Gut-brain axis: Review on the association between Parkinson's disease and plant lectins

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ABSTRACT

Gastrointestinal (GI) involvement in the pathogenesis of Parkinson's Disease (PD) has been widely recognized and supported in recent literature. Prospective and retrospective studies found non-motor symptoms within the GI, specifically constipation, precede cardinal signs and cognitive decline by almost 20 years. In 2002, Braak et al. were the first to propose that PD is a six-stage propagating neuropathological process originating from the GI tract (GIT). Aggregated α-synuclein (α-syn) protein from the GIT is pathognomonic for the development of PD. This article reviews the current literature from the past 10 years as well as original research found in PubMed on the combined effects of enteric glial cells and lectins on the development of Parkinson's Disease. Studies have found that these aggregated and phosphorylated proteins gain access to the brain via retrograde transport through fast and slow fibers of intestinal neurons. Plant lectins, commonly found within plant-based diets, have been found to induce Leaky Gut Syndrome and can activate enteric glial cells, causing the release of pro-inflammatory cytokines. Oxidative stress on the enteric neurons, caused by a chronic neuro-inflammatory state, can cause a-syn aggregation and lead to Lewy Body formation, a hallmark finding in PD. Although the current literature provides a connection between the consumption of plant lectins and the pathophysiology of PD, further research is required to evaluate confounding variables such as food antigen mimicry and other harmful substances found in our diets.

KEYWORDS: Parkinson's Disease; Enteric Nervous System; Lewy Bodies; Gastrointestinal Tract; Plant Lectin

■ 1. INTRODUCTION

1.1 Parkinson's Disease

Parkinson's Disease (PD) is the second most common neurodegenerative disease only second to Alzheimer's Disease, affecting 1-2 per 1000 persons at any time, and 1% of the population above the age of 60 [1]. It was first described by James Parkinson in 1817 when he investigated the cardinal motor signs used today to diagnose PD (i.e., bradykinesia, rigidity, and tremor) [1]. However, postural instability, a fourth cardinal sign, can help clinicians better recognize PD [2]. Indeed, PD is a clinical diagnosis relying on signs and symptoms alone and cannot be confirmed until an autopsy.

PD is characterized by its hallmark findings of dopaminergic neuron loss within the substantia nigra, the center for motor coordination, and Lewy pathology (LP) [1,3,4]. Lewy bodies (LB) and neurites (LN), or eosinophilic inclusions, are accumulations of alpha-synuclein (α-syn), a presynaptic neuronal protein that can be found in the olfactory bulb, spinal cord, peripheral autonomic ganglia, submandibular gland, cardiac nerves, red blood cells, cerebrospinal fluid, plasma, and enteric nervous system (ENS) [3,5,6]. α-syn accounts for nearly 1% of total protein in soluble cytosolic brain fractions and plays a vital role in neuronal function by modulating membrane stability and vesicular transport [6]. Its structural configuration can be segmented into three domains: 1) the alpha-helical N-terminal that anchors α-syn to membranes; 2) the central hydrophobic core with aggregating potential when forming beta-sheets; and 3) the negatively charged, proline-rich, C-terminal that is able to counteract the aggregating potential induced by the central core [6]. The physiological role of a-syn may be altered following pathological phosphorylation and aggregation-ultimately leading to disease [7]. Additionally, environmental exposures such as low pH and salts can induce structural alterations of α-syn and promote the formation of LB and LN [7].

Accumulation of these misfolded proteins outside the central nervous system (CNS) can manifest additional nonmotor symptoms such as constipation, dysphagia, sialorrhea, nausea, vomiting, early satiety, gastroparesis, and postprandial fullness [5]. Although constipation is a

well-recognized symptom of PD, the intricate connection between the CNS and GIT was not thoroughly explored until the early 2000s [5]. Since then, Hawkes and Warnecke [3,5] have found the onset of nonmotor symptoms to precede the cardinal signs and cognitive decline by almost 20 years.

While there is no cure for PD, pharmacologic agents such as Levodopa (L-dopa) are still the most effective therapeutic options [4]. The effect of L-dopa is amplified when combined with Carbidopa, which inhibits its peripheral degradation, resulting in enhanced symptomatic relief [4]. However, management becomes more challenging as the disease progresses due to "wearing-off" a phenomenon in which L-dopa's therapeutic effect is reduced before the next dose [4]. While L-dopa may provide relief for motor symptoms of PD by enhancing neuronal activity and communication within the striatum and striato-cortical motor pathway, it may also exacerbate GI discomfort [3]. Patients may also develop dysphagia (82% prevalence), leading to retention of oral medications in the pharynx and unpredictable drug absorption or clinical response [5]. Consequently, additional therapies such as dopaminergic agonists, monoamine oxidase B (MAO-B) inhibitors, and anticholinergics may also be added to alleviate on-off symptoms [4].

1.2 Gastrointestinal Tract

The GIT is a complex series of organs spanning from the mouth to the anus, which is necessary for its numerous functions, including digestion, absorption, and protection against foreign invasion. Most of these activities occur in the stomach, small intestine, and large intestine [8].

The mesentery joins the serosa to form an outer layer that lubricates and supports contractions. Contractions are carried out by two layers of smooth muscle, an inner circular layer, and an outer longitudinal layer. The lamina propria, a highly vascularized structure deep to the muscular layers that is rich in connective tissue, enables the transport of foreign antigens and rapid immune response [8]. Structures within the lamina propria, such as Peyer's patches and gut-associated lymphoid tissue (GALT), contain both adaptive and innate immune cells [8]. The selective permeability of the GIT can be credited to an epithelium composed of distinct cells bound together by tight junctions (TJ) [9]. TJs form barriers between cells that regulate the paracellular transport of ions and water [10]. Together the lamina propria and epithelium, collectively known as the mucosa, regulate absorption, but are moderated by extrinsic factors such as nerves, hormones, and cytokines [9].

