ARTICLE | OPEN ACCESS

Drug Discovery

To Cite:
Talkad MS, Javed A, Satish V, Kumar HVA, Saba K. Long-term neuroprotective efficacy of VEMSA-PD nasal drops: An innovative approach for management and mitigation of Parkinson's disease symptoms. *Drug Discovery* 2024; 18: e17dd1993
doi: https://doi.org/10.54905/dissi.v18i42.e17dd1993

Author Affiliation:
[1]R&D Centre, Vemsa Biotech Pvt. Ltd. #468, 39th cross, 9th main, 5th block Jayanagar, Bangalore 560041, Bengaluru, Karnataka, India
[2]Professor of Sericulture, Laboratory for Applied Biological Science, DVS College of arts and Science. Shimoga, Karnataka, India
[3]Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, 201002, India

[*]**Corresponding Author**
R&D Centre, Vemsa Biotech Pvt. Ltd. #468, 39th cross, 9th main, 5th block Jayanagar, Bangalore 560041, Bengaluru, Karnataka, India
Email: muralidhar@vemsabiotech.com
ORCID: 0000-0002-3306-9413

Peer-Review History
Received: 30 May 2024
Reviewed & Revised: 03/June/2024 to 05/August/2024
Accepted: 08 August 2024
Published: 16 August 2024

Peer-Review Model
External peer-review was done through double-blind method.

Drug Discovery
pISSN 2278–540X; eISSN 2278–5396

DISCOVERY SCIENTIFIC SOCIETY

Long-term neuroprotective efficacy of VEMSA-PD nasal drops: An innovative approach for management and mitigation of Parkinson's disease symptoms

Muralidhar S Talkad[1*], Aamir Javed[1], Vijay Satish[1], HV Anil Kumar[2], Kamal Saba[3]

ABSTRACT

Background and Purpose: Parkinson's Disease (PD) is a neurodegenerative disorder primarily affecting dopamine-producing neurons in the brain, leading to a spectrum of motor and non-motor symptoms. *Methods:* This study introduces VEMSA-PD nasal drops as a potential candidate, which, based on our investigations, has shown promising neuroprotective properties. VEMSA-PD nasal drops are a proprietary herbal compound enriched with micro-emulsions and bioactive components that are beneficial for the Central Nervous System (CNS) and managing PD. Our experimental model initially involved inducing PD in mice using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, administered at a dosage of 20 mg/kg (i.p) twice daily for five days. *Result:* Subsequent treatment with VEMSA-PD nasal drops revealed a significant neuroprotective effect. Notably, co-administration of MPTP with VEMSA-PD (2 drops, 3 times/day) almost restored the striatum's glutathione (GSH) levels and antioxidant enzyme activity to normal levels in the MPTP-induced group (1.33 ± 0.06). Additionally, the elevated malondialdehyde (MDA) levels seen in the MPTP-induced group (8.11 ± 0.05) were considerably reduced in the group treated with VEMSA-PD, achieving MDA levels of 2.63 ± 0.143 in the striatum and 4.45 ± 0.169 in the treated group. *Conclusion:* Human PD patients received VEMSA-PD nasal drops for six months in the research. Uric acid levels decreased significantly pre-, four-month-, and post-treatment, suggesting VEMSA-PD may manage PD-related metabolic dysregulation. In conclusion, VEMSA-PD nasal drops reduce uric acid levels in human PD patients and protect neurons in animal models of PD.

Keywords: VEMSA-PD nasal drops, MPTP, Parkinson's disease, oxidative stress, free radical, brain histopathology.

1. INTRODUCTION

Parkinson's disease (PD) is a chronic and progressive neurodegenerative disorder that poses significant challenges to medical researchers and clinicians (Ojha et al., 2016). The disease is marked by both motor and non-motor dysfunctions, the most significant of which include dopaminergic neuronal death, oxidative stress, and neuroinflammation (Mhyre et al., 2012; DeMaagd and Philip, 2015). As PD continues to affect millions of individuals worldwide, gaining a profound understanding of its pathophysiology is of paramount importance for the development of effective therapeutic strategies (Dinda et al., 2019). PD is characterized by a distinct neuropathology, predominantly involving the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) region of the brain, and the subsequent reduction of dopamine levels in the striatum (Dinda et al., 2019).

This characteristic feature of PD, combined with the presence of intraneuronal proteinaceous inclusions known as Lewy bodies, forms the pathological basis for the cardinal motor symptoms observed in the disease, which include bradykinesia, resting tremor, rigidity, and postural instability (Alexander, 2004). To explore the pathophysiological mechanisms of PD and evaluate potential therapeutic approaches, various in vivo models are employed. One of the most extensively utilized models involves the administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Alexander, 2004). This neurotoxin exerts its detrimental effects primarily on dopaminergic neurons and induces the formation of α-synuclein in the striatum and substantia nigra, recapitulating many aspects of PD pathology (Von-Bohlen, 2005; Grotemeyer et al., 2022).

α-Synuclein, a protein primarily found at presynaptic terminals, plays a significant role in the pathogenesis of PD. Under pathological conditions, it can aggregate to form insoluble fibrils, which contribute to the formation of Lewy bodies. Recent research has highlighted the role of α-synuclein in modulating neurotransmitter release, synaptic function, and plasticity, further underscoring its importance in PD pathophysiology (Stefanis, 2012). Animal models of PD, such as the MPTP model, offer essential insights into the underlying mechanisms that contribute to the broad spectrum of symptoms associated with the disease. These models serve as instrumental platforms for both fundamental and translational research (Ghavami et al., 2014). When rodents or primates are exposed to MPTP, they exhibit biochemical and cellular changes that closely resemble those observed in human cases of idiopathic PD (Duty, Jenner, 2011; Meredith and Rademacher, 2011).

Furthermore, these models help to shed light on the role of oxidative stress, apoptosis, and neuroinflammation in PD progression, even if the precise molecular underpinnings remain elusive (Imamura et al., 2003). Oxidative stress, defined as the imbalance between the production of intracellular free radicals and the efficacy of the intracellular antioxidant defence system, emerges as a crucial biochemical pathogenic component in PD (Dexter and Jenner, 2013; Dauer and Przedborski, 2003). Accumulating evidence suggests that chronic oxidative stress can lead to cellular damage and dysfunction, contributing to the degeneration of dopaminergic neurons observed in PD. Moreover, elevated levels of oxidative stress markers have been identified in the brain, cerebrospinal fluid, and peripheral tissues of PD patients, reinforcing the concept that oxidative stress is intimately involved in the pathogenesis of PD (Dias et al., 2013).

Interestingly, several studies indicate that specific compounds may exhibit neuroprotective properties against PD. For instance, administration of Catalpol, a bioactive compound isolated from the herb Rehmannia glutinosa, has been shown to mitigate MPTP-induced oxidative stress, thereby preventing chronic inflammation and neurodegeneration (Wang et al., 2019). Moreover, the herbal extract Sophora tomentosa L displayed neuroprotective effects against MPTP-induced Parkinsonism by restoring nigrostriatal dopaminergic function, suggesting a potential therapeutic role of natural compounds in PD (Hu et al., 2016). These neuroprotective mechanisms likely involve the reduction of GSK-3 phosphorylation and α-synuclein accumulation, coupled with the rejuvenation of striatal antioxidant defences (Garcia-Yague et al., 2021). Additionally, Embelin, a bioactive compound known to penetrate the blood-brain barrier, has exhibited neuroprotective effects on the central nervous system and demonstrate beneficial impact in rotenone-induced PD models (Witika et al., 2022).

Given the therapeutic potential of these natural compounds, the focus of the current study is on VEMSA-PD nasal drops. This novel treatment modality contains a proprietary blend of herbal extracts, micro-emulsions, and bioactive constituents, purported to be beneficial for the central nervous system and specifically for PD (Thomford et al., 2018). It is proposed that the neuroprotective mechanism of VEMSA-PD nasal drops against MPTP-induced Parkinsonism may involve a multifaceted approach, including restoring striatal antioxidant defenses, reestablishing nigrostriatal dopaminergic function, and reducing α-synuclein accumulation. As PD

continues to represent a significant burden for patients and healthcare systems worldwide, novel therapeutic strategies like VEMSA-PD nasal drops can potentially provide much-needed relief for those affected by this debilitating disease. The ongoing quest to better understand the pathophysiological processes underlying PD and the exploration of innovative treatment approaches underline the importance of this type of research in the field of neurodegenerative disorders.

