed at a height of 24 cm. The test was carried out in four sessions (training, basal, after PD development and after treatment). During the training session, the mice were placed in the thickest part of the bar at the farthest to the destination. It was repeated for 2 days and 3 times per day so that the animals were habituated to walk on the apparatus. During the testing sessions, a lattice with 0.5 × 0.5 cm spaces was placed on the path. Test was repeated three times at each session for each animal and video recorded. In the last session, the mice were tested at 30th, 60th, 120th minutes after drug administration. The errors (stepping on the empty spaces of the lattice) were counted. The number of errors divided by total step number is defined as the error index and used for locomotor activity.

2.2.6.4. Spontaneous activity. A transparent glass cylinder (20 × 25 cm) was placed on glass blocks with a height of 30 cm. It is important to provide a minimum stimulating test area, simple enough not to distract the mouse. The camera was placed underneath the cylinder and spontaneous activity of the mouse was recorded for 3 min.

Two independent researchers examined the records at slow motion. The grooming and rearing times were measured and the number of hindlimb and forelimb steps were counted and used to assess spontaneous activity.

2.2.6.5. Determination of blood and tissue DA levels by ELISA. Tissue samples were diluted by 1:100 (v:v) upon considering the sensitivity range of the commercial kit. Serum samples were not diluted. The protocol was applied as described by the manufacturer and the optical density was measured within 10 min at 450 nm using spectrophotometer (Spectramax M2 Orleans Drive, USA). DA concentrations (ng/ml) were calculated from the optical density values measured using the standard curve.

2.2.6.6. Statistical analysis. The minimum animal number in each group to perform statistical evaluations was determined by G-power program, one-way ANOVA was used to test the significance of the difference between the four mean values at $\alpha = 0.05$ significance and $\beta = 0.80$ power value.

The data were analyzed by IBM SPSS 23.0 statistical program. Kolmogorov–Smirnov test was used for normality analysis of data sets. Since the data did not meet the parametric conditions, it was evaluated with non-parametric tests. The comparison of the groups was carried out with Kruskal–Wallis test and pairwise comparisons were done with Mann–Whitney *U* test. All the results were shown as mean and standard error of the mean (SEM), and $p < 0.05$ was considered significant.

3. Results and discussion

3.1. Preparation and characterization of PLGA nanoparticles

The double emulsion–solvent evaporation method was used in the preparation of drug-loaded nanoparticles of biodegradable PLGA carrier containing levodopa (Zeini et al. 2012; Zhou et al. 2013; Gambaryan et al. 2014; Cortesi et al. 2017). Preparative variables such as the concentration of stabilizer, molecular weight of polymer, and polymer/drug ratio are important factors for the formation of PLGA nanoparticle size, PDI values and EE.

3.1.1. Optimizing the outer aqueous phase (S2)

The function of PVA concentration and molecular weight is an effective factor on nanoparticle size since the presence of surfactant at the aqueous to organic phase interface governs the effectiveness of emulsion formation and stabilization of the oil nanodroplets during the emulsification-solvent evaporation process. In formulations, two different concentrations of PVA, which are 2.5 and 5%, and three different molecular weights were applied. As PVA molecular weight was increased, the size of particles plateaus and then increases due to the enhanced viscosity of the aqueous phase; the viscosity enhancement reduces the net shear stress available for droplet breakdown. As in the F1, F2 and F3, it was found that the particle size of the nanoparticles increased from about approx. 350–4000 with increasing molecular weight of PVA used (Kumar and Siril 2015). It was observed that the increased viscosity of the S2 phase prevents the mixing operation required for the particles to shrink and to form. So, EE and particle size were decreased with increasing S2 phase viscosity. In general observation, smaller nanodroplets have large surface area, therefore more surfactant may be needed to stabilize the emulsion nanodroplet. When compared F2–F6 it was supported that PVA concentration increase resulted with smaller nanoparticles while F3–F5 was demonstrated opposite due to particles plateaus was negotiated (Zweers et al. 2003; Budhian et al. 2007). Encapsulation efficiency results and the size reduction effect were lower for Poloxamer 188 than all PVA types as shown in Table 1. 5% (w/v) of Poloxamer concentration was used to prepare nanoparticles, shows that much more amount of Poloxamer was necessary to form nanoparticles to achieve high EE and smaller particle size (Feczkó et al. 2008).

3.1.2. Determining internal liquid phase (S1), internal liquid phase (S1) volume, oil (O) phase, evaporation time and used device

In the next step, F1-1 was developed from F1 by using % 0.125 levodopa containing 20 mM HCl instead of % 0.5 levodopa containing TFA solution as S1 phase. The size and PDI values of F1-2 and F1-3 were 0.455–509.5 nm; 0.708–1335 nm, respectively, showed that increasing the temperature caused increased the particle size with higher % EE. Furthermore, nanoparticles that were

Table 1. The effect of outer aqueous phases (S2).

Formulation	S2, type of surfactant; molecular weight; %	Size (nm)	PDI	ZP (mV)	EE (%)
F1	PVA; 13,000–23,000; 5%	383.7 ± 66.94	0.426 ± 0.195	−20.8 ± 3.63	50.47
F2	PVA; 31,000; 5%	939.2 ± 610.8	0.728 ± 0.289	−32.7 ± 4.34	3.44
F3	PVA; 130,000; 5%	4943 ± 4750	0.828 ± 0.375	−18.8 ± 3	0
F4	PVA; 31,000; 2.5%	397.9 ± 59.05	0.315 ± 0.062	−17.7 ± 2.41	0
F5	PVA; 130,000; 2.5%	340.3 ± 96.25	0.537 ± 0.241	−11.9 ± 0.10	0
F6	Poloxamer; 188; 5%	1154 ± 533.1	0.806 ± 0.168	6.63 ± 1.98	0

formulated with fast evaporation time by using evaporator had higher EE as shown in Table 2 (Mainardes and Evangelista 2005). The effect of volume ratio (VR) of the first emulsion to external aqueous phase was determined for each experimental formulation with a S2 phase volume in the range of 20–75 ml (F1-2, F1-4, F1-5). Adebileje et al. (2017) indicated that increasing VR caused an increase in nanoparticle size. Feczkó et al. (2011) also reported that significant interaction was recognized between the two variables, effect on particle size of VR was greatly dependent on the level of PVA concentration. In our work, when VR is reduced by keeping all other conditions constant. Particle size increased considerably by using 20 ml of PVA as S2, while any change was not observed when S2 volume was reduced from 35 to 75 ml. The particles are thought to be aggregated due to the application of total energy to less volume.

Among the organic solvents, ethyl acetate and dichloromethane are frequently used for oil phase. In our study, when ethyl acetate used for oil phase nanoparticles showed larger size and higher PDI values. Dichloromethane, acetone and ethyl acetate's boiling points are 40 °C, 56 °C, 77 °C, respectively, which means that highest evaporation rate belongs to dichloromethane, while least belong to ethyl acetate. Fast evaporation of organic solvent results fast hardening of PLGA with a smaller size. But according to McCall and Sirianni (2013), smaller particles might be produced upon using acetone instead of dichloromethane. It was observed that EE was lower when the active substance was dispersed in the inner aqueous phase, and higher EE was observed upon dissolving (F1, F1-1).

3.2. In vitro *dissolution studies*

F1, F1-1 formulations were determined as optimum formulations for EE, ZP, particle size and PDI values, and *in vitro* dissolution studies were performed with these chosen formulations. In Figures 1 and 2, the graphs of the percentage of active substance released overtime at pH 4.5 and pH 7.4 have been plotted.