Ganglia networks between these muscular layers are the Myenteric (Auerbach) and Submucosal (Meissner) plexuses, together known as the intrinsic ENS [11]. The ganglia are primarily composed of enteric glial cells (EGCs). Like astrocytes in the CNS, which maintain the blood-brain barrier, EGCs receive and propagate neuronal signals and help maintain the epithelial barrier in the GIT [12]. Both are pertinent to ensuring the GIT functions properly. For example, in the Myenteric plexus, which lies between the two muscular layers, neuronal signaling by EGCs generate 'slow waves' in surrounding smooth muscle cells [8]. Regular, continuous changes in the membrane potential increase the likelihood of smooth muscle contractions, which propel food through the GIT. Likewise, EGCs in the submucosal plexus, found between the muscular layers and lamina propria, promote epithelial barrier function [13].

This role is in addition to the submucosal plexus' responsibility to maintain mucus production, uphold the pH of the lumen, and regulate blood flow [14]. The intrinsic ENS is not only pertinent to GIT functions but also so extensive that it is often referred to as the "second brain" [15]. EGCs continue to generate slow waves in smooth muscle cells even after the Vagus nerve is severed, indicating that this "second brain" is capable of functioning independently [16]. However, in addition to the VN, GI activity is significantly moderated by other extrinsic nerves and hormones [8].

Likewise, hormones including neuropeptide Y (NPY), Acetylcholine (ACh), and vasoactive intestinal peptide (VIP), regulate absorption at the epithelium [17]. VIP can also slow the rate of paracellular transport by downregulating the transcription of zona occludens, a protein that makes up tight junctions [18]. In contrast, ACh stimulates enterochromaffin-like cells and NPY upregulates a distinct TJ protein, claudin-2; which both cause an increased epithelial permeability [19]. TJ expression is also affected by diet. For example, high protein diets promote zona occludens, and occludin synthesis resulting in higher TJ expression [20]. In contrast, TJ expression is lessened in high-carb and high-fat diet [20]. However, the mucosal barrier remains impermeable to larger molecules such as sugars, amino acids, and vitamins, which must be absorbed transcellularly [21]. Transcellular transport is primarily dependent on specific protein channels, but some absorption may also be done by endocytosis [21].

Though there are many safeguards to ensure the integrity of the epithelium, the GALT is packed with primed immune cells in the lamina propria [22]. A compromise in the epithelium will result in a vigorous immune response. When activated, EGCs in both the Myenteric and Submucosal plexuses can release an array of pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-α, growth factors as glial cell-derived neurotrophic factor and nerve growth factor, and other immunomodulatory signal molecules like nitric oxide [23].

1.3 Gut-Brain Axis of Parkinson's Disease

An unknown pathogen in the gut may be responsible for the subclinical onset of PD, according to Braak et al. [24]. This hypothesis states that after traversing the mucosal barriers and brush borders of the GIT, a neurotropic pathogen could infiltrate VIP neurons in Auerbach's plexus and pass to fibers of the dorsal motor nucleus of the vagus nerve (dmX) (Fig. 1). From the dmX, Braak [24] suggests that the pathogen travels to the "gain setting system" consisting of the medullary raphe nuclei, the gigantocellular nucleus, and the locus coeruleus, which are connected directly to the amygdala via descending projections from the amygdala's central subnucleus. The central subnucleus' projections also provide the upward pathway for the pathogen, and ensuing LBs, to reach the cholinergic nuclei of the basal forebrain and the substantia nigra pars compacta, where it would begin to cause the motor symptoms characteristic of PD.

Transmission of the unknown pathogen could occur via retrograde axonal transport. The pathogen may enter neurons via receptor-mediated endocytosis at the presynaptic bouton and travel to the soma via retrograde transport [24]. Transfer to the next neuron could occur via specific receptor-mediated endocytosis at the presynaptic membrane or direct transfer via species-specific types of synapses [25-28].
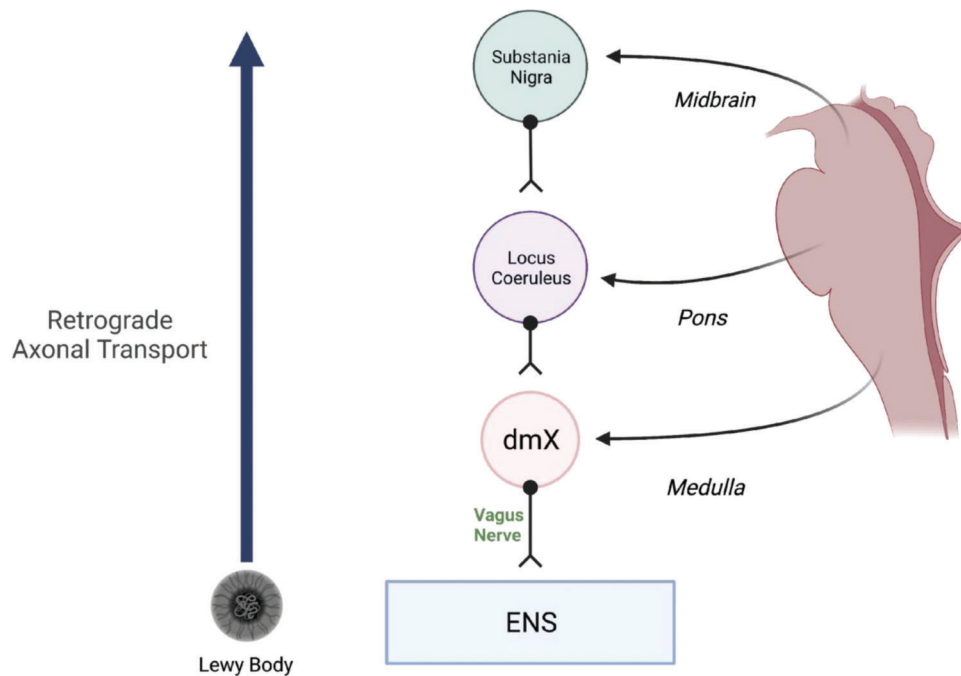
Fig. 1. This figure illustrates Braak's hypothesis: a-synuclein forms aggregates called Lewy bodies, which then travel retrogradely from the ENS to the dorsal motor nucleus (dmX) located in the medulla via the vagus nerve. From there, they continue cephalically to the locus coeruleus located in the Pons, and finally reach the substantia nigra located in the midbrain. The dopaminergic neurons found in the substantia nigra can be damaged by a Lewy body and lead to the classic neurologic manifestations of PD.