This worthwhile examine the microscopic view of the brain tissue, specifically the hippocampus region, under the influence of Parkinson's Disease (PD) and its reversal with the administration of VEMSA-PD Nasal Drops. Histopathological examinations offer a fundamental means of understanding the disease's influence at the tissue level, providing an extensive picture of the changes the disease induces within the brain (Dickson, 2012). Parkinson's Disease, a neurodegenerative disorder, prominently impacts the dopaminergic neurons in the substantia nigra and significantly affects other areas of the brain, including the hippocampus. This region plays an essential role in memory formation and spatial navigation, and any alterations in its structure can lead to cognitive and motor deficits, commonly observed in PD (Halliday et al., 2014). In the study, the hippocampal region of PD induced and non-induced mice was scrutinized. It was observed that PD led to significant pathological alterations in the hippocampus, including fatty vacuolation and neuronal hyperplasia with apoptosis and gliosis.

These changes underline the destructive nature of the disease and emphasize the importance of identifying and implementing neuroprotective strategies. The innovative VEMSA-PD Nasal Drops, designed as a non-invasive therapeutic administration method, were found to be one such neuroprotective agent. The nasal route, with its direct connection to the brain via the olfactory and trigeminal nerves, allows the drug to bypass the blood-brain barrier, ensuring more efficient drug delivery and less systemic exposure, thereby reducing potential side effects. The nasal drops contain a potent formulation of selective herbal extracts, developed to combat PD-induced oxidative stress, inflammation, and neurodegeneration. The histopathological observations of the hippocampal region following the administration of VEMSA-PD Nasal Drops revealed a significant reduction in PD-induced pathological changes. This suggests that the nasal drops not only protected the brain from further damage but also reversed some of the PD-induced modifications.

The implications of these findings extend beyond our understanding of the disease's microscopic effects on the brain's structure. They highlight the potential of VEMSA-PD Nasal Drops as a novel and effective therapeutic strategy for PD, underscoring the potential of herbal extracts in the management and treatment of neurodegenerative diseases. Recent studies have highlighted the potential role of uric acid in neurodegenerative diseases, including PD (Cutler et al., 2015). Uric acid is the final oxidation product of purine metabolism in humans and has potent antioxidant properties, which can counteract oxidative stress, a primary contributor to the development of PD (Sautin and Johnson, 2008). Interestingly, epidemiological studies have found an inverse correlation between uric acid levels and the risk of PD, implying that higher levels of uric acid may have a protective effect against PD development (Fazlollahi et al., 2022).

However, the precise role of uric acid in PD and its potential use as a therapeutic target or biomarker in PD remains unclear (Cipriani et al., 2010). In this study, we aimed to investigate the effect of VEMSA-PD nasal drops on uric acid levels in PD patients. VEMSA-PD is a proprietary herbal compound infused with micro-emulsions and bioactive components beneficial for the Central Nervous System (CNS) and managing PD. We hypothesized that the treatment might not only help with PD symptoms but also regulate uric acid levels, thereby providing a holistic approach to manage PD. This research represents an essential stride towards understanding the multifaceted mechanisms of PD and highlighting the potential of innovative therapeutic interventions. Our findings can illuminate the potential therapeutic effects of VEMSA-PD nasal drops and help establish the groundwork for larger clinical studies investigating the effectiveness of these drops as a treatment for PD, taking into consideration both the symptomatic management and metabolic modulation.

2. MATERIALS AND METHODS

Animal Preparation

ARRIVE guidelines have been followed in the preparation of the manuscript. The experimentation was carried out as per Research and Ethics (CPSEA/154/106/2022) approved experimental protocols. Two months old male, C57BL6/J mice procured from the Jackson Laboratory, USA (Stock no: 000664) were used for the study. They were housed at 22°C, 60% relative humidity and 12hrs light/dark cycle with ad libitum access to food and water. Mice were randomly divided into two groups:

MPTP Group (n=6): This group received MPTP 20mg/kg (SIGMA/Aldrich, USA) prepared in normal saline as IP once in a day for 7 consecutive days.
Control Group (n=7): This group was treated with normal saline as IP (volume: 25gm/kg) once in a day for 7 consecutive days.

Formulation and Administration of VEMSA-PD Nasal Drops

The VEMSA-PD nasal drops, a potent extract formulation, were meticulously prepared using the well-established Soxhlet extraction process. This extraction method allows for the comprehensive extraction of active constituents from the selected herbs, providing a rich and therapeutically potent formulation. The drops have been designed with a dosage concentration of 100mg/ml, optimized for ease of administration and therapeutic efficacy. The specific formulation encompasses a blend of five distinct herbal extracts, each chosen for their unique medicinal properties.

The botanical ingredients and their quantities per milliliter of the formulation are as per the ingredient given in the (Table 1). Each of these components contributes to the overall efficacy of the VEMSA-PD nasal drops. Mucuna pruriens, for example, has been found to contain naturally occurring levodopa, which may provide dopaminergic support in the treatment of PD. Similarly, Curcuma longa is known for its anti-inflammatory and antioxidant properties, both of which may be beneficial in neurodegenerative conditions like PD.

Table 1 Formulation of VEMSA-PD Nasal Drops

Botanical Name	Part Used	Form of ingredient	Qty in ml
Mucuna pruriens	Seed	Extract	2.0
Vitis vinifera	Seed	Oil	1.5
Sida cordifolia	Root	Extract	1.0
Curcuma longa	Rhizome	Extract	0.05
Prunus amigdalus	Seed	Oil	0.05

Table 1 details the individual botanical ingredients used in the formulation of VEMSA-PD Nasal Drops. Each component, including the part used, form of ingredient, and quantity per milliliter of formulation, is listed.

The safety and tolerance of VEMSA-PD nasal drops were carefully assessed via an acute toxicity LD 50 evaluation. This assessment was conducted in a controlled laboratory environment following the guidelines stipulated by the Organisation for Economic Co-operation and Development. The "LD 50" represents the lethal dose at which 50% of the tested population would be expected to succumb, hence providing a measure of the substance's acute toxicity. As per the regulatory guidelines, the maximum therapeutic dose of any drug should not exceed 1/10th of the maximum tolerance dose determined from the LD 50 evaluation.

Accordingly, the therapeutic doses for the VEMSA-PD nasal drops were judiciously set at 250 mg/kg and 750 mg/kg of body weight. These dosages have been selected to optimize the neuroprotective benefits of the formulation while ensuring its safety profile. Taken together, the methodical preparation and rigorous safety evaluation of the VEMSA-PD nasal drops highlight their potential as a promising therapeutic intervention in PD management. Further experimental investigation is warranted to explore their precise neuroprotective mechanisms and potential clinical utility in treating Parkinson's disease.

Methodology and Experimental Design

The study incorporated two separate experimental designs, each utilizing six rats and targeted at exploring the impact of VEMSA-PD Nasal Drops under different conditions.

Preparatory Phase: Evaluating Impact of VEMSA-PD on Normal Mice:

The first phase of the study involved dividing six mice into three groups, each subjected to different treatments for a duration of 21 days:

Group 1 - Normal Control: Mice in this group received drinking water in doses of 10ml/kg of body weight.

Group 2 - Low Dosage VEMSA-PD: Mice in this group were orally administered with VEMSA-PD Nasal Drops in a low dosage of 250mg/kg of body weight.

Group 3 - High Dosage VEMSA-PD: Mice in this group received the VEMSA-PD Nasal Drops orally in a high dosage of 750mg/kg of body weight.

Throughout the 21-day period, mice were monitored and their body weight was recorded at the beginning and end of the feeding process. On the final day, blood was collected from the mice to analyze the levels of Reduced Glutathione (GSH). Following the feeding process, the mice were subjected to necropsy and the weight of vital organs like the liver, kidney, and brain were recorded. These organs were then subjected to histopathological analysis.