Kura et al. (2014) has prepared Zn/Al nanocomposite and examined the *in vitro* release profiles of this formulation. At pH 4.8, 74% of the encapsulated drug was released in 6 h, while 76% of the drug was released at pH 7.4 over 8 h. The release of the active substance from the nanocomposite was slower at pH 7.4. It has been suggested that the positively charged Zn/Al nano compounds hardly release the negatively charged levodopa at pH 7.4 (Kura et al. 2014).

In this study, the formulations prepared with polymeric material showed a similar release profile at different pH levels. F1-1 formulation released 96% of the active substance at pH 4.5 and at pH 7.4 over 9 h. At the beginning, a burst release was observed in all nanoparticles. Subsequently, the active ingredient release continued in a controlled manner, resulting in a biphasic release. The active substance adsorbed onto the surface of the nanoparticle is thought to cause a burst effect in the dissolution medium. In addition, relatively small nanoparticles on the surface may cause such a result. In the first hours, the release of the active substance closer to the surface resulted in a rapid release, and then, the release of the active substance started from the PLGA polymer in the inner regions. By this way, controlled release of the active substance was observed (Choudhary et al. 2010; Seju et al. 2011; Barwal et al. 2013; Mohanraj et al. 2013; Sharma et al. 2014). Based on the results obtained, the formulation F1-1, which had the highest release of active substance with a sustained manner, was chosen for further studies.

3.3. Surface modification with WGA

Used formulation, amount of nanoparticles, incubation time with NHS/EDAC and incubation time with F-WGA were evaluated during the modification study in F1-1 formulation with optimum properties. The effect of these parameters on the binding

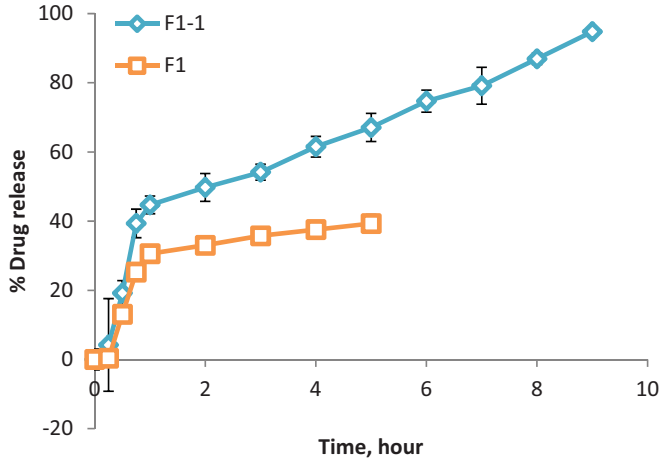

Figure 1. *In vitro* release results of F1, F1-1 formulations in pH 4.5 buffer solution.

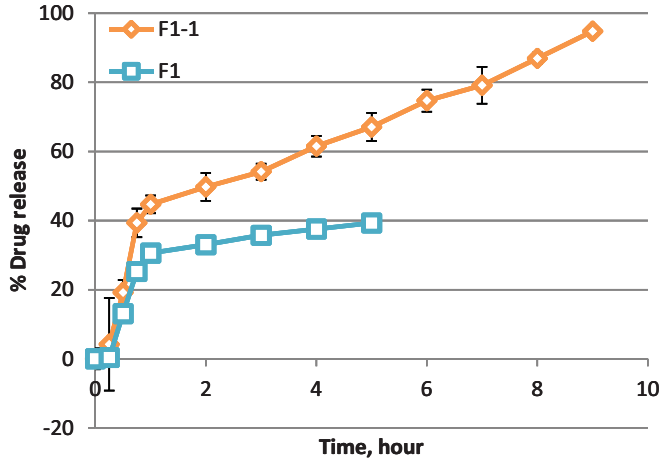

Figure 2. *In vitro* release results of F1, F1-1 formulations in pH 7.4 buffer solution.

Table 2. The effect of internal liquid phase (S1), internal liquid phase (S1) volume, Oil (O) phase, evaporation time and used device.

Formulation	S1; volume mL	O	Evaporation condition	Size (nm)	PDI	ZP (mV)	EE (%)
F1-1	20 mM HCl; 75	Dichloromethane	20 min, 40° C, evaporator	329.0 ± 188.3	0.384 ± 0.113	−4.47 ± 0.576	72.95
F1-2	20 mM HCl; 75	Dichloromethane	85 min, 1000 rpm, magnetic stirrer (25 °C)	509.5 ± 112.3	0.455 ± 0.079	−14.9 ± 6.26	19.88
F1-3	20 mM HCl; 75	Dichloromethane	85 min, 1000 rpm, magnetic stirrer (35 °C)	1335 ± 136.3	0.708 ± 0.037	−11.6 ± 2.53	25.99
F1-4	20 mM HCl; 20	Dichloromethane	85 min; 1000 rpm; magnetic stirrer (25 °C)	2006 ± 1190	0.886 ± 0.112	−19.4 ± 2.34	10.66
F1-5	20 mM HCl; 35	Dichloromethane	85 min; 1000 rpm, magnetic stirrer (25 °C)	540 ± 165.6	0.536 ± 0.148	−20.6 ± 1.85	0
F1-6	20 mM HCl; 75	Ethyl acetate	6.5 h; 1000 rpm; magnetic stirrer (25 °C)	2006 ± 1190	0.886 ± 0.112	−19.4 ± 2.34	10.66
F1-7	20 mM HCl; 75	Dichloromethane (1) / Acetone (1)	2.5 h; 1000 rpm; magnetic stirrer (25 °C)	1131 ± 383.6	0.685 ± 0.175	−10.8 ± 4.39	13.09

Table 3. The quality characteristics of nanoparticles in the result of conjugation with WGA.

Formulation	Used formulation	Amount of nanoparticles (mg)	Incubation time with NHS/EDAC	Incubation time with F-WGA (Hour)	Binding constant %	µg/mg (WGA/ Nanoparticle)	Size after modification (nm)	PDI after modification
F1-1a	F1-1	20	2	24	86.44	4.04	720.0 ± 87.39	0.672 ± 0.073
F1-1b	F1-1	20	2	18	49.78	2.49	1057 ± 875.3	0.683 ± 0.280
F1-1c	F1-1	20	0.5	24	69.41	3.47	729.3 ± 267.0	0.625 ± 0.166
F1-1d	F1-1	20	0.5	18	20.89	1.04	732 ± 124.9	0.621 ± 0.056
F1-1e	F1-1	6.5	2	24	83.11	12.79	1290 ± 703.2	0.839 ± 0.145
F1-1f	F1-1	6.5	2	18	5.33	0.82	1304 ± 623.2	0.856 ± 0.140
F1-1g	F1-1	6.5	0.5	24	68.67	10.56	1002 ± 420.1	0.752 ± 0.170
F1-1h	F1-1	6.5	0.5	18	73.11	11.25	677.6 ± 162.9	0.739 ± 0.149

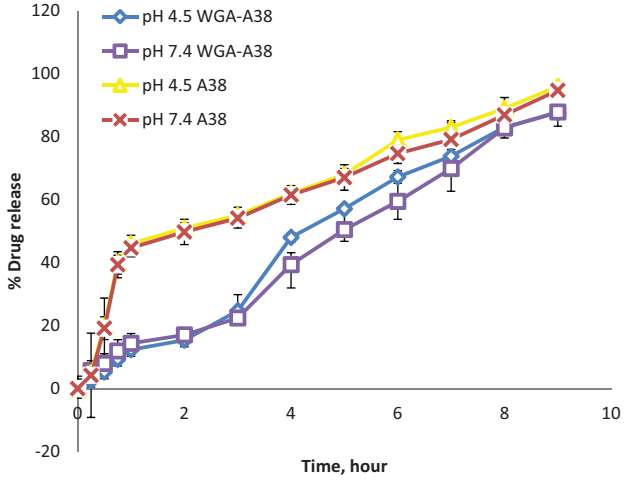

Figure 3. Levodopa release profiles from F1-1 and WGA-F1-1 at different pH levels.