Holmqvist et al. [29] injected human PD lysate containing fibrillar, monomeric, and oligomeric forms of α-syn, as well as recombinant α-syn into the duodenum and stomach of rats and were able to trace the passage of the α-syn to the VN and the dmX, providing evidence of the viability of this pathway.

Subsequent research supports a "dual-hit" hypothesis for the development of PD. The first part of the "dual-hit" is the inhalation of the pathogen into the nasal cavity from where it could enter the olfactory nerve and travel in an anterograde fashion to the temporal lobe [30]. The second "hit" would occur once the nasal secretions where they would cross the gastric lining into the axons of Meissner's plexus before traveling to the vagal nucleus [30].

Evidence of LP has been observed in nuclei of the VN and the dmX before it spreads to other parts of the CNS commonly affected in PD, such as the locus coeruleus, mesocortex, neocortex, or prefrontal cortex [24,31-34]. This suspected viral pathogen could consist of misfolded α-syn and possess prion-like abilities to induce conformational change and aggregation of nearby normal α-syn molecules, leaving behind LB/LNs as it traveled [30,34]. Svensson et al.'s [35] recent study showed that a truncal vagotomy might help reduce the long-term risk of developing PD, as this would obstruct the pathway from the ENS to the CNS. Additional evidence for the potential utility of truncal vagotomy comes from animal models. Pan-Motojo et al. [36] induced a PD-like state in experimental rats with an intragastric dose of rotenone, a pesticide known to induce LBs. Truncal vagotomy successfully prevented the effects of rotenone on the CNS.

Exposure to herbicides and pesticides has also been linked to the development of PD. Rotenone, a mitochondrial inhibitor, has been shown in rats to promote both the release of α-syn from myenteric neurons, its uptake by vagal fibers,

and its transport to the brainstem, decreasing preganglionic parasympathetic dmX neurons [36]. The α-syn accumulates in the neuron's soma where it coalesces into LBs [36]. The herbicide paraquat induces parkinsonism in experimental rats by attenuating gastric motility response to stimulation from the nigro-vagal pathway and increasing the concentration of misfolded α-syn in myenteric and dmX neurons [37,38]. The development of α-synucleopathy and parkinsonism after oral or intraperitoneal exposure to pesticides and herbicides supports Braak's hypothesis that PD can be established by a pathogen traveling from the ENS and vagal pathway to the CNS [24,39].

1.4 Plant Lectins

Lectins are carbohydrate-binding proteins with agglutination properties that are found in both the animal and plant kingdom [40]. The discovery of plant lectins started with the study done by Hermann Stillmark in 1888, which indicated that specific seed extracts from castor beans (*Ricinus communis* L.) caused agglutination of erythrocytes [41]. They chose to call this protein agglutinin, 'ricin.' Subsequently, it was discovered that ricin poisoning from castor seed oil can cause convulsions, muscle weakness, leukocytosis, and hyperemia of several organs, including the spleen, bone marrow, and liver [42]. Michaelis and Steindorff then found that ricin also caused agglutination of the epithelial cells of the mucosal barrier of the intestinal system, causing gastrointestinal disruption after ingestion at a specific dose [43]. The term 'lectin' was coined shortly after research by Watkins and Morgan found that the agglutination of lectins was dependent on the recognition and specificity of binding to carbohydrate residues on the cell surface of erythrocytes [43,44].

As plant lectins were further studied, they began to be classified via subcellular localization, molecular structure, genomic sequence, and abundance [45]. Regarding abundance, high amounts of lectins have been discovered in legumes (i.e. beans, peas, lentils, peanuts), wheat germ, tomatoes, mushrooms, cabbage, and many other common plant-based foods [40]. These findings have raised concern for several unbalanced diets such as vegetarianism or veganism that may overutilize these specific plant-based foods.

It is hypothesized that plant lectins have emerged and become more widely distributed due to their ability to defend against microorganisms (bacteria, viruses, and fungi), insects, and plant-eating animals [46]. This hypothesis has been linked to the carbohydrate-binding properties of lectins. For example, it has been found that extracellular lectins found in the roots of plants have a role in defense against bacteria by immobilizing copious quantities via carbohydrate residue recognition and agglutination [47]. In addition to their agglutinin properties, plant lectins are resistant to proteolysis both in vitro and in vivo and have been stable through a wide range of pH [40]. These characteristics help explain the mechanism in which plant lectins survive the gastric pH of plant-eating animals and cause deleterious systemic and gastrointestinal effects [48].

One of the most prominent effects that plant lectins have on the GIT is the induction of a phenomenon called leaky gut syndrome (LGS). LGS is a condition in which TJs are disrupted and gaps are formed in the intestinal wall, causing paracellular hyperpermeability [49]. This enables various hostile antigens or toxins to invade directly into the lamina propria, bloodstream, and plexus [50]. An animal study involving mammalian rats showed that when phytohemagglutinin (PHA), a plant lectin derived from kidney bean (*Phaseolus vulgaris*) seeds, was ingested, there was increased mucosal epithelial cell turnover and TJ disruption, leading to increased intestinal permeability [51]. This observation is explained by lectin's ability to bind to glycosylated residues on the gut epithelial cell surface [52]. Since carbohydrate residues on epithelial surfaces are similar between mammals,

these findings suggest that lectins may also increase gut permeability in humans [53].

