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EVALUATION AND NEUROPROTECTIVE ANALYSIS OF FAST AND SLOW-RELEASE PELLETS FOR TRIPLE COMBINATION THERAPY IN PARKINSON'S DISEASE

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ABSTRACT:
Parkinson's disease (PD) is a slowly progressive neurodegenerative disorder with the loss of dopaminergic neurons in the substantia nigra, which results in motor and non-motor dysfunction. Although the classic approach to management with Levodopa continues, its usefulness is blighted by a short half-life, pulsatile receptor stimulation and motor oscillations. The use of a combination therapy of Carbidopa and Entacapone increases bioavailability but still does not provide for stable dopaminergic tone. In this study, we investigated the neuroprotective effects of a new multiparticulate capsule comprising fast-, slow- and immediate -release pellets of Levodopa, Carbidopa and Entacapone in an MPTP-lesioned rat model of PD. Multiparticulate capsules were prepared by mixing fast-releasing pellets coated with HPMC/EC, slow-releasing pellets coated with Eudragit L100 and S100 and timed releasing pellets layered with HPMC E15. Wistar rats were grouped into five categories as, normal control, standard group, toxic group, low pellet treatment dose and high pellet treatment dose. Behavioural parameters (elevated plus maze, locomotor activity), biochemical estimations (GPx, CAT, SOD, LPO) and histopathological examination were done during the first 21 days. Pellet-based therapy produced dose-dependent improvements. The high dose of D adjusted retention transfer latency to near control values, prevented the fall in locomotor scores and enzyme activities and hyper-peroxidation. Reduced inflammatory accumulation and preserved neuronal integrity in comparison with toxic control were histopathologically confirmed. The results show that pellet triple therapy provides long-term dopaminergic stimulation and neuroprotection, representing a non-invasive alternative to infusion or implant systems. This formulation has the potential to enhance compliance, decrease motor fluctuations and contribute to a more patient-centered approach of PD.

KEYWORDS: Parkinson's disease, Multiparticulate Capsules, Neuroprotection, Oxidative stress, MPTP model

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder that causes the selective degeneration of dopaminergic neurons in the substantia nigra pars compacta, with subsequent motor symptoms including tremor, rigidity, bradykinesia and postural instability [1-2]. Apart from motor features, cognitive impairment, mood alterations and autonomic failure result in loss of quality of life [3-4]. Although the pathophysiology of PD has been studied for decades, dopaminergic replacement therapy continues to be the foundation of treatment and Levodopa is still the most potent drug for symptomatic control [5-6]. Nevertheless, long-term Levodopa treatment often leads to the emergence of motor fluctuations and dyskinesia resulting from its short plasma half-life and pulsatile delivery of stimulation of dopamine receptors [7]. However, other therapies have been used in combination with Levodopa, such as Carbidopa (a peripheral decarboxylase inhibitor) and Entacapone (a catechol-O-methyltransferase [COMT] inhibitor). This triple combination enhances central bioavailability of L-dopa, increases its duration of treatment and decreases peripheral metabolism [8]. However, drugs in the class are not administered by a conventional formulation that provides for a consistent dopaminergic tone of drug, resulting in blood levels of the drug contained therein that rise and fall with half-life, which compromises patient compliance. Bestowed with these valuable features, sustained and controlled drug delivery systems are increasingly being investigated to provide the advantage of continuous dopaminergic input to patients while eliminating side effects, thereby maximizing therapeutic results [9].

Multi-particulate drug systems, especially pellet-filled capsules, provide several advantages over the single-unit dosage form. By co-formulation of fast-release, slow-release and delayed-release pellets in a capsule it is possible to obtain a customized release profile that more closely approximates physiological dopamine replacement therapy [10]. These systems can offer prompt alleviation of symptoms, prolonged therapeutic delivery and site-specific drug delivery to manage not only motor but also non-motor symptoms in PD. Furthermore, multiparticulate capsules provide dose uniformity, and decrease inter- and intra-patient variability as well as increase compliance [11].