constant and WGA/nanoparticle (µ/mg) ratio was investigated, and the results are listed in Table 3. It was found in the formulation F1-1a (WGA-F1-1) that 86.44% F-WGA were conjugated to the surface of the nanoparticles. As shown in Table 3, when the incubation time of WGA was increased from 18 to 24 h, independent of the incubation time with a crosslinker, the conjugation ratio of WGA to nanoparticles was increased. There was a linear relationship between the amount of nanoparticles initially added and the binding constant. When the nanoparticle amount was increased from 6.5 to 20 mg, the conjugated amount of WGA increased significantly, but the WGA/nanoparticle ratio was decreased (Table 3). In contrast to the data from literature, binding constant decreased significantly when incubation time of crosslinking agents with the nanoparticles was reduced. After binding nanoparticles with WGA, their size increased to 1304 nm. In addition, PDI values were also increased after binding process (Mo and Lim 2004; Weissenbock et al. 2004; Wang et al. 2010). Since the drug release from nanoparticles was not pH dependent, same release characteristics were observed at different pH values. It was assumed that small nanoparticles and the adsorbed drug on their surface were released during the adhesion process, and burst release disappeared as shown in Figure 3.

3.4. DSC and TEM analysis results

As shown in Figure 4, Levodopa, WGA and PLGA, which are used in the formulation, have endothermic peaks at 304.12 °C, 296.32 °C, 330.24 °C, respectively. Levodopa showed an endothermic process at 304° C which is compatible with the findings of the literature, gives us information about the crystal structure of the active substance (Luinstra et al. 2015). The used polymer melts in the temperature range of 53–59 °C, in which endothermic peaks were observed in the DSC thermogram (Maleki et al. 2017). Also, polymers peaked in the temperature range of 300–400 °C. In F1-1 formulation, the active substance peak completely disappeared and the polymer's peaks at 300 °C–400 °C partially disappeared. This indicates that the active substance in the formulation becomes amorphous or irregular crystalline, trapped in the polymer and partially adsorbed on the surface. WGA shows an endothermic peak at 296.32 °C, which is shown in WGA-F1-1, meaning that WGA has been conjugated with nanoparticles.

As shown in Figure 5(A), F1-1 has a smooth surface and round shape. All drug content was incorporated with the polymer. As shown in Figure 5(B), the drug content closer to the surface is believed to be released during the surface bonding process. Therefore, the sides near the surface are shown whiter upon comparing a. and b. As shown in Figure 5(C), the surface was decorated with WGA.

3.4. FT-IR analysis

In F1-1 formulation, NH amide vibration caused by the active substance at 3100–3500 cm^{-1} is quite light. Therefore, it is considered that there was not any active substance on the surface of F1-1. F1-1 contains peaks originating from the carbonyl group of the polymer, which is seen at 1700 cm^{-1}. This indicates that the active substance did not bind to the carboxylic acid group, and is, therefore, suitable for the surface modification of the nanoparticles (Figure 6).

The conjugation of WGA to F1-1 was confirmed by the appearance of predominant amide A peak at 3274 cm^{-1}, amide I peak at 1750 cm^{-1} which is a peak specific for WGA, amide II peak at 1451 cm^{-1}, and amide III peak at 1235 cm^{-1}, formed by covalent bonding between the acid group of levodopa and the amine group of WGA. The absence of a peak corresponding to the carboxylic group of PLGA in the spectra of WGA-F1-1 points out the successful conjugation of WGA on the surface of nanoparticles (Figure 6; Pooja et al. 2015).

3.5. Cell viability in PC-12 neural-like cells

MTT assay was conducted to evaluate the cytotoxicity of the produced nanoparticles containing levodopa on PC-12 cell line as an *in vitro* model, a widely used cell line with neuronal characteristics for PD (Kura et al. 2014). Some studies show that levodopa accelerates the process and apoptosis of the disease because it accelerates the death of dopaminergic neurons. Apoptosis is an evolutionary process that differs from ordinary cell death, and it is a pathophysiological event underlying neurological diseases (Sadigh-Eteghad et al. 2013). Therefore, the cells were exposed to different concentrations of WGA-grafted and non-grafted; levodopa-loaded and levodopa-free nanoparticles over a period of 24 h, and at the end of the exposure period, cell proliferation assay showed

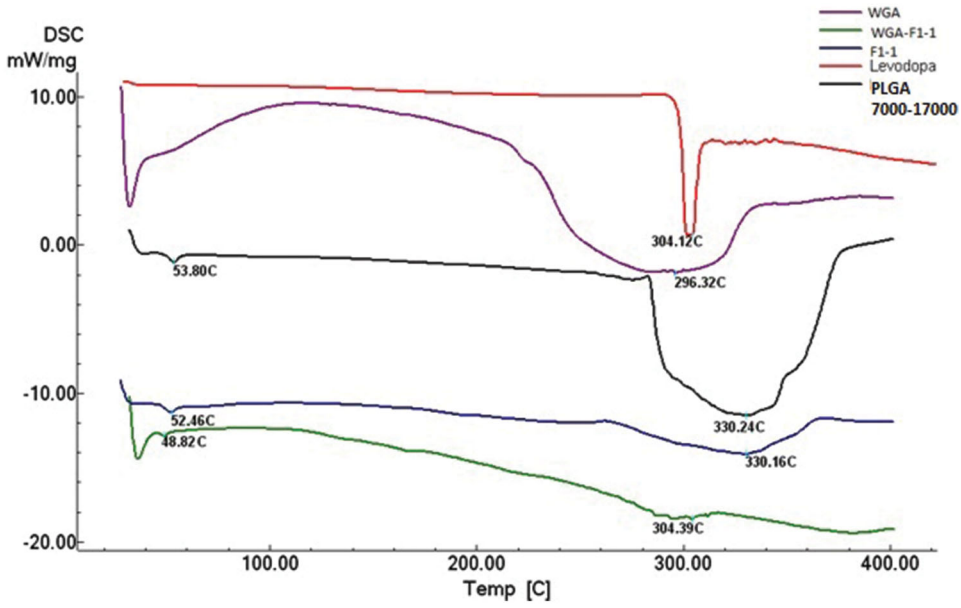

Figure 4. DSC analysis results.

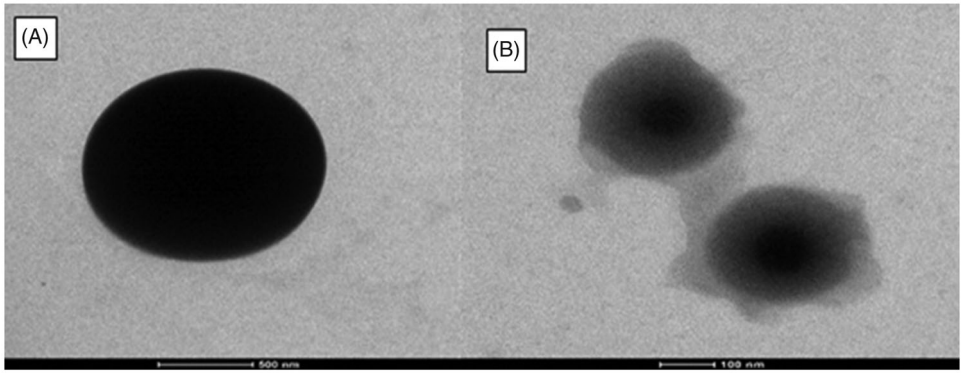

Figure 5. TEM analysis results; (A) TEM image of F1-1 nanoparticles, (B) TEM image of WGA-F1-1.

sustained cell survival even at higher doses (Figure 7). MTT assay results were calculated as the percentage of living cells in treated cultures compared with negative control cultures. No significant cell death (cytotoxicity) was observed after the exposure to all agents within the dose range of 6.25–100 μg/mL compared to control (untreated wells) (Figures 7 and 8). Upon comparing levodopa-free nanoparticle formulation (levodopa free F1-1) with levodopa-loaded formulation (F1-1), it can be stated that the nanoparticles reduced levodopa toxicity at higher doses (Sadigh-Eteghad et al. 2013).