1.5 Combined Effects of Immune cells and Lectins on the development of PD

Tight junctions (TJ) in the GIT are imperative for a physiologic divide between luminal contents and underlying mucosa. Disruption of TJs allows foreign antigens found within the lumen to enter the lamina propria, allowing immune cells, like EGCs, to mount a response (Fig. 2) [54,55]. Disruption of TJs may occur secondary to environmental exposures, including toxins, lectins, and macromolecules—leading to LGS [51,56].

Wheat germ agglutinin (WGA), a plant lectin commonly found in breakfast cereals, is known to activate EGCs and trigger a subsequent immune response [57,58]. For example, neutrophil NADP-oxidase activity and basophilic release of IL-4 and IL-13 allow direct access to the surrounding vasculature at the basolateral surface [59,60]. Plant lectins were recently discovered to activate NLRP3 inflammasomes, which are responsible for the maturation and release of proinflammatory cytokines, such as IL-1b and IL-18 [61,62]. In conjunction with pro-inflammatory cytokine release, inflammasome activation leads to pyroptosis, a necrosis-like programmed cell death [63]. Thus, when indigestible plant lectins pass through the leaky barrier, they can immunologically prime EGCs and inflammasomes to promote a cyclic enteric neuroinflammatory response [23]. This multifaceted immune response contributes to the breakdown of TJs, death of enterocytes, and persistent oxidative injury to the "second brain."

Enteric neurons exposed to oxidative stress and neuroinflammation lead to phosphorylation and aggregation of α-syn [64,65]. Since a-syn is found in the presynaptic apparatus of enteric neurons, the ongoing oxidative stress and neuroinflammation from EGC activation can lead to the pathological formation of LBs [65,66]. The most likely explanation is that oxidative stress creates free radicals that disrupt mitochondrial function [65]. This may cause a
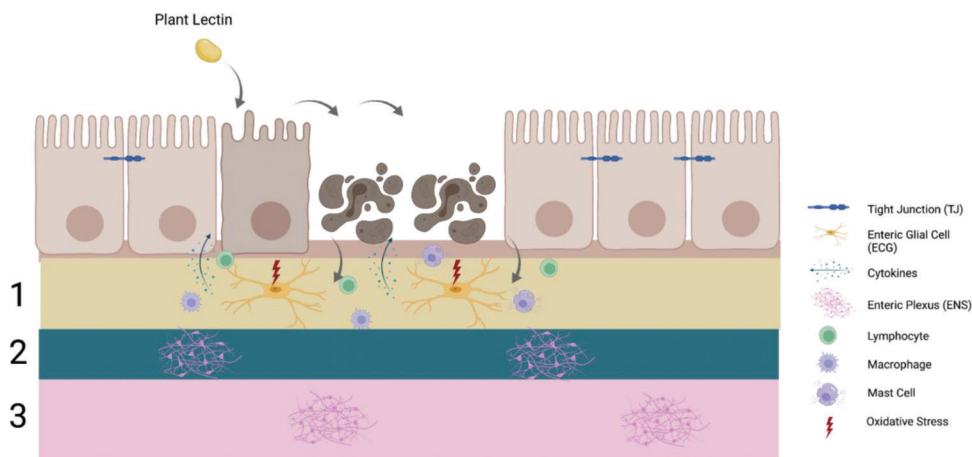
Fig. 2. Directly beneath the basement membrane lies the lamina propria and muscularis mucosae (1), followed by the submucosa (2), which houses the submucosal plexus, and muscularis propria (3), which houses the Auerbach's plexus. Plant lectins can downregulate and interrupt TJ stability causing leaky gut syndrome. This can result in foreign particles and viruses traversing the epithelium and enter the lamina propria and enteric plexus (ENS). Plant lectins can also activate the EGC, releasing pro-inflammatory cytokines from the lymphocytes, macrophages, and mast cells, continuing a positive feedback loop of inflammation. This inflammatory response can lead to oxidative stress, resulting in a-syn structural changes and accumulation within the ENS.

reduction in degradation and an accumulation of intracellular α-syn, predisposing LB formation [67]. It may also alter folding and post-transcriptional modification of a-syn causing aggregation [65]. Ultimately, lectin-induced activation of EGC and inflammasome formation results in a cyclic oxidative injury to the enteric neurons leading to the accumulation of intracellular a-syn and LB formation.

As mentioned, constipation is the earliest and most crucial prodromal symptom of PD [68-70]. PD pathogenesis is thought to be a result of EGC alteration and activation, as this has been shown to cause changes in GIT motility both in vivo and in vitro [71]. Plant lectins persist in the GIT since they can withstand the harsh environment and avoid expulsion due to constipation, which adds to the chronic inflammatory state and a constant insult to the ENS. Considering Braak's hypothesis, it is evident the LBs formed in the ENS due to indirect injury from plant lectins, have the means to travel retrogradely towards the substantia nigra and cause PD.

■ 2. FUTURE PERSPECTIVES

Aside from neuroinflammation and oxidative stress, lectins have been recently linked to other mechanisms in the etiology of PD. Vojdani et al. [72] recently discovered high peptide sequence homology between α-syn and many food antigens such as yeast, soybean agglutinin, WGA, pea lectins, and lentil lectins through cross-reactivity with recombinant monoclonal α-syn antibodies. With this research, they inferred that once past the mucosal barrier, these food antigens that resembled a-syn would aggregate within the enteric cells and then migrate cephalically towards the substantia nigra. There is limited literature directly linking lectins to a-syn antibodies, and further studies are needed to expand our understanding of this pathophysiology.

Though current research is exploring the connection between plant lectins and LGS and between LGS and PD, future longitudinal studies should evaluate the direct correlation between plant lectins and PD. Recently, a 4-year longitudinal cohort study involving participants from the Memory and Aging Project (MAP) assessed diet via questionnaire [73]. The study showed that greater adherence to a MIND (Mediterranean-DASH Diet Intervention for Neurodegenerative Delay) diet, which emphasizes foods such as legumes, nuts, whole grains, and poultry, was associated with lower incidence and progression rates of parkinsonian symptoms [73]. The argument for the utilization of this diet comes from the research that shows that plant-based foods reduce oxidative stress and inflammation, thus reducing α-syn aggregation [74,75]. However, many of the foods in the MIND diet such as legumes and whole grains contain lectins [40], raising the need for investigation into the direct relationship between lectins and PD.