To assess the neuroprotection effect of some recently developed multiparticulate capsule formulation containing Levodopa, Carbidopa and Entacapone in fast-release (FR), slow-release (SR) and immediate-release (IR) pellet formulations, respectively. Using an MPTP rat model of PD, the study examined behavioral performance, oxidative stress biomarkers and histological changes in brain. Through combining the pharmacological and neuroprotective evaluations, this study is designed to provide evidence on pellet-based drug delivery to serve as a potential regimen for long-term treatment of PD [12].

MATERIALS AND METHODS

Formulation of multiparticulate capsules

Multiparticulate capsules were prepared from three types of pellets for controlled drug release. Fast-release (FR) pellets were prepared with Levodopa, Carbidopa and Entacapone (20%) in combination microcrystalline cellulose (MCC-PH101), mannitol and sodium starch glycolate as excipients. These pellets were then applied with hydroxypropyl methylcellulose and ethyl cellulose in predetermined proportions to control the release pattern [13]. A higher level of drug loading (60% w/w) was used in SR pellets, with MCC as the main excipient, coated with methacrylic acid copolymers (Eudragit S100) to give pH dependent release profiles. Immed release (IR) pellets were prepared by coating the triple drug HPMC E15 as binder and designed for immediate drug delivery at the target site. The FR, SR and IR pellets were mixed together and filled into size "00" hard gelatin capsules using a weight of 111.5 mg to ensure the consistency of trials [14].

In vivo Evaluation

Experimental protocol

The present experimental study was performed in the laboratory animal house, approved by the IAEC with approval no. CPCSEA/1657/IAEC/CMRCP/COL-26/28. Adult healthy Wistar rats of either sex weighing 180-220 g were obtained from authorized animal breeding colony and acclimatized for 1 week prior to experimentation [15-16]. The animals were kept in polypropylene cages at standard laboratory conditions with a 12 h light/dark cycle, controlled temperature (22±2°C) and humidity (50–60%), and free access to standard pellet diet and water ad libitum. The animals were randomly divided into five groups with six rats in each group. Group I (normal) received normal saline (10 ml/kg, p.o.). Group II (standard) received Levodopa + Carbidopa intraperitoneally at a dose of 25 mg of Levodopa with 6.25 mg of Carbidopa in the ratio 4:1. Group III was treated with MPTP (20 mg/kg, i.p.) to induce Parkinson-like symptoms and placed as toxic control. Groups IV and V were treated with 100 mg/kg and 250 mg/kg pellets, respectively per oral as low-dose and high dose pellets formulation. Test formulations and saline were orally administered daily at one time a day, and MPTP was injected intraperitoneally by the procedure used in each animal model [17]. Treatment was administered for 21 successive days, and behavioural studies were performed on the 14th and 21st days after treatment, followed by sacrifice, brains removed for biochemical and histopathological analysis. All experimental protocols were conducted in strict accordance with the principles of laboratory animal care and experiments causing pain to the animals were carried out in such a way as to minimize suffering [18].

Behavioral Assessments

Elevated Plus Maze Test

Cognitive function and anxiety behavior in the rats were evaluated on an elevated plus maze. The equipment was four openers and two enclosed in the configuration of a plus on the floor. Each rat was positioned alone in the center of the apparatus, facing one of the open arms, and ITL (time to enter a closed arm) was then determined [19]. Retrieval transfer latency (RTL) was recorded on successive trials to test memory retention and learning performance. Reduction in transfer latency in the retention trials indicated enhanced memory function and anxiolytic effect. This testing offered a useful insight to MPTP-induced neurotoxicity and the neuroprotective possibility of test preparations [20].

Locomotor Activity

Spontaneous motor function and neurologic status were tested by locomotor activity measurement. Each rat was maintained alone in an automated activity monitoring chamber for a 10-minute period of free locomotor activity. The overall locomotor activity score was assessed at day 14 and day 21 of treatment [21]. This latter test was also valuable to evaluate the effect of MPTP on motor coordination and the extent of protection provided by pellet-based formulation. Alterations of hindlimb locomotor scores among groups mirrored the therapeutic effect of the intervention in recovering motor function [22].