Despite the extensive literature on polymer-based nanoparticles, reports about the toxicity of nanoparticles on the brain are very rare. Although PLGA is degradable under physiological conditions, several weeks may be needed to eliminate the nanoparticles transported into the brain, which is likely to cause damage to brain tissue. Moreover, the damage may be enhanced after WGA modification, which was reported to induce toxicity to pancreatic cancer cells *in vitro* (Liu et al. 2011).

PLGA nanoparticles were thought to be non-toxic because of the biocompatible characteristics of PLGA polymer. However, the preparation method could lead to toxicity due to the use of organic solvent. F1-1 nanoparticles showed very good cell viability, which could be explained with the fact that the preparation method did not cause toxicity.

The anticancer effect of WGA could result in toxicity on living healthy cells. Therefore, in our study, WGA-grafted and non-grafted nanoparticles were compared to determine the toxicity effect of WGA alone. WGA-grafted and non-grafted F1-1 nanoparticles showed similar cell viability. It was proved that WGA conjugated nanoparticles were a safe carrier system for brain delivery.

3.6. Stability of nanoparticles

Physical stability was determined with the key parameters like particle size, PDI and ZP for WGA-F1-1 nanoparticles after 6 months of storage at 4–8 °C. As shown in Table 4, nanoparticles maintained their quality parameters after 6 months.

3.7. In vivo tests

The body weights (BW) of the tested animals were 25.43 ± 0.91 g at the beginning of the experiment. The BW was 27.02 ± 0.56 g for oral levodopa group, 21.33 ± 0.80 g for nasal levodopa group, 22.50 ± 0.88 g for nasal F1-1 nanoparticle group, and 22.54 ± 0.76g for nasal WGA-F1-1 nanoparticle group at the last day of the protocol before being sacrificed. There was no significant difference between the experimental groups in terms of BW before and after the protocol.

The results of motor tests revealed that the employed model resulted in PD in all of the animals. Both spontaneous and locomotor activity changed significantly after MPTP injections.

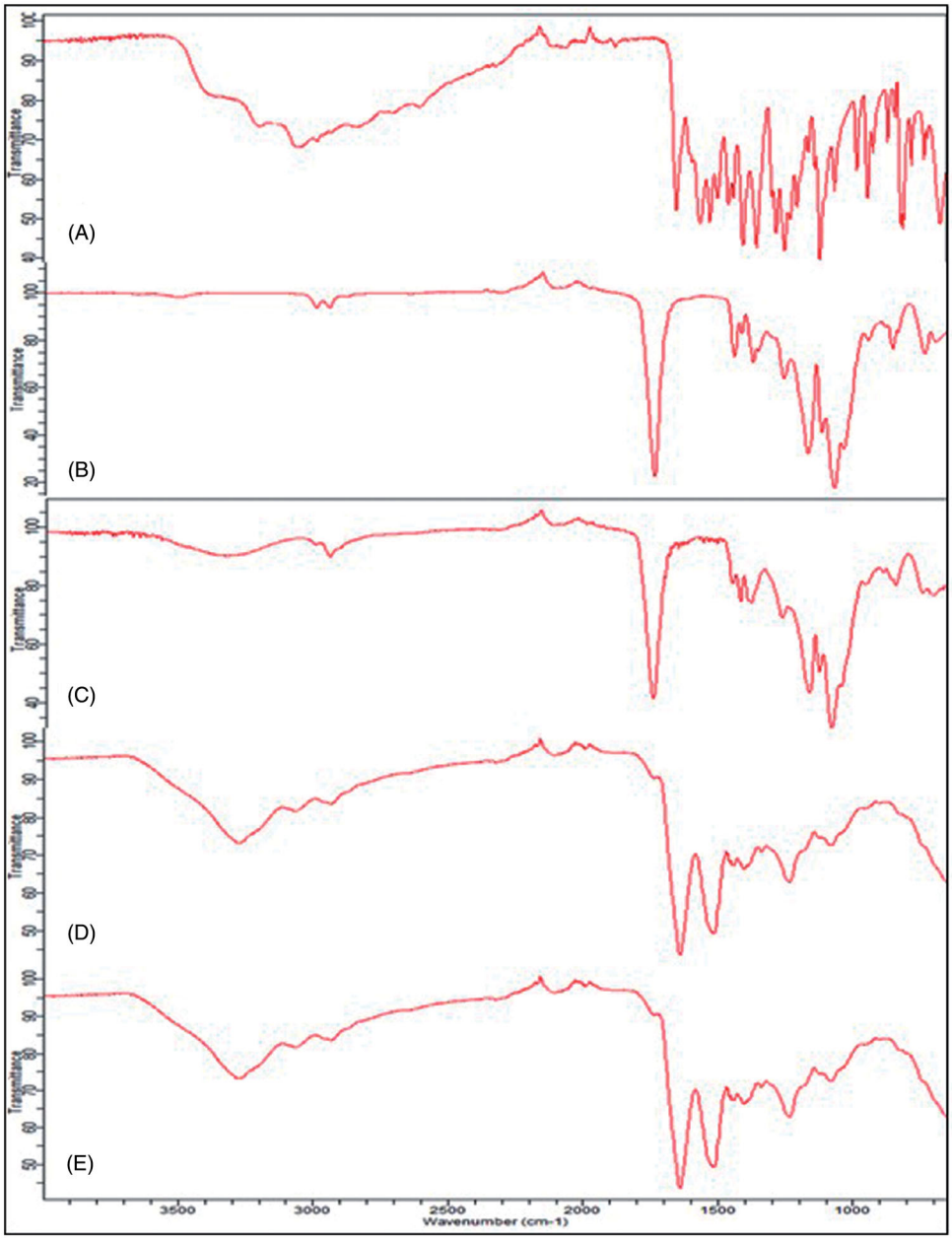

Figure 6. (A) FT-IR analysis of levodopa, (B) FT-IR analysis of PLGA (MW = 7000–17 000), (C) FT-IR analysis of F1-1, (D) FT-IR analysis of WGA, (E) FT-IR analysis of WGA-F1-1.

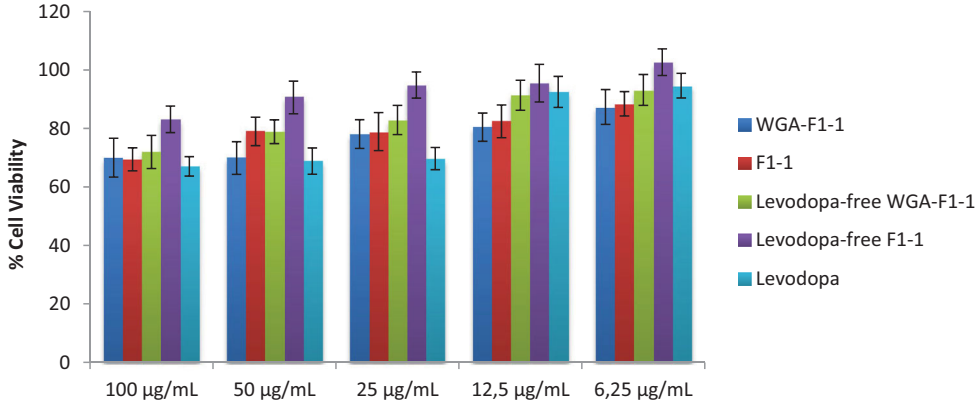

Figure 7. Dose and formulation dependent viability changes in PC12 cells at 24 h.