■ CONCLUSIONS

Through EGC-induced cyclic neuroinflammation and oxidative stress, plant lectins may lead to pathological Lewy body formation in the GIT. The Lewy body can then travel retrogradely via the VN to the substantia nigra and contribute to the classic neurologic manifestations of PD. Although the current literature provides a connection between the consumption of plant lectins and the pathophysiology of PD, further research is required to evaluate confounding variables such as food antigen mimicry and other harmful substances found in our diets.

■ REFERENCES

1. Elbaz A, Carcaillon L, Kab S, Moisan F. Epidemiology of Parkinson's disease. *Rev Neurol (Paris)*. 2016;172(1):14-26. PubMed PMID: 26718594. doi: 10.1016/j.neurol.2015.09.012.

2. Gelb DJ, Oliver E, Gilman S. Diagnostic criteria for Parkinson disease. *Arch Neurol.* 1999;56(1):33-9. PubMed PMID: 9923759. doi: 10.1001/archneur.56.1.33.

3. Travagli RA, Browning KN, Camilleri M. Parkinson disease and the gut: new insights into pathogenesis and clinical relevance. *Nat Rev Gastroenterol Hepatol.* 2020;17(11):673-85. PubMed PMID: 32737460. doi: 10.1038/s41575-020-0339-z.

4. Katzenschlager R, Lees AJ. Treatment of Parkinson's disease: levodopa as the first choice. *J Neurol.* 2002;249 Suppl 2:II19-24. PubMed PMID: 12375059. doi: 10.1007/s00415-002-1204-4.

5. Warnecke T, Schafer KH, Claus I, Del Tredici K, Jost WH. Gastrointestinal involvement in Parkinson's disease: pathophysiology, diagnosis, and management. *NPJ Parkinsons Dis.* 2022;8(1):31. PubMed PMID: 35332158; PubMed Central PMCID: PMC8948218. doi: 10.1038/s41531-022-00295-x.

6. Malek N, Swallow D, Grosset KA, Anichtchik O, Spillantini M, Grosset DG. Alpha-synuclein in peripheral tissues and body fluids as a biomarker for Parkinson's disease - a systematic review. *Acta Neurol Scand.* 2014;130(2):59-72. PubMed PMID: 24702516. doi: 10.1111/ane.12247.

7. Uversky VN, Li J, Souillac P, Millett IS, Doniach S, Jakes R, et al. Biophysical properties of the synucleins and their propensities to fibrillate: inhibition of alpha-synuclein assembly by beta- and gamma-synucleins. *J Biol Chem.* 2002;277(14):11970-8. PubMed PMID: 11812782. doi: 10.1074/jbc.M109541200.

8. Yoo BB, Mazmanian SK. The Enteric Network: Interactions between the Immune and Nervous Systems of the Gut. *Immunity.* 2017; 46(6):910-26. PubMed PMID: 28636959; PubMed Central PMCID: PMC5551410. doi: 10.1016/j.immuni.2017.05.011.

9. Suzuki T. Regulation of intestinal epithelial permeability by tight junctions. *Cell Mol Life Sci.* 2013;70(4):631-59. PubMed PMID: 22782113. doi: 10.1007/s00018-012-1070-x.

10. Turner JR. Intestinal mucosal barrier function in health and disease. *Nat Rev Immunol.* 2009;9(11):799-809. PubMed PMID: 19855405. doi: 10.1038/nri2653.

11. Furness JB. The enteric nervous system and neurogastroenterology. *Nat Rev Gastroenterol Hepatol.* 2012;9(5):286-94. PubMed PMID: 22392290. doi: 10.1038/nrgastro.2012.32.

12. Yu YB, Li YQ. Enteric glial cells and their role in the intestinal epithelial barrier. *World J Gastroenterol.* 2014;20(32):11273-80. PubMed PMID: 25170211; PubMed Central PMCID: PMC4145765. doi: 10.3748/wjg.v20.i32.11273.

13. Cirillo C, Sarnelli G, Esposito G, Turco F, Steardo L, Cuomo R. S100B protein in the gut: the evidence for enteroglial-sustained intestinal inflammation. *World J Gastroenterol.* 2011;17(10):1261-6. PubMed PMID: 21455324; PubMed Central PMCID: PMC3068260. doi: 10.3748/wjg.v17.i10.1261.

14. Furness JB, Kunze WA, Bertrand PP, Clerc N, Bornstein JC. Intrinsic primary afferent neurons of the intestine. *Prog Neurobiol.* 1998; 54(1):1-18. PubMed PMID: 9460790. doi: 10.1016/S0301-0082(97) 00051-8.

15. Lebouvier T, Chaumette T, Paillusson S, Duyckaerts C, Bruley des Varannes S, Neunlist M, et al. The second brain and Parkinson's disease. *Eur J Neurosci.* 2009;30(5):735-41. PubMed PMID: 19712093. doi: 10.1111/j.1460-9568.2009.06873.x.

16. Miyano Y, Sakata I, Kuroda K, Aizawa S, Tanaka T, Jogahara T, et al. The role of the vagus nerve in the migrating motor complex and ghrelin- and motilin-induced gastric contraction in suncus. *PLoS One.* 2013;8(5):e64777. PubMed PMID: 23724093; PubMed Central PMCID: PMC3665597. doi: 10.1371/journal.pone.0064777.

17. Keast JR, Furness JB, Costa M. Different substance P receptors are found on mucosal epithelial cells and submucous neurons of the guinea-pig small intestine. *Naunyn Schmiedebergs Arch Pharmacol.* 1985;329(4):382-7. PubMed PMID: 2412139. doi: 10.1007/BF00496372.