Biochemical Assays

Glutathione Peroxidase (GPx) Assay

Glutathione peroxidase activity was measured in brain homogenates by assay of hydrogen peroxide as substrate. The reaction mixture consisted of phosphate buffer (0.1 M, pH 7.4), reduced glutathione (1 mM), sodium azide (1 mM) and hydrogen peroxide (0.2 mM). The substrate tissue supernatant was added to the enzymes, and the reaction proceeded at 37°C for 10 min. Remaining glutathione was then reacted with Ellman's Reagent (DTNB, 5, 5'-dithiobis-(2-nitrobenzoic acid)) and measured at 412nm. GPx activity expressed as nmol of GSSG oxidized per minute per mg protein [23].

Catalase (CAT) Assay

The activity of the catalase was determined as rate of decomposition of H2O2. The reaction mixture was made up of phosphate buffer (50 mM, pH 7.0) and hydrogen peroxide (10 mM). The tissue homogenate was added to start the reaction, and OD at 240 nm for 3 min was measured by a UV–Visible spectrophotometer. Enzyme activities were expressed as μmol H_2O_2 reduction per minute per mg protein [24].

Superoxide Dismutase (SOD) Assay
Superoxide dismutase activity was assayed by its capacity to inhibit the auto-oxidation of pyrogallol. The assay mixture was 50 mM Tris-HCl buffer (pH 8.2) containing 1 mM EDTA. Pyrogallol (0.2 mM) was added to start the reaction and the rate of auto-oxidation was recorded at 420 nm. The inhibition in the rate of auto-oxidation of pyrogallol by tissue homogenate was used for SOD activity with one unit being defined as that amount of enzyme required to inhibit the rate of pyrogallol auto-oxidation by 50 % [25].

Lipid Hydroperoxide (LPO) Assay
MDA, a stable product of LPO, was analyzed to assess lipid peroxidation according to the thiobarbituric acid reactive substances test (TBARS). Brains were homogenized in phosphate buffer (0.1 M, pH 7.4). 1 ml of 10% TCA and 1 ml of 0.67% TBA was added to 0.5 mL of homogenate. The mixture was boiled at 95°C in a water bath for 15 min, cooled and centrifuged at 3,000 g for l0min. The supernatant was measured for absorbance at 532 nm. The concentration of MDA was determined with an absorptivity coefficient of 1.56×10^5 M^{-1} cm^{-1} and expressed as nmol/mg protein, indicating lipid hydroperoxides in the tissue [26-27].

Tissue Collection
At the end of the experimental period, animals were sacrificed by anesthetic overdose (ketamine: 80 mg/kg, i.p.; xylazine: 10 mg/kg, i.p.) to guarantee deep anesthesia. At the end of behavioural testing, rats were anaesthetized and killed by cervical dislocation and their brains were rapidly removed. The tissues were rinsed with ice-cold saline to remove blood clots and divided into two parts. One was homogenize in phosphate buffer (0.1 M, pH 7.4) for biochemical assay and the other one was fixed in 10% neutral buffered formalin for histopathological study. All tissues were processed by sterile techniques to avoid contamination and frozen at –80°C for subsequent biochemical assay [28].

Histopathological Analysis
Tissues were fixed in formalin and embedded in paraffin wax for histopathological assessment. 5 µm thick sections were cut with a rotary microtome and mounted on glass slides. The sections were stained with Hematoxylin and Eosin (H&E) for seeing general histoarchitecture. The sections were examined under a light microscope at various magnifications to evaluate neuronal integrity, inflammatory infiltrate, necrosis, vacuolization and edema. The observations were compared among groups: the control group displayed good architecture, while the disease control showed severe neuronal degeneration and leukocyte invasion, and other treated groups had their range of neuroprotection in different extents. Photomicrographs were taken for documentation and comparative study [29].

Statistics
Data was shown as mean ± SEM. Statistical analysis One-way analysis of variance (ANOVA) was followed by Tukey's multiple comparison post hoc test to evaluate the statistical significance between groups. Comparison was done between toxic control and normal control, as well as among treatment groups and the toxic control group. An association with a *p-value of < 0.05 was considered as significant. GraphPad Prism (v. 5.0) was used for statistical analysis and the generation of graphical data. There were six animals per group for the heart homogenate which produced sufficient power to observe any significant differences in behavioral, biochemical, and histopathological parameters.