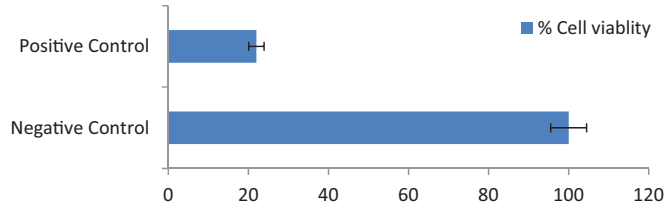

Figure 8. Negative and positive control groups viability at 24 h.

Table 4. Stability results of WGA-F1-1 nanoparticles.

Formulation (time)	Size (nm)	PDI	ZP (mV)
WGA-F1-1 (0 month)	720.0 ± 87.39	0.672 ± 0.073	−2.86 ± 0.440
WGA-F1-1 (6 months)	652.0 ± 67.7	0.463 ± 0.115	−3.15 ± 0.435

All the measures of spontaneous activity indicated decreased motor function. Hindlimb step counts were significantly different between healthy and diseased animals ($p = 0.000$) (Figure 9(A)). The ratio of forelimb steps to hindlimb steps also increased significantly after MPTP injection ($p = 0.002$) (Figure 9(B)). Moreover, rearing time decreased significantly in PD animals ($p = 0.017$) (Figure 9(D)). The grooming time was lower in PD animals, but as it did not reach statistical significance ($p = 0.294$) (Figure 9(C)), the evaluation in terms of grooming time scores was not included in further analysis.

The error index, as the measure of locomotor activity, were significantly higher after MPTP treatment (0.31 ± 0.01) compared to basal (0.12 ± 0.01) values ($p = 0.000$) (Figure 10).

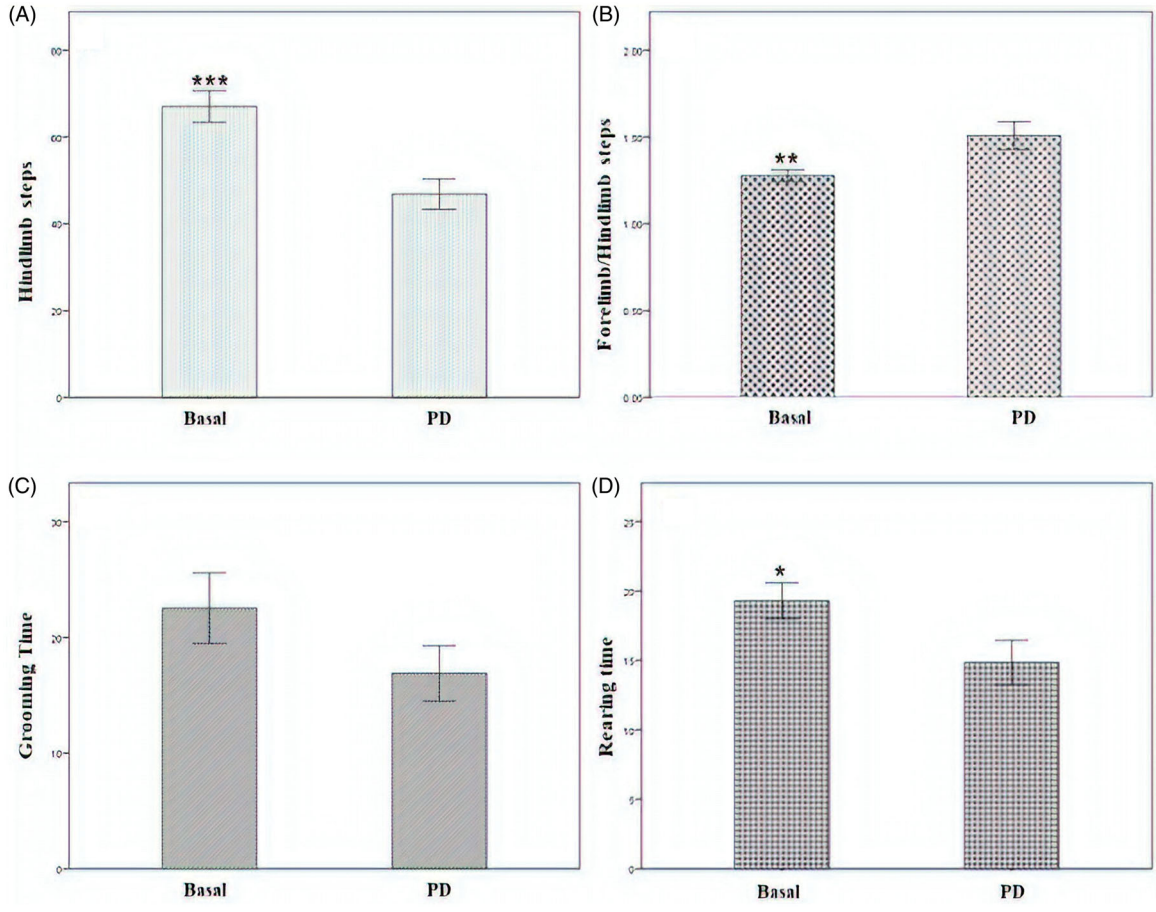

Figure 9. Spontaneous activity of the animals before (basal) and after PD development; (A) Hindlimb step number (***$p < 0.001$), (B) Ratio of forelimb/hindlimb steps (**$p < 0.01$), (C) Grooming time, (D) rearing time (*$p < 0.05$).

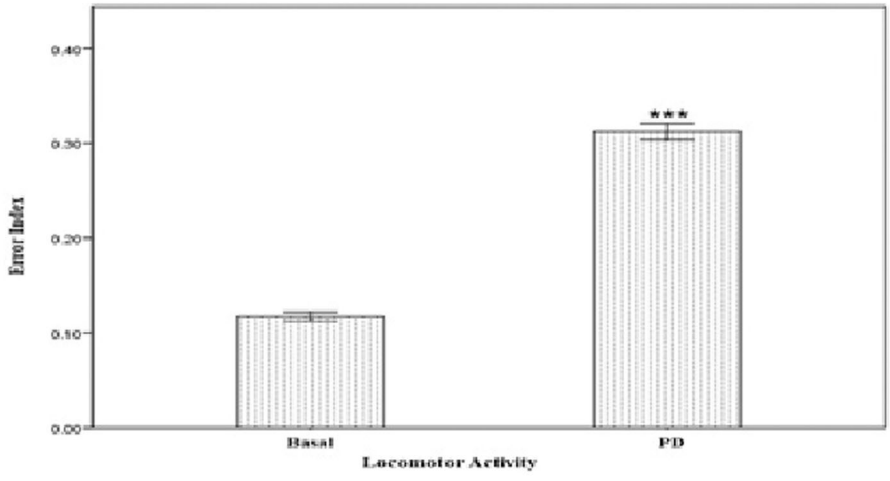

Figure 10. Locomotor activity of the animals presented by error index before (basal) and after PD development. ***$p < 0.001$.