Experimental Phase: Evaluating Therapeutic Response in PD-Induced Chemical Mice Model

The second phase of the study involved an examination of the therapeutic response of VEMSA-PD Nasal Drops on a PD-induced model. In this phase, six mice were divided into five groups:

Group 1 - Normal Control: Mice in this group were not subjected to any specific treatment.

Group 2 - MPTP-induced: Mice in this group were given 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to induce Parkinson's disease-like symptoms.

Group 3 - Standard Levodopa: This group received standard Levodopa treatment at a dosage of 7.5mg/kg of body weight orally.

Group 4 - Low Dosage VEMSA-PD with MPTP: These mice received MPTP along with two drops of VEMSA-PD Nasal Drops (extracts), administered twice a day.

Group 5 - High Dosage VEMSA-PD with MPTP: Mice in this group were given MPTP and received three drops of VEMSA-PD Nasal Drops (extracts) three times a day.

VEMSA-PD Nasal Drops were administered via the nasal route for 15 consecutive days until behavioral observation. Meanwhile, MPTP (20 mg/kg, intraperitoneally) was administered twice daily, with a 4-hour interval, for five days until behavioral observation. The activities of antioxidant enzymes in the brain striatum, including glutathione peroxidase, glutathione reductase, glutathione, and malondialdehyde, were evaluated. Tissue samples from the brain of each group were collected and subjected to histopathological evaluation.

Histological Examination of Brain Tissues

For histological analysis, brain tissues were initially fixed using 4% paraformaldehyde in phosphate-buffered saline (PBS) for a duration of 24 hours. Post-fixation, tissues were then cryoprotected in 30% sucrose at 4°C until they descended. Following this, the tissues were incorporated in OCT compound (Sakura Finetek) and sectioned at 20μm on a cryostat. The subsequent tissue sections were assembled onto gelatin-coated slides. To begin with the staining procedure, the slides were initially rinsed with PBS to expel the OCT compound. The tissue slides were then prepared for staining with hematoxylin and eosin (H & E), which is a common staining procedure used to examine the morphology and pathology of the tissue.

The stained tissue sections were then visualized under a microscope, and images were obtained for further analysis. The H & E staining provided detailed information on the general structure and form of the brain tissue. This allowed for in-depth scrutiny of any pathological changes in the brain tissue associated with Parkinson's disease. All steps were meticulously performed to maintain the tissue's integrity and provide accurate histopathological findings.

Human Subject Preparation and Study Design

Under this subheading, the details of the study design involving human participants are described. The study involved a cohort of 162 PD patients who were diagnosed according to the UK Brain Bank criteria. Participants were between the ages of 45 and 70 and were not on any other treatment regimen for PD during the study duration. Patients were randomly assigned into three equal groups:

VEMSA-PD Nasal Drops Treated Group (n=54): This group received VEMSA-PD nasal drops, two drops thrice a day for a period of 6 months.

Non-Treated Control Group (n=54): This group did not receive any form of treatment and served as a control group. The patients were closely monitored during the study.

Placebo Group (n=54): Patients in this group were given placebo nasal drops, administered in the same way as the treated group.

Blood samples were collected from all patients at three different time points: before the start of treatment (Pre-treatment), at the end of 4 months (Treatment - 4 Months), and at the end of 6 months (post-treatment). The uric acid levels in the blood samples were measured using an enzymatic colorimetric method, which was the outcome measure for this study.

The data were analyzed using two-way ANOVA for repeated measures, followed by Tukey's multiple comparisons test. The significance level was set at $p<0.05$. The statistical analysis was performed using GraphPad Prism software. This study was approved by the Institutional Ethics Committee and was conducted. Informed lawful consent (PDL-88-89) was procured from all participants. Applicable approval was received from the ethical committee of the PD Labs (Neelanjana Ayurveda Specialty clinic), Department of Medicine, Bangalore, India (approval No. PDL/PAR-88-RI-89). The datasets used and/or analyzed during the current study are available from the corresponding author upon a reasonable request.

Statistics

During our study, we utilized non-parametric one-way analysis of variance (ANOVA), a statistical method that's effective for handling biological data without the need for normal distribution or equal variances assumptions. This technique enabled us to compare the effectiveness of VEMSA-PD nasal drops across multiple treatment groups and the control group, providing clear insights into the formulation's potential in mitigating PD symptoms in MPTP-treated mice. It's important to note that while this method helps identify patterns and trends, the interpretation and application of these results to biological or clinical significance necessitate further investigations and a deeper understanding of the underlying biological mechanisms. Therefore, despite the strong statistical evidence, we underscore the need for continued research to validate the neuroprotective effects of VEMSA-PD nasal drops and to uncover the precise molecular mechanisms that support these effects.

3. RESULTS

Analysis of Biochemical Parameters

The biochemical markers of interest in this study included Glutathione (GSH), Glutathione Reductase (GR), Glutathione Peroxidase (GPx), Superoxide Dismutase (SOD), Catalase, and Malondialdehyde (MDA). These markers play crucial roles in our body's antioxidant defenses and oxidative stress status (Younus, 2018). GSH is a potent antioxidant found in most cells, protecting them against free radicals and peroxides. GR and GPx are enzymes that, together with GSH, constitute the glutathione-ascorbate cycle, a system that eliminates damaging peroxides (Kurutas, 2016).

SOD and Catalase are additional critical antioxidant enzymes. SOD facilitates the conversion of superoxide radicals into either ordinary molecular oxygen or hydrogen peroxide, which is then broken down by catalase into water and oxygen, thus protecting the cells from the harmful effects of these radicals (Mukhopadhyay et al., 2009). MDA, on the other hand, is a marker of oxidative stress and is produced as a result of lipid peroxidation. Increased levels of MDA signify enhanced oxidative stress and damage to cell membranes.

Glutathione (GSH) Levels

The Normal Control group exhibited a mean GSH level of 2.55 ± 0.13 nmoL/mg of protein. The GSH level was significantly reduced to 1.33 ± 0.06 nmoL/mg of protein in the MPTP group, indicating the induction of oxidative stress. Levodopa treatment moderately increased the GSH level to 1.91 ± 0.10 nmoL/mg of protein. The VEMSA-PD treatment showed a substantial increase in GSH levels, reaching 2.06 ± 0.05 nmoL/mg of protein in the low-dosage group (2 drops/2 times) and an even higher level of 2.63 ± 0.143 nmoL/mg of protein in the high-dosage group (2 drops/3 times) (Table 2).

Glutathione Reductase (GR) and Glutathione Peroxidase (GPx) Activities

The MPTP group exhibited reduced levels of both GR (144.5 ± 2.39 mU/mg of protein) and GPx (3.11 ± 0.05 mU/mg of protein) compared to the Normal Control group (GR: 204.23 ± 3.60 mU/mg of protein, GPx: 4.45 ± 0.16 mU/mg of protein). Levodopa treatment resulted in a slight increase in the GR (149.83 ± 1.58 mU/mg of protein) and GPx (3.67 ± 0.04 mU/mg of protein) activities. The VEMSA-PD treated groups demonstrated a significant restoration in both GR and GPx activities. In particular, the high-dosage VEMSA-PD group showed remarkable improvements with GR reaching 248 ± 3.307 mU/mg of protein and GPx reaching 5.63 ± 0.161 mU/mg of protein (Table 2).