RESULTS
Formulation of multiparticulate capsules
Formulation trials were performed designed to develop a multicomponent (Levodopa, Carbidopa and Entacapone) unidirectional release profiles system in a rational way based on stress controlled though immediate and delayed approach. The coated of pellets using controlled release coating with HPMC and Eudragit in different proportions were carried out in our previous experiments [30]. Slow-Release Pellets (60 % w/w label claim) were prepared with MCC as the major excipient for drug blend mix. The pellets were coated with methacrylic acid copolymers (Eudragit L100 and S100) in different combinations to generate pH dependent release characteristics. Immediate release drug pellets were also prepared by coating the triple combination of drugs onto the delayed release beads using HPMC E15 as a binder for rapid disintegration of solid fraction. The FR and IR pellets were blended with SR-pellets at the end to obtain the mixture of all pellets (FR, SR and IR) which was then filled into size "00" hard gelatin capsules (avg wt per capsule-115 mg; overall fill weight = 638.750 mg) as multiparticulate capsules. The average capsule weigh was found to be 753.750 mg in all trials indicating uniformity of formulation design.

Elevated plus maze test
The results of the elevated plus maze test demonstrated significant differences in cognitive performance among the experimental groups. Normal control rat had a baseline initial transfer latency (ITL) of 59.23 ± 1.05 seconds and retention transfer latencies (RTL) of 20.2 ± 0.90 and 21.30 ± 0.60 seconds demonstrating intact learning and memory. Comparison of ITL (63.1 ± 1.04 seconds) and RTL (18.3 ± 0.62 and 17.5 ± 0.67 seconds, *$p < 0.05$) demonstrated similar ITL values but significantly decreased RTL values compared to controls, associated with cognitive retention within the standard drug group (Levodopa + Carbidopa). However, toxic control rats treated with MPTP presented vastly reduced RTL values (81.10 ± 1.40 and 80.3 ± 1.46 seconds), corresponding to a complete memory and learning impairment caused by the dopaminergic neurotoxicity (Table 1). Animals which received treatments with the pellet

formulations exhibited dose-dependent increases. The RTL values of the group with the high dose (250 mg/kg) were 30.1 ± 0.98 and 33.1 ± 1.02 seconds, respectively (*p < 0.05) and equal to that of the group with the standard drug. These findings indicate that the pellet-based combination therapy provided significant neuroprotection against MPTP-induced cognitive deficits, with higher doses showing greater efficacy in improving memory retention and learning ability.

Table 1 Assessment of Cognitive performance in MPTP treated rats

Groups	Treatment	Mean transfer latency (sec)		
		ITL	1st RTL	2nd RTL
Group I	Normal control (10ml/kg. N. Saline)	59.23±1.05	20.2±0.90	21.30±0.60
Group II	Standard group (25 mg Levodopa with 6.25 mg of Carbidopa)	63.1±1.04	18.3±0.62*	17.5±0.67*
Group III	Toxic group (20mg/kg of MPTP)	67.12±1.21	81.10±1.40	80.3±1.46
Group IV	Low dose treatment (100mg/kg pellets)	64.16±0.52	46.23±1.02*	43.2±1.04*
Group V	High dose treatment group (250mg/kg pellets)	63.9±1.02	30.1±0.98*	33.1±1.02*

*Values are expressed as mean±SEM (n=6), *p<0.05 compared to control treated group*

Locomotor activity

Significant differences in locomotor activity scores during the 10-min time intervals were observed between the experimental groups. The normal control animals remained constant in the activity levels with scores of 220.00 ± 11.35 on the 14th day and 219.00 ± 10.11 at *p<0.05 on the third week, an indicative of unaltered motor performance (normal). In comparison, the MPTP-treated Parkinsonian toxic control group had lower activity (205.1 ± 10.45 and 212.6 ± 11.20) demonstrating their motor impairment because of dopaminergic neurotoxicity. Dose-dependent recovery was achieved by the pellet forms during healing (Table 2). A moderate recovery was noted after treatment with low dose (100 mg/kg) group on day 14 and 21 at the locomotor scores as of 210.1 ± 9.26 and 218.4 ±10.6 (*p<0.05), which indicates a partial motor function recovery. The effect was even better in the high-dose (250 mg/kg) group with a score of 234.1 ± 10.1 and 240.1 ±11.3 on day 14th and day 21st respectively, which were like that of standard drug treated group. These results suggest that the pellet-based therapy reduced motor deficits following MPTP lesion, and the high dose therapy was more effective on locomotor activity recovery.