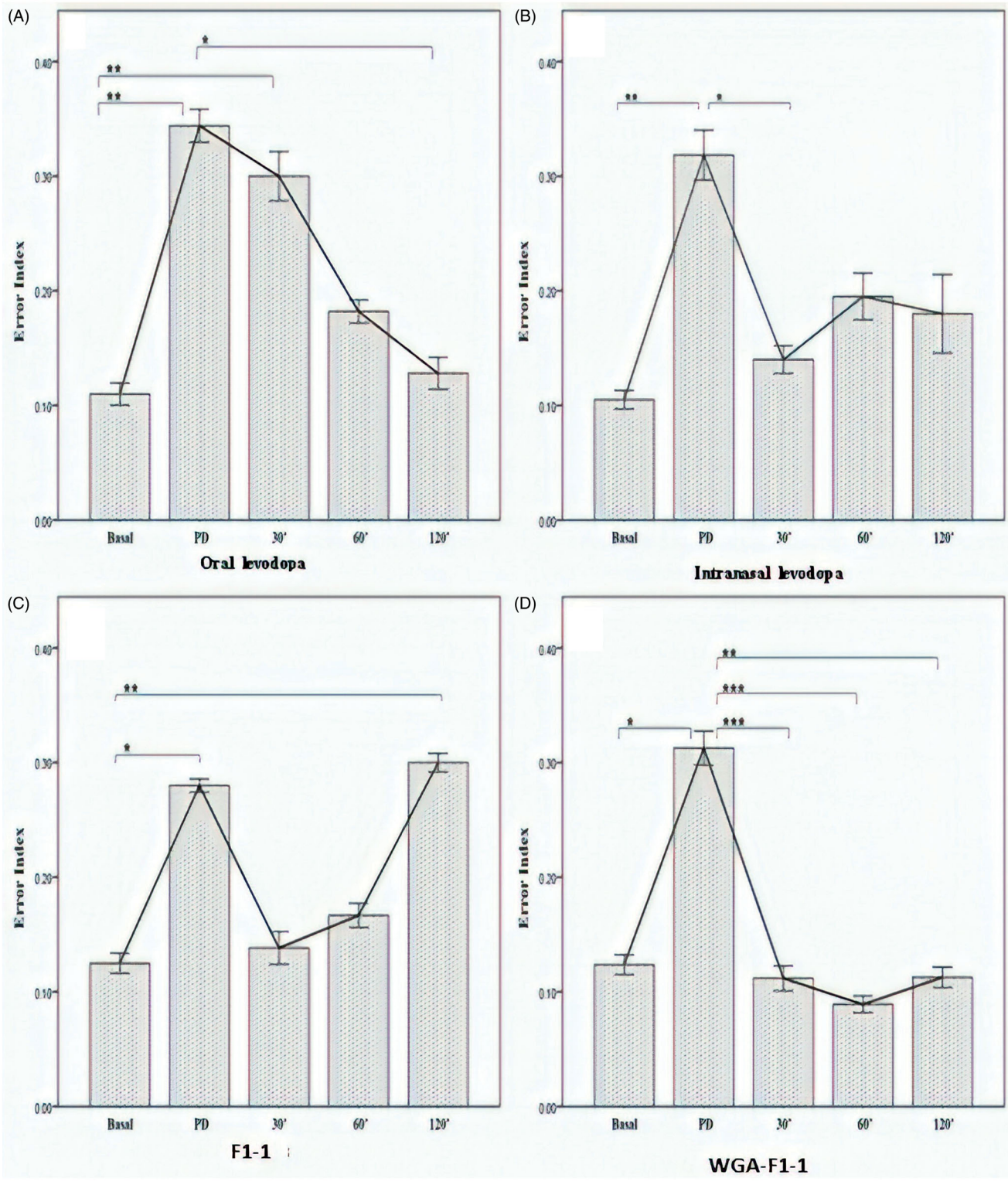

Figure 11. Locomotor activity of the animals presented by error index following corresponding form of levodopa application. (A) Oral levodopa, (B) intranasal levodopa, (C) intranasal F1-1 nanoparticles, (D) intranasal WGA-F1-1 nanoparticles (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Although primates and rodents were frequently preferred for experimental PD models, due to the ethical concerns rodent (mice or rat) models are the first choice and recently use of mice dominates the field of behavioral research (Betarbet et al. 2002). In this study, male C57/BL6 mice highly sensitive to the selected PD model have been employed (Chesselet and Richter 2011). The fact that MPTP-induced PD has a neurotoxic effect on the nigrostriatal region identical to the one observed in PD, is one of the most powerful aspects of this model (Blesa et al. 2015). The acute model rapidly causes dopaminergic neuronal loss and effectively develop a PD picture (Meredith and Rademacher 2011) as also supported by the behavioral tests in this study.

The symptomatic effect of treatment groups over time after application was presented in Figure 11(A–D). The error index

Table 5. The parameters to evaluate spontaneous activity before and after PD development and the effect of levodopa treatment at different pharmaceutical forms.

Experimental groups	Basal			After PD			Time after treatment								
							30th minute			60 th minute			120 th minute		
	H	F/H	R	H	F/H	R	H	F/H	R	H	F/H	R	H	F/H	R
Oral levodopa	88.80 ± 6.28	1.44 ± 0.07	20.80 ± 1.86	51.60 ± 7.46*	1.40 ± 0.11	18.40 ± 5.20	58.80 ± 3.83*	1.28 ± 0.05	15.80 ± 4.02	47.40 ± 8.50*	1.38 ± 0.17	13.20 ± 2.31	48.40 ± 7.37*	1.51 ± 0.16	14.40 ± 1.75
Intranasal levodopa	71.50 ± 5.48	1.22 ± 0.02	19.83 ± 2.75	50.50 ± 5.06*	1.52 ± 0.06	14.50 ± 3.49	55.83 ± 8.46*	1.18 ± 0.07	14.17 ± 1.96	51.67 ± 9.87*	1.50 ± 0.26	14.00 ± 1.88	63.33 ± 8.95**	1.22 ± 0.06	16.00 ± 2.99
F1-1	63.33 ± 4.60**	1.23 ± 0.04	17.33 ± 1.78	49.67 ± 5.46*	1.20 ± 0.08	13.33 ± 2.14	56.83 ± 5.17	1.25 ± 0.11	14.00 ± 1.57*	62.00 ± 7.66**	1.11 ± 0.08	16.00 ± 2.94	66.17 ± 7.77**	1.13 ± 0.05	20.33 ± 2.54**
WGA-F1-1	65.40 ± 6.01**	1.26 ± 0.08	21.80 ± 2.34	31.90 ± 4.95*	1.74 ± 0.18*	12.20 ± 2.10*	51.00 ± 10.38	1.95 ± 0.42*	15.45 ± 2.64*	63.18 ± 9.67**	1.63 ± 0.25	17.45 ± 2.20**	64.09 ± 6.36**	1.36 ± 0.15	19.82 ± 2.37**

F/H: Ratio of forelimb/hindlimb steps; H: Number of hindlimb steps; R: rearing time in seconds. The data is presented as mean ± SEM.
*$p < 0.05$; compared to basal value. **$p < 0.05$; compared to oral levodopa treatment at the corresponding time point.

Table 6. Serum and brain dopamine levels *$p < 0.05$ compared to oral group, **$p < 0.005$ compared to intranasal and F1-1 group.

Group	Blood (ng/ml)	Brain (ng/ml)
Oral levodopa	0.104 ± 0.007	1.868 ± 0.804
Intranasal levodopa	0.098 ± 0.004	2.131 ± 0.254*
F1-1	0.098 ± 0.017	2.469 ± 0.552*
WGA-F1-1	0.087 ± 0.004	4.177 ± 1.427*,**

(errors per step), the indicator of locomotor activity was significantly lower in healthy animals compared to the diseased animals in all groups. The effect of different pharmaceutical forms of levodopa application revealed different results; so that the effect of oral levodopa treatment became significantly prominent at the 120th minute of application (p_{PD-120}= 0.023) (Figure 11(A)). However, intranasal levodopa application relieved motor symptoms as early as 30th minute ($p_{Basal-PD}$=0.001, p_{PD-30}=0.013), indicating the rapid delivery of the drug to the desired location. But this effect disappeared rapidly and the results from 60th minute on even though still lower, were comparable to pre-treatment values (Figure 11(B)). The locomotor activity of F1-1 nanoparticle-treated group (Figure 11(C)) was improved in diseased animals through the measurements at 30th, 60th and 90th time points. The only difference compared to basal locomotor activity measured in healthy animals was at the 120th minute of treatment ($p_{Basal-120}$ = 0.001). These results show that the clinical effect of the F1-1 nanoparticle showed the most significant improvement at 30th minute (p_{PD-30} = 0.044), while gradually decreasing and losing its effect completely at 2 h.