Table 2 Evaluation of Antioxidant Enzymes and Oxidative Stress Markers in Different Experimental Groups

Sl.no	Groups	GSH (nmoL/mgof Protein)	GR (mU/mg of Protein)	GPx (mU/mgof Protein)	SOD (U/mg ofProtein)	Catalase (U/mg of Protein)	MDA (nmoL/mgof Protein)
1	Normal Control	2.55± 0.13	204.23± 3.60	4.45± 0.16	21.46± 0.49	11.91± 0.30	4.40± 0.15
2	MPTP	1.33± 0.06	144.5± 2.39	3.11± 0.05	12.08± 0.05	4.68± 0.09	8.11± 0.05
3	MPTP + Std Levodopa: 7.5 mg/kg (P.O)	1.91±0.10 **	149.83± 1.58	3.67± 0.04	14.99± 0.08	5.50± 0.06	7.30± 0.17**
4	MPTP + VEMSA-PD 2 drops /2 times	2.06± 0.05**	179.5± 2.28	4.21± 0.07 **	17.11± 0.12	8.06± 0.09**	4.77± 0.07**
5	MPTP + VEMSA-PD 2 drops /3 times	2.63± 0.143*	248*± 3.307	5.63± 0.161	26.18 *± 0.290	12.1± 0.127*	4.45± 0.169*

Table 2 illustrates the quantification of key oxidative stress and antioxidant parameters, including Reduced Glutathione (GSH), Glutathione Reductase (GR), Glutathione Peroxidase (GPx), Superoxide Dismutase (SOD), Catalase, and Malondialdehyde (MDA) across different experimental groups. The units of measure are given in nanomoles per milligram of protein (nmoL/mg of Protein) for GSH and MDA, and milliunits or units per milligram of protein (mU/mg of Protein or U/mg of Protein) for GR, GPx, SOD, and Catalase. Group 1 represents the Normal Control, Group 2 signifies the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced model, Group 3 encompasses animals treated with standard Levodopa (7.5mg/kg, administered perorally - P.O.) after MPTP induction, while Group 4 and Group 5 include animals treated with low (2 drops twice a day) and high (2 drops thrice a day) dosages of VEMSA-PD Nasal Drops (Vedica Herbals' extract formulations for Parkinson's Disease) following MPTP induction, respectively. The values displayed are means ± standard deviation. Statistical significance from the MPTP group is denoted by asterisks: **p<0.01, *p<0.05.

Superoxide Dismutase (SOD) and Catalase Activities

The MPTP group exhibited lower levels of SOD (12.08 ± 0.05 U/mg of protein) and catalase (4.68 ± 0.09 U/mg of protein) compared to the Normal Control group (SOD: 21.46 ± 0.49 U/mg of protein, Catalase: 11.91 ± 0.30 U/mg of protein). Levodopa moderately increased the activities of both SOD (14.99 ± 0.08 U/mg of protein) and catalase (5.50 ± 0.06 U/mg of protein). Remarkably, VEMSA-PD treatment demonstrated a substantial increase in SOD and catalase activities. Especially, the high-dosage VEMSA-PD group showed SOD reaching 26.18 ± 0.290 U/mg of protein and catalase reaching 12.1 ± 0.127 U/mg of protein (Table 2).

Malondialdehyde (MDA) Levels

The MPTP group exhibited a significantly higher MDA level (8.11 ± 0.05 nmoL/mg of protein) than the Normal Control group (4.40 ± 0.15 nmoL/mg of protein), suggesting increased lipid peroxidation. Levodopa treatment only slightly decreased the MDA level to 7.30 ± 0.17 nmoL/mg of protein. Both VEMSA-PD treatment groups showed a marked reduction in MDA levels, with the high-dosage VEMSA-PD group exhibiting an MDA level of 4.45 ± 0.169 nmoL/mg of protein, comparable to the Normal Control group (Table 2). Overall, these results suggest that VEMSA-PD Nasal Drops effectively enhance antioxidant enzyme activities and reduce oxidative stress in the MPTP-induced PD model, providing further evidence for its potential neuroprotective effects.

Histopathological Examination of MPTP-Induced Changes and Therapeutic Effects of VEMSA-PD Nasal Drops

In our study, we conducted detailed histopathological assessments to investigate the neuroprotective effects of VEMSA-PD nasal drops against MPTP-induced Parkinson's disease in mice. The histopathological evaluations focused on the hippocampal region of the brain, as this area is often significantly affected in Parkinson's disease. In the control group, normal morphology was observed in the hippocampus (refer to Figures A1 and A2), providing a baseline reference for subsequent comparisons. In contrast, the group induced with MPTP (G2) demonstrated severe morphological changes, including 4+ fatty vacuolation and moderate 3+ neuronal hyperplasia. These changes were accompanied by notable levels of apoptosis and gliosis (Figures B1 and B2), providing clear evidence of MPTP-induced neuronal damage.

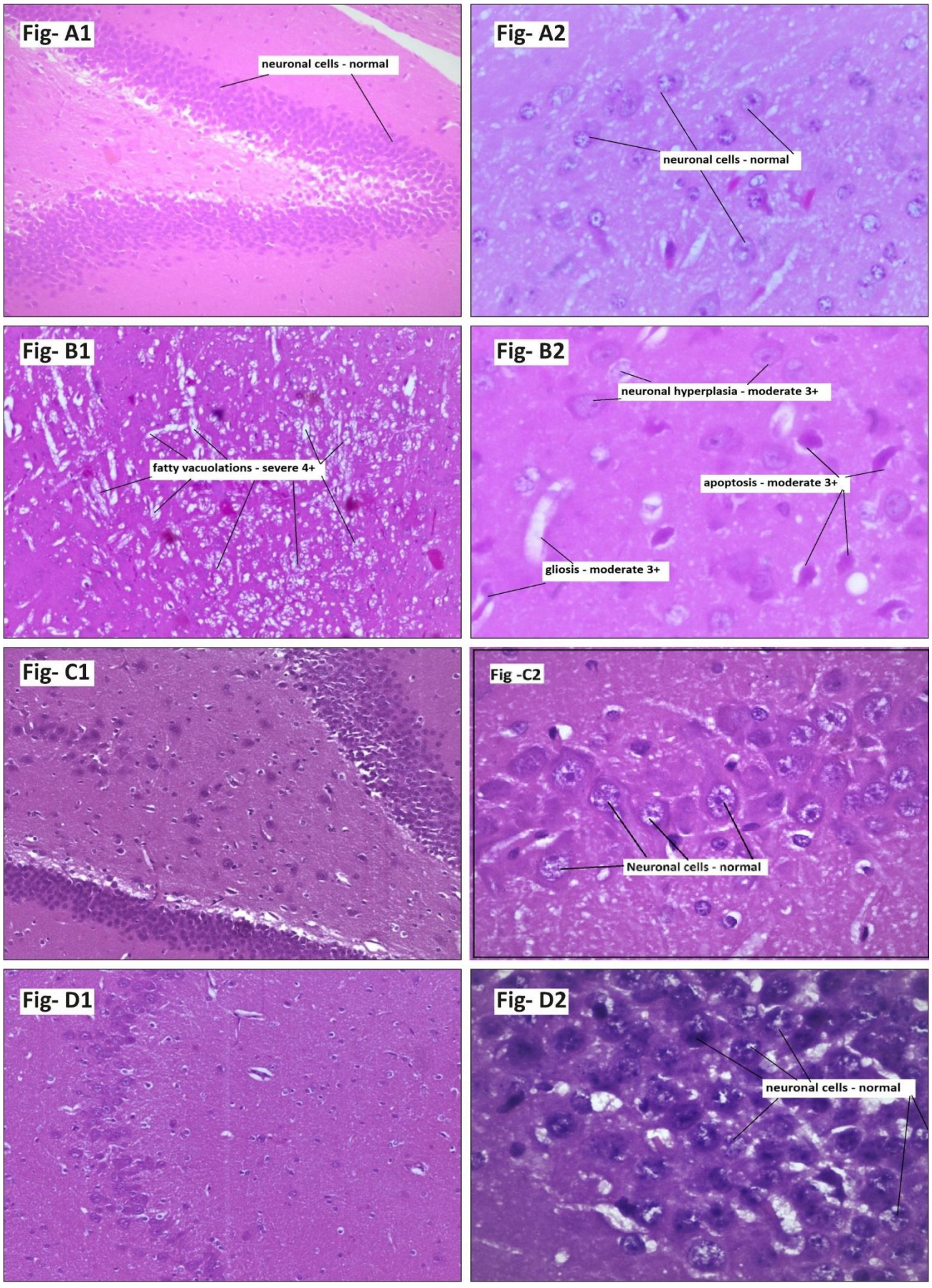
Fig- A1
neuronal cells - normal
Fig- A2
neuronal cells - normal
Fig- B1
fatty vacuolations - severe 4+
Fig- B2
neuronal hyperplasia - moderate 3+
apoptosis - moderate 3+
gliosis - moderate 3+
Fig- C1
Fig -C2
Neuronal cells - normal
Fig- D1
Fig- D2
neuronal cells - normal