Table 2 Locomotor activity of different treatment groups

Groups	Treatment	Locomotor activity (score) in 10min±SEM	
		14th day	21st day
Group I	Normal Control	220±11.35	219.00±10.11
Group II	Standard group	238.1±11.34*	242.40±10.45*
Group III	Toxic group	205.1±10.45	212.6±11.20
Group IV	Low dose treatment group	210.1±9.26	218.4±10.6*
Grozup V	High dose treatment group	234.1±10.1*	240.1±11.3*

Biochemical characterization of rat brain homogenate

The biochemical profile in rat brain homogenates exhibited marked differences according to group. In the normal control group, GPx activity was 21.1 ± 1.09 nmol/min/mg protein, CAT was 47.55 ± 1.04 µM/mg protein, SOD was 33.43 ± 1.13 units/mg protein and LPO remained at a lower level of 3.01 nmol/mg-protein representing normal oxidative equilibrium in tissues. The normal group standard drug treatment (Levodopa + Carbidopa) showed a little enhancement in antioxidant defense with GPx 22.16±1.23, CAT 52.45 ±1.24, SOD 32.01±1.01 and low LPO level of 2.34±0.20 and were protecting against the oxidative stress. In contrast, toxic control (MPTP-treated) group exhibited dramatic decrease in the activities of antioxidant enzymes to 11.16 ± 0.83 for GPx, 30.34 ± 0.67 for CAT and 9.52 ± 0.54 SOD and significant increase was recorded in the level of LPO (4.34 ± 0.15), which resulted in profound oxidation damage (Table 3). Dose-dependent responses were obtained when the animals were treated with pellet formulations. GPx, CAT and SOD were partially restored to 21.33 ± 1.43, 43.43 ± 1.65 and 20.14 ± 1.34 respectively in low-dose group (100 mg/kg), with decrease in LPO levels to 2.45±0.15 indicating partial restoration of antioxidant status of the kidney. Efficacy was even more notable at the high dose group (250 mg/kg), with GPx 20.01 ± 1.34, CAT 46.19 ± 1.04, SOD 22.02 ±0.27 and LPO 2.21±0.20 were nearly restored back to normal control levels. Altogether, the results

proved that pellet-based combination therapy efficiently counter MPTP-induced oxidative stress and high dose treatment possessed stronger neuroprotective capability via restoration of antioxidant enzyme activities and inhibition of lipid peroxidation.

Table 3 Biochemical estimation in homogenate of brain tissues

Groups	Glutathione Peroxidase GPx(nmol/min/mg of protein)	Catalase CAT(µM/mg of protein)	Superoxide dismutase (units/mg of protein)	Lipid hydroperoxide LPO (nmol/mg of protein)
Group I	21.1±1.09	47.55±1.04	33.43±1.13	3.01±0.10
Group II	22.16±1.23*	52.45±1.24*	32.01±1.01*	2.34±0.20*
Group III	11.16±0.83	30.34±0.67	9.52±0.54	4.34±0.15
Group IV	21.33±1.43*	43.43±1.65	20.14±1.34*	2.45±0.15
Group V	20.01±1.34*	46.19±1.04*	22.02±0.27*	2.21±0.20*

Data are expressed as mean ± SEM (n=6), *p<0.05: significantly different from control group.

Histopathological images

Figure 1 showing histopathological image of treated animals.