18. Neunlist M, Toumi F, Oreschkova T, Denis M, Leborgne J, Laboisse CL, et al. Human ENS regulates the intestinal epithelial barrier permeability and a tight junction-associated protein ZO-1 via VIPergic pathways. *Am J Physiol Gastrointest Liver Physiol.* 2003; 285(5):G1028-36. PubMed PMID: 12881224. doi: 10.1152/ajpgi. 00066.2003.

19. Chandrasekharan B, Jeppsson S, Pienkowski S, Belsham DD, Sitaraman SV, Merlin D, et al. Tumor necrosis factor-neuropeptide Y cross talk regulates inflammation, epithelial barrier functions, and colonic motility. *Inflamm Bowel Dis.* 2013;19(12):2535-46. PubMed PMID: 24108115; PubMed Central PMCID: PMC4180268. doi: 10.1097/01.MIB.0000437042.59208.9f.

20. Cersosimo MG, Benarroch EE. Neural control of the gastrointestinal tract: implications for Parkinson disease. *Mov Disord.* 2008;23(8): 1065-75. PubMed PMID: 18442139. doi: 10.1002/mds.22051.

21. Kiela PR, Ghishan FK. Ion transport in the intestine. *Curr Opin Gastroenterol.* 2009;25(2):87-91. PubMed PMID: 19528875; PubMed Central PMCID: PMC4427515. doi: 10.1097/MOG.0b013e328326 0900.

22. Ahluwalia B, Magnusson MK, Ohman L. Mucosal immune system of the gastrointestinal tract: maintaining balance between the good and the bad. *Scand J Gastroenterol.* 2017;52(11):1185-93. PubMed PMID: 28697651. doi: 10.1080/00365521.2017.1349173.

23. Seguella L, Sarnelli G, Esposito G. Leaky gut, dysbiosis, and enteric glia activation: the trilogy behind the intestinal origin of Parkinson's disease. *Neural Regen Res.* 2020;15(6):1037-8. PubMed PMID: 31823880; PMC7034261. doi: 10.4103/1673-5374.270308.

24. Braak H, Rub U, Gai WP, Del Tredici K. Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. *J Neural Transm (Vienna).* 2003;110(5):517-36. PubMed PMID: 12721813. doi: 10.1007/ s00702-002-0808-2.

25. Prusiner SB. Prions. *Proc Natl Acad Sci U S A.* 1998;95(23):13363-83. PubMed PMID: 9811807; PubMed Central PMCID: PMC33918. doi: 10.1073/pnas.95.23.13363.

26. Wadsworth JD, Jackson GS, Hill AF, Collinge J. Molecular biology of prion propagation. *Curr Opin Genet Dev.* 1999;9(3):338-45. PubMed PMID: 10377292. doi: 10.1016/S0959-437X(99)80051-3.

27. Brown P. The pathogenesis of transmissible spongiform encephalopathy: routes to the brain and the erection of therapeutic barricades. *Cell Mol Life Sci.* 2001;58(2):259-65. PubMed PMID: 11289307. doi: 10.1007/PL00000853.

28. Nicotera P. A route for prion neuroinvasion. *Neuron.* 2001;31(3): 345-8. PubMed PMID: 11516392. doi: 10.1016/S0896-6273(01)00385-3.

29. Holmqvist S, Chutna O, Bousset L, Aldrin-Kirk P, Li W, Bjorklund T, et al. Direct evidence of Parkinson pathology spread from the gastrointestinal tract to the brain in rats. *Acta Neuropathol.* 2014;128 (6):805-20. PubMed PMID: 25296989. doi: 10.1007/s00401-014-1343-6.

30. Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: a dual-hit hypothesis. *Neuropathol Appl Neurobiol.* 2007;33(6):599-614. PubMed PMID: 17961138; PubMed Central PMCID: PMC7194308. doi: 10.1111/j.1365-2990.2007.00874.x.

31. Del Tredici K, Rub U, De Vos RA, Bohl JR, Braak H. Where does parkinson disease pathology begin in the brain? *J Neuropathol Exp Neurol.* 2002;61(5):413-26. PubMed PMID: 12030260. doi: 10.1093/ jnen/61.5.413.

32. Bloch A, Probst A, Bissig H, Adams H, Tolnay M. Alpha-synuclein pathology of the spinal and peripheral autonomic nervous system in neurologically unimpaired elderly subjects. *Neuropathol Appl Neurobiol.* 2006;32(3):284-95. PubMed PMID: 16640647. doi: 10.1111/ j.1365-2990.2006.00727.x.

33. Halliday G, Hely M, Reid W, Morris J. The progression of pathology in longitudinally followed patients with Parkinson's disease. *Acta Neuropathol.* 2008;115(4):409-15. PubMed PMID: 18231798. doi: 10.1007/s00401-008-0344-8.

34. Liautard JP. Are prions misfolded molecular chaperones? *FEBS Lett.* 1991;294(3):155-7. PubMed PMID: 1756852. doi: 10.1016/0014-5793 (91)80657-O.

35. Svensson E, Horvath-Puho E, Thomsen RW, Djurhuus JC, Pedersen L, Borghammer P, et al. Vagotomy and subsequent risk of Parkinson's disease. *Ann Neurol.* 2015;78(4):522-9. PubMed PMID: 26031848. doi: 10.1002/ana.24448.

36. Pan-Montojo F, Schwarz M, Winkler C, Arnhold M, O'Sullivan GA, Pal A, et al. Environmental toxins trigger PD-like progression via increased alpha-synuclein release from enteric neurons in mice. *Sci Rep.* 2012;2:898. PubMed PMID: 23205266; PubMed Central PMCID: PMC3510466. doi: 10.1038/srep00898.

37. Anselmi L, Toti L, Bove C, Hampton J, Travagli RA. A Nigro-Vagal Pathway Controls Gastric Motility and Is Affected in a Rat Model of Parkinsonism. *Gastroenterology.* 2017;153(6):1581-93. PubMed PMID: 28912019; PubMed Central PMCID: PMC5705565. doi: 10.1053/ j.gastro.2017.08.069.