Figure 1 Neuroprotective Effects of VEMSA-PD Nasal drops on the Histopathological Profile of Mice Brain in MPTP-Induced Parkinson's Disease. The figure comprises eight micrographs (Fig-A1 to Fig-D2), illustrating the histopathological changes in the hippocampus region of mice brains across different experimental conditions. Fig-A1 & Fig-A2: Represent the hippocampus region of a normal control mouse brain, revealing no abnormalities in the cellular morphology. Images were captured at 100x (Fig-A1) and 400x (Fig-A2) magnification. Fig-B1 & Fig-B2: Demonstrate severe and moderate histopathological alterations respectively in MPTP-induced mouse brains. The hippocampus region exhibits fatty vacuolation (severity grade: 4+) at 100x magnification (Fig-B1) and neuronal hyperplasia with apoptosis and gliosis (severity grade: 3+) at 400x magnification (Fig-B2). Fig-C1 & Fig-C2: Display the hippocampus region of mice brains treated with standard Levodopa (7.5mg/kg of body weight - PO), showing recovery to normal morphology. These images were captured at 100x (Fig-C1) and 400x (Fig-C2) magnification. Fig-D1 & Fig-D2: Represent the hippocampus region of mice brains treated with high dosage (2 drops three times) of MPTP + VEMSA-PD. The histological evaluation shows normal morphology, indicative of no abnormal detection (NAD+), as observed at 100x (Fig-D1) and 400x (Fig-D2) magnification. Collectively, these micrographs underscore the neuroprotective potential of VEMSA-PD nasal drops, as evidenced by the restoration of normal hippocampal morphology in MPTP-induced mice following treatment.

Intriguingly, treatment results varied significantly. For the group treated with Levodopa, a standard Parkinson's disease medication, near-normal hippocampal morphology was restored (Figures C1 and C2). This indicates the successful alleviation of MPTP-induced neuronal damage, which is consistent with the well-documented efficacy of Levodopa in Parkinson's disease treatment. Of particular note, however, was the group treated with high-dose VEMSA-PD nasal drops (2 drops administered 3 times daily). Here, the histological architecture of the brain showed remarkable improvement, nearly returning to the normal morphology seen in the control group (Figures D1 and D2). These findings underscore the potential of VEMSA-PD nasal drops as a therapeutic intervention against MPTP-induced Parkinson's disease, warranting further investigation.

Effectiveness of VEMSA-PD Nasal Drops in Modulating Uric Acid Levels: A Time-Series Analysis on PD patients

The data consists of age information for three different patient groups: VEMSA-PD Treated, Non-Treated, and Placebo Treated. Each group contains data for 54 patients. For the VEMSA-PD Treated group, the average age is approximately 60.69 years with a standard deviation of 7.34 years. The youngest patient in this group is 29 years old, while the oldest is 78. The median age, or the age at which half the patients are younger and half are older, is 61 years. The non-treated group has an average age of 58.56 years with a standard deviation of 4.54 years. The age range in this group is narrower than in the VEMSA-PD Treated group, with the youngest patient being 51 years old and the oldest being 71. The median age in this group is 58.5 years. In the Placebo Treated group, the average age is nearly 59.96 years with a standard deviation of 6.15 years. The youngest patient is 49 years old and the oldest is 76. The median age in this group is 59.5 years.

The age distributions for all three groups appear to be approximately normal, although the VEMSA-PD Treated group shows a slightly wider age range than the other groups (Figure 2). A two-way ANOVA was conducted to understand the differential effects of VEMSA-PD Nasal Drops, non-treatment, and placebo on uric acid levels over time. The interaction between the treatment modalities and time was significant [$F(4, 477) = 24.08$, $p < .0001$], contributing to approximately 11.59% of the total variance in uric acid levels. The main effect of treatment was also significant [$F(2, 477) = 112.6$, $p < .0001$], accounting for 27.10% of the total variation. Likewise, the main effect of time emerged as significant [$F(2, 477) = 16.13$, $p < .0001$], explaining around 3.884% of the total variation.

Multiple Comparison Analysis: Pre-Treatment, Treatment at 4 Months and Post-Treatment

Group Differences

A post-hoc Tukey's multiple comparisons test was applied to identify pairwise differences between the treatment groups at different time points.

Pre-Treatment

At the outset, no significant differences were observed in baseline uric acid levels between the VEMSA-PD Nasal Drops group, the non-treated group, and the Placebo group (all $p > .05$), indicating an equitable starting point for all groups (Figure 2).

Treatment at 4 Months

After 4 months of treatment, the VEMSA-PD Nasal Drops group showed a significant decrease in uric acid levels as compared to both the Non-Treated group (Mean difference = -2.632, $p < .0001$) and the Placebo group (Mean difference = -1.832, $p < .0001$). Interestingly, a significant difference emerged between the Non-Treated and the Placebo groups as well (Mean difference = 0.7998, $p = .0404$). This

suggests that there might be a possible placebo effect influencing uric acid levels, albeit the change is notably less pronounced than the change induced by the VEMSA-PD Nasal Drops (Figure 3).