WGA-F1-1 nanoparticles improved locomotor activity significantly at all measurement points after drug application (p_{PD-30} = 0.001, p_{PD-60} = 0.000, p_{PD-120}= 0.002) (Figure 11D) so that they become comparable with the healthy animals.

The parameters of spontaneous activity assessment are presented in Table 5. Although the attenuated spontaneous activity in PD animals also improved with levodopa treatment regardless of application form or route, the best results were obtained with WGA-F1-1 nanoparticles.

The results of spontaneous and locomotor activity revealed that the intranasal route provides a better delivery, and faster response. Moreover, the prepared formulation of levadopa-loaded WGA-F1-1 resulted in rapid action and long-lasting symptomatic improvement (Table 5).

The brain tissue and blood levels of dopamin are measured to compare the achieved concentration following treatment with different pharmaceutical formulations and passage routes (Table 6). In all the groups where levodopa is applied intranasally, the brain DA concentration was significantly higher than the blood level. The highest concentration in brain accompanied by lowest blood concentration is measured in WGA-F1-1 nanoparticles.

The efficiency of the developed formulation is tested by comparing the conventional and still in use treatment approach, i.e. oral levodopa. As expected, the significant symptomatic improvement was observed in all groups but at different time scale. When all results were evaluated together, it was observed that the effect of levodopa was alleviated by WGA-F1-1 nanoparticle formulation, and the effect of this formulation was significantly higher in mice both with regard to amount and time compared to oral or nasal levodopa administration.

Conclusion

PD is a progressive neurological disorder characterized by a large number of motor and non-motor features. Effective and well-

tolerated DA replacement agent levodopa, a DA precursor, is the first option in treatment for PD. Dyskinesia and psychiatric problems are the treatment-related complications due to intermittent delivery of DA-replacing drugs to the brain may eventually limit the clinical use of levodopa. In this study, F1-1 formulation was developed to extend the release of levodopa up to 9 h. Consequently, frequent stimulation of the receptors which causes levodopa-induced dyskinesia was prevented. F1-1 surface was modified with WGA to enhance nasal adsorption of nanoparticles. WGA-F1-1 reached high target tissue concentration with the low DA level in blood and showed high clinical efficacy. The nanoparticle formulation was also found to be well tolerated in mice and showed lower cytotoxicity compared to levodopa alone. Taken together, these results indicate that drug-loaded PLGA nanoparticles could be a potential alternative to the existing levodopa therapy in PD.

Disclosure statement

The authors declare that there is no conflict of interest regarding the publication of this article.

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References

Abbott NJ. 2013. Blood–brain barrier structure and function and the challenges for CNS drug delivery. J Inherit Metab Dis. 36(3): 437–449.

Adebileje T, Valizadeh A, Amani A. 2017. Effect of formulation parameters on the size of PLGA nanoparticles encapsulating bovine serum albumin: a response surface methodology. J Contemp Med Sci. 3(12):306–312.

Aricioglu F, Yigit G. 2015. Pharmacological treatment approach for Parkinson's diseases in today and the future. MUSBED. 5(4):1.

Barwal I, Sood A, Sharma M, Singh B, Yadav SC. 2013. Development of stevioside Pluronic-F-68 copolymer based PLA-nanoparticles as an antidiabetic nanomedicine. Colloids Surf, B. 101:510–516.

Betarbet R, Sherer TB, Greenamyre JT. 2002. Animal models of Parkinson's disease. Bioessays. 24(4):308–318.

Blesa J, Lanciego JL, Obeso JA. 2015. Parkinson's disease: cell vulnerability and disease progression. Front Neuroanat. 9:125.

Brime B, Ballesteros M, Frutos P. 2000. Preparation and *in vitro* characterization of gelatin microspheres containing Levodopa for nasal administration. J Microencapsul. 17(6):777–784.

Budhian A, Siegel SJ, Winey KI. 2007. Haloperidol-loaded PLGA nanoparticles: systematic study of particle size and drug content. Int J Pharm. 336(2):367–375.

Cakmur R. 2011. Parkinson Hastalığı ve Medikal Tedavisi. Klinik Gelişim. 23(1):53–61.

Chesselet M-F, Richter F. 2011. Modelling of Parkinson's disease in mice. Lancet Neurol. 10(12):1108–1118.

Choudhary H, Agrawal A, Malviya R, Yadav S, Jaliwala Y, Patil U. 2010. Evaluation and optimization of preparative variables for controlled-release floating microspheres of levodopa/carbidopa. Die Pharmazie. 65(3):194–198.

Cortesi R, Esposito E, Drechsler M, Pavoni G, Cacciatore I, Sguizzato M, Di Stefano A. 2017. L-dopa co-drugs in nanostructured lipid carriers: a comparative study. Mater Sci Eng C Mater Biol Appl. 72:168–176.

Feczkó T, Tóth J, Dósa G, Gyenis J. 2011. Influence of process conditions on the mean size of PLGA nanoparticles. Chem Eng Process. 50(8):846–853.

Feczkó T, Tóth J, Gyenis J. 2008. Comparison of the preparation of PLGA–BSA nano-and microparticles by PVA, poloxamer and PVP. Colloids Surf A. 319(1-3):188–195.

Gabathuler R. 2010. Approaches to transport therapeutic drugs across the blood-brain barrier to treat brain diseases. Neurobiol Dis. 37(1):48–57.

Gambaryan P, Kondrasheva I, Severin E, Guseva A, Kamensky A. 2014. Increasing the efficiency of Parkinson's disease treatment using a poly (lactic-co-glycolic acid)(PLGA) based L-DOPA delivery system. Exp Neurobiol. 23(3):246–252.

Gao H. 2016. Progress and perspectives on targeting nanoparticles for brain drug delivery. Acta Pharmaceutica Sinica B. 6(4): 268–286.

Gao X, Tao W, Lu W, Zhang Q, Zhang Y, Jiang X, Fu S. 2006. Lectin-conjugated PEG–PLA nanoparticles: preparation and brain delivery after intranasal administration. Biomaterials. 27(18):3482–3490.

Garbayo E, Ansorena E, Blanco-Prieto MJ. 2013. Drug development in Parkinson's disease: from emerging molecules to innovative drug delivery systems. Maturitas. 76(3):272–278.

Hurtig HI. 1997. Problems with current pharmacologic treatment of Parkinson's disease. Exp Neurol. 144(1):10–16.

Illum L. 2003. Nasal drug delivery—possibilities, problems and solutions. J Control Release. 87(1-3):187–198.

Karanth H, Murthy R. 2008. Nanotechnology in brain targeting. Int J Pharm Sci Nanotechnol. 1(1):9–34.

Khan AR, Liu M, Khan MW, Zhai G. 2017. Progress in brain targeting drug delivery system by nasal route. J Control Release. 268: 364–389.

Kim TK, Kang W, Chun IK, Oh SY, Lee YH, Gwak HS. 2009. Pharmacokinetic evaluation and modeling of formulated levodopa intranasal delivery systems. Eur J Pharm Sci. 38(5): 525–532.

Kondrasheva IG, Antipova TA, Barsegyan GG, Gambaryan PY, Guseva AA, Kamensky AA. 2012. Efficacy and safety of nasal administration of "Na-no-L-DOPA" based on PLGA nanoparticles. Engineering. 04(10):27–29.