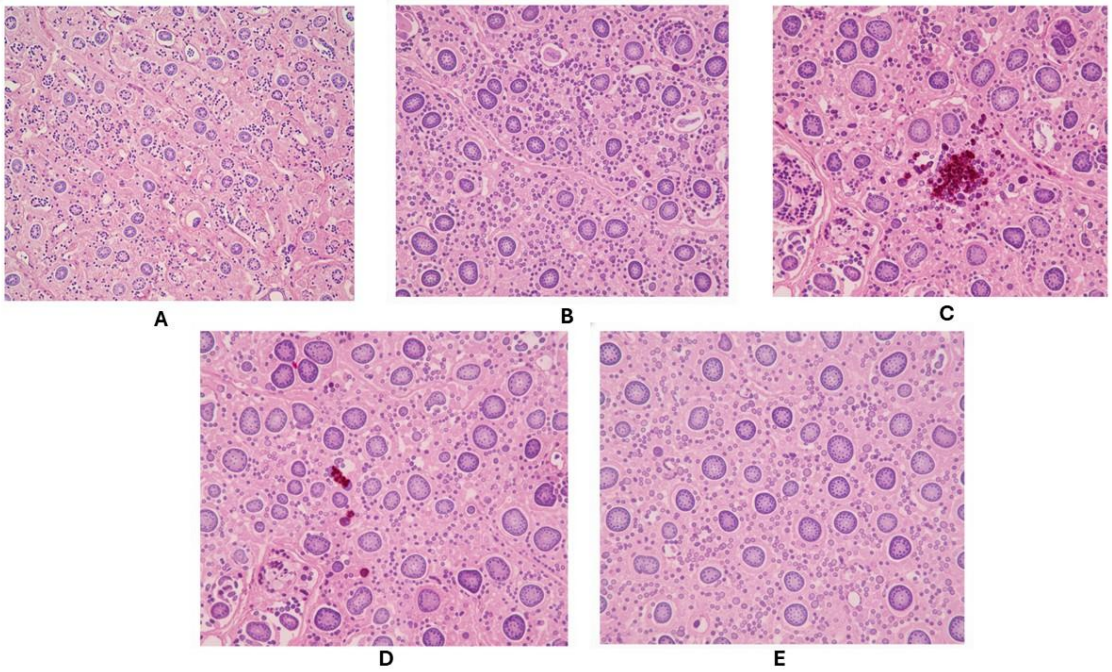

Figure 1 Histopathological slides of brain tissues given formulated pellets
A: Normal control, B: Standard group C. Toxic groups, D Low dose treatment E: High dose treatment

DISCUSSION

This study shows that PPCI with Levodopa, Carbidopa and Entacapone delivers pronounced neuroprotective action in an MPTP rat model of PD. Results showed that cognitive task, locomotor activity, antioxidant enzyme recovery and histopathological preservation got better. In this regard the current data, which exhibit simultaneous locomotion and cognitive benefits, is consistent because pellet therapy was shown to improve beyond motor control [31]. also underlined that although conventional therapy with Levodopa is successful, it suffers from its short half-life and pulsatile receptor activation. The pellet technology developed here potentially circumvents this problem by using fast-release, slow-release and delayed-release pellets to resemble physiological dopamine restoration [32].

The comparison with IV administered therapies is highly informative in this context. Santos-García et al. found that LECIG infusion allowed continuous intrajejunal drug delivery, which in turn resulted in more stable plasma concentrations and a better control of motor fluctuations in patients with advanced PD. This is consistent with our results, where the high dose pellet treated group brought levels of both locomotor scores and antioxidant enzyme activity to those similar to the standard drug [33]. Infusion-based systems are labour-intensive and require a dedicated environment, whereas the capsule is a minimally invasive disposable option which can easily be taken orally, making this more suited to low-resource settings. This difference highlights the clinical benefit of pellets with respect to infusion treatments such as those reported by [34].

Oxidative stress is a major mechanism in the pathogeneses of Parkinson's disease and biochemical assays indicated that pellet therapy normalized glutathione peroxidase, catalase and superoxide dismutase activities while decreasing lipid peroxidation [35]. observed that methoxsalen alleviate neurotoxicity via antioxidant and anti-inflammatory mechanisms, whereas [36]. emphasized the therapeutic value of furocoumarins on oxidative stress and inflammation. The current findings echo these accounts, indicating that pellet therapy does not merely offer dopaminergic replacement but also has potential to restore oxidative balance. This dual action separates it from antioxidant-only treatments that

are found to be devoid of any dopaminergic property, and emphasizes the need to juxtapose symptomatic improvement with neuroprotective approaches [37].