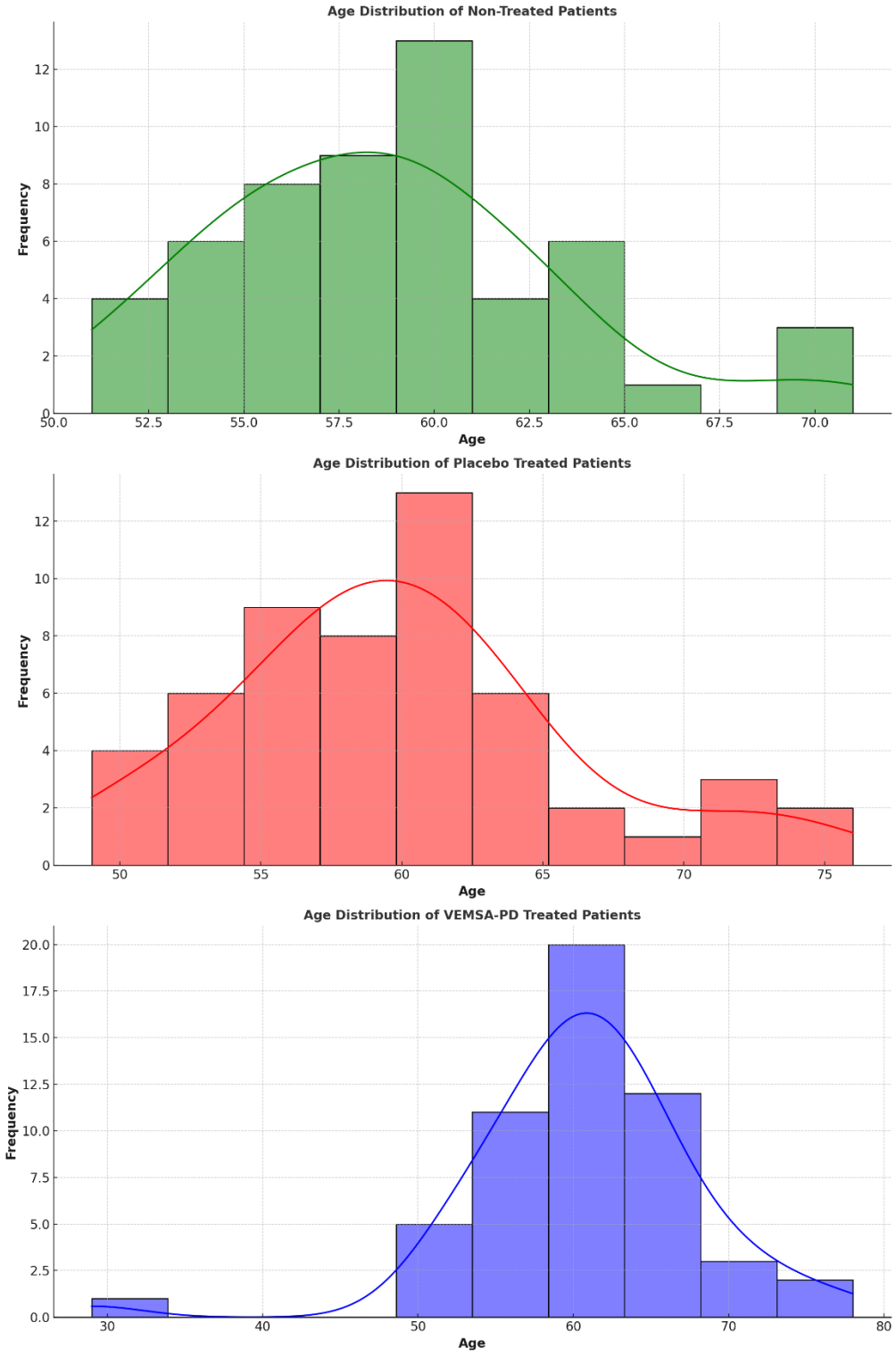

Figure 2 Age Distribution Across Different Treatment Groups. The histograms above show the age distribution for three patient groups: Non-Treated (top, green), Placebo treated (middle, red), and VEMSA-PD treated (bottom, blue). Each group appears to follow an approximately normal distribution. The y-axis represents the frequency of patients in each age bin, while the x-axis represents age. The mean age is around 60 for all groups, with the VEMSA-PD treated group exhibiting a slightly broader age range.

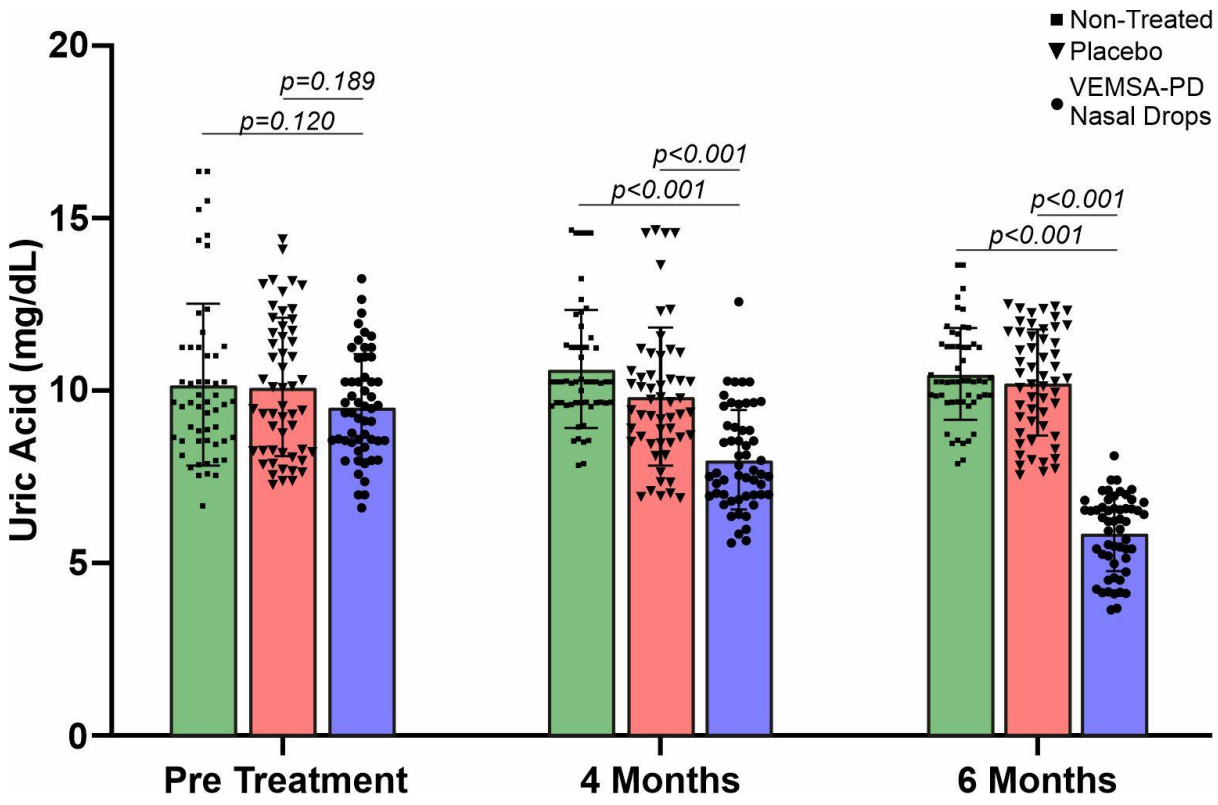

Figure 3 Impact of VEMSA-PD Nasal Drops, Non-Treatment, and Placebo on Uric Acid Levels Across Time. Illustrates the uric acid levels (in mg/dl) trajectories for the three groups (VEMSA-PD Nasal Drops, Non-Treated, and Placebo) at three time points (Pre-Treatment, 4-Months Treatment, and 6-Months Post-Treatment). The y-axis represents uric acid levels, while the x-axis signifies the time points. Error bars indicate the standard error of the mean.

Post-Treatment-6 Months

At the 6-months post-treatment mark, the VEMSA-PD Nasal Drops continued to manifest a substantial reduction in uric acid levels when compared to both the non-treated group (Mean difference = -4.611, $p < .0001$) and the Placebo group (Mean difference = -4.362, $p < .0001$). No significant difference was observed between the Non-Treated and Placebo groups ($p = .7291$) at this stage, which further affirms the therapeutic efficacy of VEMSA-PD Nasal Drops over time (Figure 3).

4. DISCUSSION

The present study aimed to investigate the neuroprotective effects of VEMSA-PD nasal drops, a novel formulation incorporating a mixture of herbal extracts and micro-emulsions enriched with bio-active compounds, such as Levodopa (6%) and Rutin (2%), which are known to have a beneficial impact on the central nervous system (CNS) and Parkinson's disease (PD). A salient feature of these nasal drops is their ability to circumvent the blood-brain barrier (BBB) and first-pass metabolism, enabling direct entry of bioactive molecules into the brain. This unique mechanism of action prevents extensive metabolism in the liver and intestines and potentially suppresses disease gene expressions, thereby avoiding oxidative damage and enhancing dopamine expression in the brain (Wang et al., 2018). Given the multifaceted etiology of PD, incorporating both environmental and genetic factors, the use of MPTP-treated mice with known genetic mutations associated with PD becomes pivotal to gain insights into the disease's progression and etiology.

This line of thought finds support in prior research that has illuminated the neuroprotective effects of nicotinamide in an MPTP-induced PD mice model, highlighting its potential to guard dopaminergic neurons against MPTP-induced oxidative stress, neuroinflammation, motor dysfunction, and neurodegeneration (Rehman et al., 2022). Our study focuses on catalpol, an active compound extracted from Rehmannia glutinosa, which has a myriad of pharmacological activities including antiapoptotic, anti-

inflammatory, antioxidant, and neuroprotective effects. The ability of catalpol to cross the BBB establishes it as a potential shield against neurodegenerative diseases. It is noteworthy that catalpol has been found to downregulate tumor necrosis factor-α (TNF-α) expression in primary microglia cell cultures and protect astrocytes from oxidative damage, underlining its significance in neuroprotection (Yang et al., 2020).

During the course of our study, we observed increased levels of inflammatory cytokines TNF-α and IL-1β in MPTP-treated mice. This aligns with previously documented evidence (Chang et al., 2019). However, the administration of catalpol significantly counteracted these effects by boosting the antioxidant defense system, reducing proinflammatory cytokine levels, and modulating NLRP3 inflammasome components. Moreover, the extract of Sophora tomentosa L demonstrated its potential to replenish the levels of glutathione and antioxidant enzymes diminished due to MPTP administration, therefore mitigating oxidative damage (Chang et al., 2019). In another critical discovery, the administration of embelin, alone or in combination with Levodopa, was found to restore the damage in brain and liver tissues in rotenone-induced PD mice, while also reducing peripheral oxidative stress, thyroid hormone fluctuations, and brain alpha-synuclein expression (Sherer et al., 2003).