Kumar R, Singh A, Sharma K, Dhasmana D, Garg N, Siril PF. 2020. Preparation, characterization and in vitro cytotoxicity of fenofibrate and nabumetone loaded solid lipid nanoparticles. Mater Sci Eng C. 106:110184.

Kumar R, Siril PF. 2015. Controlling the size and morphology of griseofulvin nanoparticles using polymeric stabilizers by evaporation-assisted solvent–antisolvent interaction method. J Nanopart Res. 17(6):256.

Kura AU, Ain NM, Hussein MZ, Fakurazi S, Hussein-Al-Ali SH. 2014. Toxicity and metabolism of layered double hydroxide intercalated with levodopa in a Parkinson's disease model. IJMS. 15(4): 5916–5927.

Kura AU, Hussein Al Ali SH, Hussein MZ, Fakurazi S, Arulselvan P. 2013. Development of a controlled-release anti-parkinsonian nanodelivery system using levodopa as the active agent. Int J Nanomed. 8:1103–1110.

Kura AU, Hussein-Al-Ali SH, Hussein MZ, Fakurazi S. 2014. Preparation of Tween 80-Zn/Al-levodopa-layered double hydroxides nanocomposite for drug delivery system. Scientif World J. 2014:1–10.

Leyva-Gomez G, Cortes H, Magana JJ, Leyva-Garcia N, Quintanar-Guerrero D, Floran B. 2015. Nanoparticle technology for treatment of Parkinson's disease: the role of surface phenomena in reaching the brain. Drug Discov Today. 20(7):824–837.

Liu Q, Shao X, Chen J, Shen Y, Feng C, Gao X, Zhao Y, Li J, Zhang Q, Jiang X. 2011. *In vivo* toxicity and immunogenicity of wheat germ agglutinin conjugated poly (ethylene glycol)-poly (lactic acid) nanoparticles for intranasal delivery to the brain. Toxicol Appl Pharmacol. 251(1):79–84.

Luinstra M, Grasmeijer F, Hagedoorn P, Moes JR, Frijlink HW, de Boer AH. 2015. A levodopa dry powder inhaler for the treatment of Parkinson's disease patients in off periods. Eur J Pharm Biopharm. 97(Pt A):22–29.

Mainardes RM, Evangelista RC. 2005. PLGA nanoparticles containing praziquantel: effect of formulation variables on size distribution. Int J Pharm. 290(1-2):137–144.

Maleki H, Dorkoosh F, Adabi M, Khosravani M, Arzani H, Kamali M. 2017. Methotrexate-loaded plga nanoparticles: preparation, characterization and their cytotoxicity effect on human glioblastoma U87MG cells. Int J Med Nano Res. 4(1):020.

Masserini M. 2013. Nanoparticles for brain drug delivery. ISRN Biochem. 2013:1–18.

McCall RL, Sirianni RW. 2013. PLGA nanoparticles formed by single- or double-emulsion with vitamin E-TPGS. J Vis Exp. (82): 51015.

Meredith GE, Rademacher DJ. 2011. MPTP mouse models of Parkinson's disease: an update. J Parkinsons Dis. 1(1):19–33.

Mistry A, Stolnik S, Illum L. 2009. Nanoparticles for direct nose-to-brain delivery of drugs. Int J Pharm. 379(1):146–157.

Mo Y, Lim LY. 2004. Mechanistic study of the uptake of wheat germ agglutinin-conjugated PLGA nanoparticles by A549 Cells. J Pharm Sci. 93(1):20–28.

Mohanraj K, Sethuraman S, Krishnan UM. 2013. Development of poly(butylene succinate) microspheres for delivery of levodopa in the treatment of Parkinson's disease. J Biomed Mater Res B Appl Biomater. 101(5):840–847.

Özsoy Y. 2007. Administration of drug via nasal route. J Faculty Pharmacy Ankara. 36(4):83–267.

PD Med Collaborative Group; Gray R, Ives N, Rick C, Patel S, Gray A, Jenkinson C, McIntosh E, Wheatley K, Williams A, Clarke CE. 2014. Long-term effectiveness of dopamine agonists and monoamine oxidase B inhibitors compared with levodopa as initial treatment for Parkinson's disease (PD MED): a large, open-label, pragmatic randomised trial. The Lancet. 384(9949):1196–1205.

Pires PC, Santos AO. 2018. Nanosystems in nose-to-brain drug delivery: a review of non-clinical brain targeting studies. J Control Release. 270:89–100.

Pooja D, Tunki T, Kulhari H, Reddy BB, Sistla R. 2015. Characterization, biorecognitive activity and stability of WGA grafted lipid nanostructures for the controlled delivery of Rifampicin. Chem Phys Lipids. 193:11–17.

Portaccio M, Menale C, Diano N, Serri C, Mita DG, Lepore M. 2015. Monitoring production process of cisplatin-loaded PLGA nanoparticles by FT-IR microspectroscopy and univariate data analysis. J Appl Polym Sci. 132(3):1–9.

Sadigh-Eteghad S, Talebi M, Farhoudi M, Mahmoudi J, Reyhani B. 2013. Effects of Levodopa loaded chitosan nanoparticles on cell viability and caspase-3 expression in PC12 neural like cells. Neurosciences. 18(3):281–283.

Seju U, Kumar A, Sawant K. 2011. Development and evaluation of olanzapine-loaded PLGA nanoparticles for nose-to-brain delivery: *in vitro* and *in vivo* studies. Acta Biomater. 7(12):4169–4176.

Sharma S, Lohan S, Murthy R. 2014. Formulation and characterization of intranasal mucoadhesive nanoparticulates and thermoreversible gel of levodopa for brain delivery. Drug Dev Ind Pharm. 40(7):869–878.

Shin M, Kim HK, Lee H. 2014. Dopamine-loaded poly (d, l-lactic-co-glycolic acid) microspheres: New strategy for encapsulating small hydrophilic drugs with high efficiency. Biotechnol Progress. 30(1):215–223.

Wang X, Chi N, Tang X. 2008. Preparation of estradiol chitosan nanoparticles for improving nasal absorption and brain targeting. Eur J Pharm Biopharm. 70(3):735–740.

Wang C, Ho PC, Lim LY. 2010. Wheat germ agglutinin-conjugated PLGA nanoparticles for enhanced intracellular delivery of paclitaxel to colon cancer cells. Int J Pharm. 400(1-2):201–210.

Weissenbock A, Wirth M, Gabor F. 2004. WGA-grafted PLGA-nanospheres: preparation and association with Caco-2 single cells. J Control Release. 99(3):383–392.

Wen Z, Yan Z, Hu K, Pang Z, Cheng X, Guo L, Zhang Q, Jiang X, Fang L, Lai R. 2011. Odorranalectin-conjugated nanoparticles: preparation, brain delivery and pharmacodynamic study on Parkinson's disease following intranasal administration. J Control Release. 151(2):131–138.

Yang X, Zheng R, Cai Y, Liao M, Yuan W, Liu Z. 2012. Controlled-release levodopa methyl ester/benserazide-loaded nanoparticles ameliorate levodopa-induced dyskinesia in rats. Int J Nanomed. 7:2077–2086.

Zeini D, Khoshkenar P, Rabiee M. 2012. The influence of formulation and process parameters on the morphology, size and release profile of L-dopa-loaded poly lactic-co-glycolic acid (PLGA) microspheres. Open Conf Proc J. 3:1–7.

Zhou YZ, Alany RG, Chuang V, Wen J. 2013. Optimization of PLGA nanoparticles formulation containing L-DOPA by applying the central composite design. Drug Dev Ind Pharm. 39(2):321–330.

Zweers ML, Grijpma DW, Engbers GH, Feijen J. 2003. The preparation of monodisperse biodegradable polyester nanoparticles with a controlled size. J Biomed Mater Res. 66(2):559–566.