Behavioral evaluations in this study such as the elevated plus maze and locomotor activity, exhibited an improvement of cognitive performance and motor function with a dose-dependent [38]. highlighted that behavioral tests are well-suited to evaluate the effects of dopamine neurotoxins pointing out that these tests offer a trusted measure of the potency of neuroprotective drugs. The findings of the current study, which demonstrated that high-dose pellet treated animals were similar in level of function to the standard Levodopa–Carbidopa group, are similar to those described by Tillerson and co-workers [39]. also supported the construct validity of the 6-hydroxydopamine model as a means for testing neuroprotective strategies and our results in MPTP-treated rats are consistent with their assertion that behavioral endpoints are important for preclinical models.

Histopathological examination demonstrated that pellet-treated groups had an intact neuronal structure and less inflammatory infiltration compared to the toxic control group in this study [7]. demonstrated early one to one relationships with dopamine depletion and neuronal loss, while subsequent work has showed that continuous delivery systems ameliorate histological damage. The retention of neuronal architecture described in this study provides clear evidence that the structure neuroprotection occurs with multiparticulate therapy, as well as its functional recovery[40]. reviewed the contribution of glutamate-induced neurotoxicity to neurodegeneration, emphasizing interventions aiming at preventing excitotoxic damage. The therapy of choice which involve the implantation of pellets, reestablishing redox status and decreasing lipid peroxidation indirectly modulates excitotoxic mechanisms in addition to being in agreement with the histopathological presentations [41].

The merits of multiparticulate capsules as compared to traditional tablets are their programmable release kinetics, decreased inter-patient variability, enhanced compliance, and dose-adjustability [42]. reported the effectiveness of nanoemulsion formulations in enhancing bioavailability of drugs, while discussed drug delivery applications of nanoemulsions. While these methods vary in the type of formulation design, the theory with regards to improving drug bioavailability and sustained release is based on similar principles to that of the pellet-based system reported here. The uniformity and robustness observed in our formulation studies also argue for the dependability of this platform technology when translated into the clinical setting [43].

The integrative view provided by this study is supportive of the emerging paradigm for CDS. Powerful as they are, infusion gels and implants can be invasive and expensive. The pellet based system is a scalable and patient-friendly alternative that allows immediate pain relief in combination with sustained neuroprotection. Both symptomatic and pathogenic mechanisms of Parkinson's disease are targeted by the formulation through the restoration of behavioral performance, antioxidant status and histological integrity [44]. This double action places multiparticulate capsules as a new promising step toward the management of Parkinson disease in between classical PO therapy and invasive continuous delivery systems. The results highlight the potential impact of pellet capsules for PD treatment, providing a non-invasive, effective and patient-oriented regime. Further research and clinical validation will be essential to translate these transformative product concepts into helpful medicines for people with Parkinson's [45].

CONCLUSION

The current work further suggests that the multiparticulate pellet-form triple therapy could be a potential breakthrough in the treatment of PD, since it was also effective (in dose-related manner) against MPTP-induced neuroprotection by restoring behavior, antioxidant and histopathological markers. In addition to confirming the therapeutic value of Levodopa, Carbidopa and Entacapone in a controlled-release formulation, this work marks a new horizon for oral delivery systems aimed at mimicking continuous dopaminergic stimulation. Such a device could potentially be improved by the addition of "smart" polymers responsive to circadian rhythms or neural biomarkers, permitting tailored drug release timed with patient-specific variations in motor and cognitive symptoms. Those advances could enable treatment for Parkinson's disease to evolve from a fixed dosing schedule into adaptive, self-titrating therapies. In conclusion, results of this study not only illustrate the potential in vivo therapeutic efficacy of pellet-based triple therapy, but also provide guidance toward future personalized, adaptive and multi-functional drug delivery ecosystems. As they converge across pharmacology, material science and digital health these types of systems can transform the therapeutic landscape of PD, getting closer to the notion of sustained neuroprotection and personalized medicine.

DECLARATION

Authors declared no conflict of interest.

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