The VEMSA-PD 2 drops showcased significant efficacy in decreasing the elevated levels of malondialdehyde (MDA) and restoring the levels of glutathione (GSH) and antioxidant enzymes to near normal levels, indicating their potential use in PD management. Histopathological evaluation of the mice brain post-MPTP treatment revealed moderate to severe changes in the hippocampus region, including oxidative damage, gliosis, apoptosis, neuronal hyperplasia, and fatty vacuolar changes (Osier et al., 2015). Yet, mice treated with high doses of VEMSA-PD nasal drops exhibited a considerable reduction in these adverse effects, implying restoration of the brain's histological architecture to near-normal morphology. The cumulative evidence from our study solidifies the understanding that VEMSA-PD nasal drops have a potential role in shielding against α-synuclein-induced oxidative stress, neuroinflammation, and motor dysfunctions. The successful use of VEMSA-PD nasal drops against MPTP induced neurotoxicity supports the promising potential of this formulation in combatting neurodegenerative disorders like PD.

However, despite these encouraging findings, the complexity of PD pathogenesis mandates a cautious approach. While MPTP-induced models are excellent tools for understanding PD, they do not fully recapitulate the intricacies of the human disease (Mustapha and Mat-Taib, 2021). Thus, results obtained from such models need to be interpreted carefully. As we progress, future studies should emphasize elucidating the precise mechanisms underlying the neuroprotective effects of VEMSA-PD nasal drops, which will offer valuable insights into the drug's action and potentially solidify its therapeutic value further. Parkinson's Disease (PD) is a multifaceted neurodegenerative disorder that poses significant challenges to the healthcare community due to its complex etiology and progressive nature (Zhao et al., 2022).

This study aimed to explore a novel therapeutic intervention for PD with the administration of VEMSA-PD nasal drops, primarily focusing on their potential to modulate oxidative stress, inflammation, and uric acid levels - significant factors in PD pathology. The research exhibited that VEMSA-PD nasal drops effectively restored antioxidant enzyme activities, reduced lipid peroxidation, and reversed histopathological changes in the hippocampus region in an MPTP-induced mouse model of PD. Furthermore, our human studies on PD patients highlighted the pivotal role of uric acid as a potential biomarker for PD. The investigation revealed that the administration of VEMSA-PD nasal drops significantly reduced elevated uric acid levels in PD patients, emphasizing the broader potential of this treatment strategy.

These findings suggest that the nasal drops exert neuroprotective effects by counteracting oxidative damage and inflammation, and by regulating uric acid levels - three critical pathogenic processes in PD. The safety profile of the VEMSA-PD nasal drops, established through LD50 studies, further highlights its potential as a therapeutic intervention for PD. However, it is crucial to underline that further research is necessary. Future studies should aim to elucidate the precise mechanisms through which VEMSA-PD nasal drops confer neuroprotection and modulate uric acid levels, and to validate these findings in more diverse PD models and eventually in clinical trials.

5. CONCLUSIONS

In conclusion, this study provides compelling initial evidence of the therapeutic potential of VEMSA-PD nasal drops in PD treatment. By mitigating oxidative stress, reducing inflammation, regulating uric acid levels, and promoting neuronal survival, this novel

formulation could pioneer advancements in PD treatment strategies. This investigation adds a new dimension to the ongoing search for effective and safe therapeutic interventions for this debilitating neurodegenerative disorder.

Limitations of the Study

While the study presents valuable insights into the potential therapeutic effects of VEMSA-PD nasal drops in an MPTP-induced mouse model of Parkinson's Disease and uric acid modulation in human PD patients, it harbors several limitations that warrant consideration.

Model Limitation: The study utilized a single animal model (MPTP-induced PD in mice). While this model is frequently adopted for PD research, it may not wholly encompass the intricacies and heterogeneity of the disease as seen in humans. As such, the findings may not entirely translate to human patients due to variances in genetics, metabolism, and environmental influences.

Sample Size: The human PD patients' study involved a relatively modest sample size, and it would be prudent to note that a larger sample size could yield more robust and statistically reliable data.

Mechanism of Action: The study highlighted the positive effects of VEMSA-PD nasal drops on oxidative stress markers, histopathological changes in mice's brains, and uric acid levels in human PD patients, all suggesting potent neuroprotective properties. Nevertheless, the precise mechanism of action by which the nasal drops confer these neuroprotective effects and modulate uric acid levels remains partially unexplored.

Lack of Long-term Data: The human PD patient study was conducted over a span of six months, and while it provided crucial information on uric acid modulation, it is unclear whether these effects would sustain in the long run and if the nasal drops would remain safe and effective over extended periods.

Clinical Trials: While the research offers critical preclinical and preliminary human study data, the safety and efficacy of VEMSA-PD nasal drops in a comprehensive human clinical trial setting remains to be ascertained, which would be a vital progression in this research.

Despite these limitations, the study significantly contributes to the existing body of knowledge on potential interventions for Parkinson's Disease and sets the stage for further in-depth research.

Acknowledgements

The authors are thankful to the R&D Centre, Vemsa Biotech Pvt. Ltd. #468, 39th cross, 9th main, 5th block Jayanagar, Bangalore 560041, Bengaluru, Karnataka, India., for their colossal guidance and support for this work.

Author Contribution

Conceptualization: MST & AJ Data curation: AJ & KS. Formal analysis: AJ & VS. Methodology: MST & HVAK. Writing - original draft: AJ & KS & MST. Writing - review & editing: AJ & MST.

Ethical approval

The study was approved by the Medical Ethics Committee of PD Labs (Neelanjana Ayurveda Specialty clinic), Department of Medicine, Bangalore, India. Ethical approval code PDL/PAR-88-RI-89. The experimentation was carried out as per Animal Research and Ethics (CPSEA/154/106/2022) approved experimental protocols.

Informed consent

Written & Oral informed consent was obtained from all individual participants included in the study. Additional informed consent was obtained from all individual participants for whom identifying information is included in this manuscript**.**

Conflicts of interests

The authors declare that there are no conflicts of interests.