38. Naudet N, Antier E, Gaillard D, Morignat E, Lakhdar L, Baron T, et al. Oral Exposure to Paraquat Triggers Earlier Expression of Phosphorylated alpha-Synuclein in the Enteric Nervous System of A53T Mutant Human alpha-Synuclein Transgenic Mice. *J Neuropathol Exp Neurol.* 2017;76(12):1046-57. PubMed PMID: 29040593; PubMed Central PMCID: PMC5939863. doi: 10.1093/jnen/nlx092.

39. Goedert M, Spillantini MG, Del Tredici K, Braak H. 100 years of Lewy pathology. *Nat Rev Neurol.* 2013;9(1):13-24. PubMed PMID: 23183883. doi: 10.1038/nrneurol.2012.242.

40. Vasconcelos IM, Oliveira JT. Antinutritional properties of plant lectins. *Toxicon.* 2004;44(4):385-403. PubMed PMID: 15302522. doi: 10.1016/j.toxicon.2004.05.005.

41. Polito L, Bortolotti M, Battelli MG, Calafato G, Bolognesi A. Ricin: An Ancient Story for a Timeless Plant Toxin. *Toxins (Basel).* 2019; 11(6). PubMed PMID: 31174319; PubMed Central PMCID: PMC6628454. doi: 10.3390/toxins11060324.

42. Balint GA. Ricin: the toxic protein of castor oil seeds. *Toxicology.* 1974;2(1):77-102. PubMed PMID: 4823740. doi: 10.1016/0300-483X (74)90044-4.

43. Watkins WM, Morgan WT. Neutralization of the anti-H agglutinin in eel serum by simple sugars. *Nature.* 1952;169(4307):825-6. PubMed PMID: 14941057. doi: 10.1038/169825a0.

44. Boyd WC, Shapleigh E. Specific Precipitating Activity of Plant Agglutinins (Lectins). *Science.* 1954;119(3091):419. PubMed PMID: 17842730. doi: 10.1126/science.119.3091.419.a.

45. Tsaneva M, Van Damme EJM. 130 years of Plant Lectin Research. *Glycoconj J.* 2020;37(5):533-51. PubMed PMID: 32860551; PubMed Central PMCID: PMC7455784. doi: 10.1007/s10719-020- 09942-y.

46. Chrispeels MJ, Raikhel NV. Lectins, lectin genes, and their role in plant defense. *Plant Cell.* 1991;3(1):1-9. PubMed PMID: 1824332; PubMed Central PMCID: PMC159974. doi: 10.1105/tpc.3.1.1.

47. Lerouge P, Roche P, Faucher C, Maillet F, Truchet G, Prome JC, et al. Symbiotic host-specificity of Rhizobium meliloti is determined by a sulphated and acylated glucosamine oligosaccharide signal. *Nature.* 1990;344(6268):781-4. PubMed PMID: 2330031. doi: 10.1038/ 344781a0.

48. Rudiger H. Plant lectins - more than just tools for glycoscientists: occurrence, structure, and possible functions of plant lectins. *Acta Anat (Basel).* 1998;161(1-4):130-52. PubMed PMID: 9780355. doi: 10.1159/000046454.

49. Camilleri M. Leaky gut: mechanisms, measurement and clinical implications in humans. *Gut.* 2019;68(8):1516-26. PubMed PMID: 31076401; PubMed Central PMCID: PMC6790068. doi: 10.1136/ gutjnl-2019-318427.

50. Kinashi Y, Hase K. Partners in Leaky Gut Syndrome: Intestinal Dysbiosis and Autoimmunity. *Front Immunol.* 2021;12:673708. PubMed PMID: 33968085; PubMed Central PMCID: PMC8100306. doi: 10.3389/fimmu.2021.673708.

51. Ovelgonne JH, Koninkx JF, Pusztai A, Bardocz S, Kok W, Ewen SW, et al. Decreased levels of heat shock proteins in gut epithelial cells after exposure to plant lectins. *Gut.* 2000;46(5):679-87. PubMed PMID: 10764712; PubMed Central PMCID: PMC1727920. doi: 10.1136/gut.46.5.680.

52. Otte JM, Chen C, Brunke G, Kiehne K, Schmitz F, Folsch UR, et al. Mechanisms of lectin (phytohemagglutinin)-induced growth in small intestinal epithelial cells. *Digestion.* 2001;64(3):169-78. PubMed PMID: 11786665. doi: 10.1159/000048858.

53. Baintner K, Jakab G, Gyori Z, Kiss P. Binding of FITC-labelled lectins to the gastrointestinal epithelium of the rat. *Pathol Oncol Res.* 2000; 6(3):179-83. PubMed PMID: 11033457. doi: 10.1007/BF03032370.

54. Sun MF, Shen YQ. Dysbiosis of gut microbiota and microbial metabolites in Parkinson's Disease. *Ageing Res Rev.* 2018;45:53-61. PubMed PMID: 29705121. doi: 10.1016/j.arr.2018.04.004.

55. Wallen ZD, Appah M, Dean MN, Sesler CL, Factor SA, Molho E, et al. Characterizing dysbiosis of gut microbiome in PD: evidence for overabundance of opportunistic pathogens. *NPJ Parkinsons Dis.* 2020;6:11. PubMed PMID: 32566740; PubMed Central PMCID: PMC7293233. doi: 10.1038/s41531-020-0112-6.

56. Suzuki T. Regulation of the intestinal barrier by nutrients: The role of tight junctions. *Anim Sci J.* 2020;91(1):e13357. PubMed PMID: 32219956; PubMed Central PMCID: PMC7187240. doi: 10.1111/asj.13357.

57. Nachbar MS, Oppenheim JD, Thomas JO. Lectins in the U.S. Diet. Isolation and characterization of a lectin from the tomato (Lycopersicon esculentum). *J Biol Chem.* 1980;255(5):2056-61. PubMed PMID: 7354077. doi: 10.1016/S0021-9258(19)85992-6.