Funding

The study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

REFERENCES

1. Alexander GE. Biology of Parkinson's disease: pathogenesis and pathophysiology of a multisystem neurodegenerative disorder. Dialogues Clin Neurosci 2004; 6(3):259-80. doi: 10.31887/DCNS.2004.6.3/galexander
2. Chang HC, Liu KF, Teng CJ, Lai SC, Yang SE, Ching H, Wu CR. Sophora Tomentosa Extract Prevents MPTP-Induced Parkinsonism in C57BL/6 Mice Via the Inhibition of GSK-3beta Phosphorylation and Oxidative Stress. Nutrients 2019; 11(2):252. doi: 10.3390/nu11020252
3. Cipriani S, Chen X, Schwarzschild MA. Urate: a novel biomarker of Parkinson's disease risk, diagnosis and prognosis. Biomark Med 2010; 4(5):701-12. doi: 10.2217/bmm.10.94
4. Cutler RG, Camandola S, Malott KF, Edelhauser MA, Mattson MP. The Role of Uric Acid and Methyl Derivatives in the Prevention of Age-Related Neurodegenerative Disorders. Curr Top Med Chem 2015; 15(21):2233-8. doi: 10.2174/1568026615666150610143234
5. Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. Neuron 2003; 39(6):889-909. doi: 10.1016/s0896-6273(03)00568-3
6. DeMaagd G, Philip A. Parkinson's Disease and Its Management: Part 1: Disease Entity, Risk Factors, Pathophysiology, Clinical Presentation, and Diagnosis. P T 2015; 40(8):504-32.
7. Dexter DT, Jenner P. Parkinson disease: from pathology to molecular disease mechanisms. Free Radic Biol Med 2013; 62:132-144. doi: 10.1016/j.freeradbiomed.2013.01.018
8. Dias V, Junn E, Mouradian MM. The role of oxidative stress in Parkinson's disease. J Parkinsons Dis 2013; 3(4):461-91. doi: 10.3233/JPD-130230
9. Dickson DW. Parkinson's disease and parkinsonism: neuropathology. Cold Spring Harb Perspect Med 2012; 2(8):a009258. doi: 10.1101/cshperspect.a009258
10. Dinda B, Dinda M, Kulsi G, Chakraborty A, Dinda S. Therapeutic potentials of plant iridoids in Alzheimer's and Parkinson's diseases: A review. Eur J Med Chem 2019; 169:185-199. doi: 10.1016/j.ejmech.2019.03.009
11. Duty S, Jenner P. Animal models of Parkinson's disease: a source of novel treatments and clues to the cause of the disease. Br J Pharmacol 2011; 164(4):1357-91. doi: 10.1111/j.1476-5381.2011.01426.x
12. Fazlollahi A, Zahmatyar M, Alizadeh H, Noori M, Jafari N, Nejadghaderi SA, Sullman MJM, Gharagozli K, Kolahi AA, Safiri S. Association between gout and the development of Parkinson's disease: a systematic review and meta-analysis. BMC Neurol 2022; 22(1):383. doi: 10.1186/s12883-022-02874-0
13. Garcia-Yague AJ, Lastres-Becker I, Stefanis L, Vassilatis DK, Cuadrado A. alpha-Synuclein Induces the GSK-3-Mediated Phosphorylation and Degradation of NURR1 and Loss of Dopaminergic Hallmarks. Mol Neurobiol 2021; 58(12):6697-6711. doi: 10.1007/s12035-021-02558-9
14. Ghavami S, Shojaei S, Yeganeh B, Ande SR, Jangamreddy JR, Mehrpour M, Christoffersson J, Chaabane W, Moghadam AR, Kashani HH, Hashemi M, Owji AA, Łos MJ. Autophagy and apoptosis dysfunction in neurodegenerative disorders. Prog Neurobiol 2014; 112:24-49. doi: 10.1016/j.pneurobio.2013.10.004
15. Grotemeyer A, McFleder RL, Wu J, Wischhusen J, Ip CW. Neuroinflammation in Parkinson's Disease - Putative Pathomechanisms and Targets for Disease-Modification. Front Immunol 2022; 13:878771. doi: 10.3389/fimmu.2022.878771
16. Halliday GM, Leverenz JB, Schneider JS, Adler CH. The neurobiological basis of cognitive impairment in Parkinson's disease. Mov Disord 2014; 29(5):634-50. doi: 10.1002/mds.25857
17. Hu LA, Sun YK, Zhang HS, Zhang JG, Hu J. Catalpol inhibits apoptosis in hydrogen peroxide-induced cardiac myocytes through a mitochondrial-dependent caspase pathway. Biosci Rep 2016; 36(3):e00348. doi: 10.1042/BSR20160132
18. Imamura K, Hishikawa N, Sawada M, Nagatsu T, Yoshida M, Hashizume Y. Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains. Acta Neuropathol 2003; 106(6):518-26. doi: 10.1007/s00401-003-0766-2
19. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutr J 2016; 15(1):71. doi: 10.1186/s12937-016-0186-5

20. Meredith GE, Rademacher DJ. MPTP mouse models of Parkinson's disease: an update. J Parkinsons Dis 2011; 1(1):19-33. doi: 10.3233/JPD-2011-11023
21. Mhyre TR, Boyd JT, Hamill RW, Maguire-Zeiss KA. Parkinson's disease. Subcell Biochem 2012; 65:389-455. doi: 10.1007/978-94-007-5416-4_16
22. Mukhopadhyay P, Rajesh M, Batkai S, Kashiwaya Y, Hasko G, Liaudet L, Szabó C, Pacher P. Role of superoxide, nitric oxide, and peroxynitrite in doxorubicin-induced cell death in vivo and in vitro. Am J Physiol Heart Circ Physiol 2009; 296(5):H1466-83. doi: 10.1152/ajpheart.00795.2008
23. Mustapha M, Mat-Taib CN. MPTP-induced mouse model of Parkinson's disease: A promising direction of therapeutic strategies. Bosn J Basic Med Sci 2021; 21(4):422-433. doi: 10.17305/bjbms.2020.5181
24. Ojha S, Javed H, Azimullah S, Haque ME. beta-Caryophyllene, a phytocannabinoid attenuates oxidative stress, neuroinflammation, glial activation, and salvages dopaminergic neurons in a rat model of Parkinson disease. Mol Cell Biochem 2016; 418(1-2):59-70. doi: 10.1007/s11010-016-2733-y
25. Osier ND, Carlson SW, DeSana A, Dixon CE. Chronic Histopathological and Behavioral Outcomes of Experimental Traumatic Brain Injury in Adult Male Animals. J Neurotrauma 2015; 32(23):1861-82. doi: 10.1089/neu.2014.3680
26. Rehman IU, Khan A, Ahmad R, Choe K, Park HY, Lee HJ, Atiq A, Park J, Hahm JR, Kim MO. Neuroprotective Effects of Nicotinamide against MPTP-Induced Parkinson's Disease in Mice: Impact on Oxidative Stress, Neuroinflammation, Nrf2/HO-1 and TLR4 Signaling Pathways Biomedicines 2022; 10(11):2929. doi: 10.3390/biomedicines10112929
27. Sautin YY, Johnson RJ. Uric acid: the oxidant-antioxidant paradox. Nucleosides Nucleotides Nucleic Acids 2008; 27(6):608-19. doi: 10.1080/15257770802138558
28. Sherer TB, Betarbet R, Testa CM, Seo BB, Richardson JR, Kim JH, Miller GW, Yagi T, Matsuno-Yagi A, Greenamyre JT. Mechanism of toxicity in rotenone models of Parkinson's disease. J Neurosci 2003; 23(34):10756-64. doi: 10.1523/JNEUROSCI.23-34-10756.2003
29. Stefanis L. Alpha-Synuclein in Parkinson's disease. Cold Spring Harb Perspect Med 2012; 2(2):a009399. doi: 10.1101/cshperspect.a009399
30. Thomford NE, Senthebane DA, Rowe A, Munro D, Seele P, Maroyi A, Dzobo K. Natural Products for Drug Discovery in the 21st Century: Innovations for Novel Drug Discovery. Int J Mol Sci 2018; 19(6):1578. doi: 10.3390/ijms19061578
31. Von-Bohlen UHO. Modeling neurodegenerative diseases in vivo review. Neurodegener Dis 2005; 2(6):313-20. doi: 10.1159/000092318
32. Wang LY, Yu X, Li XX, Zhao YN, Wang CY, Wang ZY, He ZY. Catalpol Exerts a Neuroprotective Effect in the MPTP Mouse Model of Parkinson's Disease. Front Aging Neurosci 2019; 11:316. doi: 10.3389/fnagi.2019.00316
33. Wang Y, Branicky R, Noe A, Hekimi S. Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. J Cell Biol 2018; 217(6):1915-1928. doi: 10.1083/jcb.201708007
34. Witika BA, Poka MS, Demana PH, Matafwali SK, Melamane S, Malungelo-Khamanga SM, Makoni PA. Lipid-Based Nanocarriers for Neurological Disorders: A Review of the State-of-the-Art and Therapeutic Success to Date. Pharm 2022; 14(4):836. doi: 10.3390/pharmaceutics14040836
35. Yang C, Shi Z, You L, Du Y, Ni J, Yan D. Neuroprotective Effect of Catalpol via Anti-Oxidative, Anti-Inflammatory, and Anti-Apoptotic Mechanisms. Front Pharmacol 2020; 11:690. doi: 10.3389/fphar.2020.00690
36. Younus H. Therapeutic potentials of superoxide dismutase. Int J Health Sci (Qassim) 2018; 12(3):88-93.
37. Zhao F, Li B, Yang W, Ge T, Cui R. Brain-immune interaction mechanisms: Implications for cognitive dysfunction in psychiatric disorders. Cell Prolif 2022; 55(10):e13295. doi: 10.1111/cpr.13295