58. Ortega-Barria E, Ward HD, Keusch GT, Pereira ME. Growth inhibition of the intestinal parasite Giardia lamblia by a dietary lectin is associated with arrest of the cell cycle. *J Clin Invest.* 1994;94(6):2283-8. PubMed PMID: 7989583; PubMed Central PMCID: PMC330055. doi: 10.1172/JCI117591.

59. Karlsson A. Wheat germ agglutinin induces NADPH-oxidase activity in human neutrophils by interaction with mobilizable receptors. *Infect Immun.* 1999;67(7):3461-8. PubMed PMID: 10377127; PubMed Central PMCID: PMC116532. doi: 10.1128/IAI.67.7.3461-3468.1999.

60. Haas H, Falcone FH, Schramm G, Haisch K, Gibbs BF, Klaucke J, et al. Dietary lectins can induce in vitro release of IL-4 and IL-13 from human basophils. *Eur J Immunol.* 1999;29(3):918-27. PubMed PMID: 10092096. doi: 10.1002/(SICI)1521-4141(199903)29:03<918::AID-IMMU918>3.0.CO;2-T.

61. Martinon F, Mayor A, Tschopp J. The inflammasomes: guardians of the body. *Annu Rev Immunol.* 2009;27:229-65. PubMed PMID: 19302040. doi: 10.1146/annurev.immunol.021908.132715.

62. Gong T, Wang X, Yang Y, Yan Y, Yu C, Zhou R, et al. Plant Lectins Activate the NLRP3 Inflammasome To Promote Inflammatory Disorders. *J Immunol.* 2017;198(5):2082-92. PubMed PMID: 28087670. doi: 10.4049/jimmunol.1600145.

63. Lee KH, Kang TB. The Molecular Links between Cell Death and Inflammasome. *Cells.* 2019;8(9). PubMed PMID: 31509938; PubMed Central PMCID: PMC6769855. doi: 10.3390/cells8091057.

64. Hashimoto M, Hsu LJ, Xia Y, Takeda A, Sisk A, Sundsmo M, et al. Oxidative stress induces amyloid-like aggregate formation of NACP/alpha-synuclein in vitro. *Neuroreport.* 1999;10(4):717-21. PubMed PMID: 10208537. doi: 10.1097/00001756-199903170-00011.

65. Scudamore O, Ciossek T. Increased Oxidative Stress Exacerbates alpha-Synuclein Aggregation In Vivo. *J Neuropathol Exp Neurol.* 2018;77(6):443-53. PubMed PMID: 29718367. doi: 10.1093/jnen/nly024.

66. Bottner M, Fricke T, Muller M, Barrenschee M, Deuschl G, Schneider SA, et al. Alpha-synuclein is associated with the synaptic vesicle apparatus in the human and rat enteric nervous system. *Brain Res.* 2015;1614:51-9. PubMed PMID: 25896939. doi: 10.1016/j.brainres.2015.04.015.

67. Wong YC, Krainc D. alpha-synuclein toxicity in neurodegeneration: mechanism and therapeutic strategies. *Nat Med.* 2017;23(2):1-13. PubMed PMID: 28170377; PubMed Central PMCID: PMC8480197. doi: 10.1038/nm.4269.

68. Abbott RD, Petrovitch H, White LR, Masaki KH, Tanner CM, Curb JD, et al. Frequency of bowel movements and the future risk of Parkinson's disease. *Neurology.* 2001;57(3):456-62. PubMed PMID: 11502913. doi: 10.1212/WNL.57.3.456.

69. Gao X, Chen H, Schwarzschild MA, Ascherio A. A prospective study of bowel movement frequency and risk of Parkinson's disease. *Am J Epidemiol.* 2011;174(5):546-51. PubMed PMID: 21719744; PubMed Central PMCID: PMC3202149. doi: 10.1093/aje/kwr119.

70. Ross GW, Abbott RD, Petrovitch H, Tanner CM, White LR. Pre-motor features of Parkinson's disease: the Honolulu-Asia Aging Study experience. *Parkinsonism Relat Disord.* 2012;18 Suppl 1: S199-202. PubMed PMID: 22166434. doi: 10.1016/S1353-8020(11)70062-1.

71. Aube AC, Cabarrocas J, Bauer J, Philippe D, Aubert P, Doulay F, et al. Changes in enteric neurone phenotype and intestinal functions in a transgenic mouse model of enteric glia disruption. *Gut.* 2006; 55(5):630-7. PubMed PMID: 16236773; PubMed Central PMCID: PMC1856141. doi: 10.1136/gut.2005.067595.

72. Vojdani A, Lerner A, Vojdani E. Cross-Reactivity and Sequence Homology Between Alpha-Synuclein and Food Products: A Step Further for Parkinson's Disease Synucleinopathy. *Cells.* 2021;10(5). PubMed PMID: 34063062; PubMed Central PMCID: PMC8147930. doi: 10.3390/cells10051111.

73. Agarwal P, Wang Y, Buchman AS, Holland TM, Bennett DA, Morris MC. MIND Diet Associated with Reduced Incidence and Delayed Progression of ParkinsonismA in Old Age. *J Nutr Health Aging.* 2018;22(10):1211-5. PubMed PMID: 30498828; PubMed Central PMCID: PMC6436549. doi: 10.1007/s12603-018-1094-5.

74. Asemi Z, Samimi M, Tabassi Z, Shakeri H, Sabihi SS, Esmaillzadeh A. Effects of DASH diet on lipid profiles and biomarkers of oxidative stress in overweight and obese women with polycystic ovary syndrome: a randomized clinical trial. *Nutrition.* 2014;30(11-12):1287-93. PubMed PMID: 25194966. doi: 10.1016/j.nut.2014.03.008.

75. Tiwari, P.C. and Pal, R. The potential role of neuroinflammation and transcription factors in Parkinson disease. *Dialogues Clin Neurosci,* 2017;19(1):71-80. doi: 10.31887/DCNS.2017.19.1/rpal.