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Original Article 1

Modulation of neuroinflammation by inulin and magnesium in a rotenone-induced Parkinson's disease model

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Background
Neuroinflammation is a recognized mechanism in the development and pathogenesis of Parkinson's disease (PD). Chronic pesticide exposure has been linked to neuroinflammation and neuronal loss. Emerging evidence suggests that targeting inflammatory pathways through natural compounds and dietary interventions may help manage PD symptoms.
Objective
This study aims to elucidate the effects of inulin and/or magnesium supplementation on rotenone-induced PD in mice.
Materials and methods
Sixty Swiss adult male mice (10-13 weeks old) weighing 25±5 g were used. They were divided into healthy and Parkinson's disease groups, the latter was further subdivided into 5 groups: control-PD, PD treated with Sinemet, PD supplemented with inulin, PD supplemented with magnesium, and PD supplemented with both inulin and magnesium. Biochemical assessments of oxidative stress, inflammation, apoptosis, and mitochondrial dysfunction were conducted, along with evaluations of intestinal short-chain fatty acids and microscopic examination of brain tissue.
Results and conclusion
Data were analyzed using the statistical package for the social sciences (SPSS) version 16.0 for Microsoft windows, provided by SPSS Inc. The obtained results showed that rotenone injection significantly altered brain function and structure, inducingneuro-oxidative stress, neuroinflammation, and apoptosis. This was markedly alleviated by individual supplementation with either inulin or magnesium, while combined supplementation showed less improvement. Thus, individual oral supplementation with inulin or magnesium could enhance the gut-brain axis and offer neuroprotection against PD progression in mice, while the combination is less preferred.

Keywords Parkinson's disease, neuroinflammation, inulin, magnesium, rotenone, oxidative stress, apoptosis, mitochondrial dysfunction, gut-brain axis, neuroprotection.

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Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide, with increasing incidence rates [1]. It presents with motor symptoms like tremors, bradykinesia, and balance issues, along with non-motor symptoms such as emotional changes, urinary problems, and skin disorders [2]. Pathologically, PD is characterized by Lewy bodies (LB) formed by α-synuclein (α-syn) aggregation and dopaminergic neuron degeneration in the substantianigra pars compacta [3]. Genetic, aging, immunity, microbial, and environmental factors contribute to its onset [2].

Neuroinflammation is a critical mechanism in PD development [4]. Microglia, the central nervous system's immune cells, regulateneuroinflammation and maintain homeostasis. Its prolonged activation causes inflammation, contributing to PD and other neurodegenerative conditions [5]. Microglia's efforts to clear pathological α-syn trigger neuroinflammation through pro-inflammatory

mediators and reactive oxygen species (ROS), which worsen α-syn propagation and further activate reactive microglia [6].

The PI3K/AKT/mTOR pathway is significant in PD development. Oxidative stress or inflammation inhibits this signaling cascade, contributing to PD pathogenesis. Activating PI3K and Akt improves cell survival, reduces injury, limits inflammation, and protects neurons [7]. Akt modulates apoptosis via the mTOR pathway [8], while its decreased activity in PD increases glycogen synthase kinase 3 (GSK-3) and caspase-3 levels, leading to dopaminergic neuron apoptosis [9].

Microglia detect danger signals via NOD-like receptor protein 3 (NLRP3), a sensor for the inflammasome complex. NF-κB activation enhances NLRP3 transcription, activating caspase-1 and promoting neurodegeneration in PD [10]. Chronic pesticide exposure also activates pro-inflammatory cytokines, disrupting redox balance, aggregating proteins, and causing cell death [11]. Rotenone, a fat-soluble insecticide, induces oxidative stress, dopaminergic cell death, α-synuclein buildup, and LB formation, mimicking PD [12]. Rotenone-based PD models show microglia amplify NLRP3 signaling, exacerbating neurodegeneration [10].

Current PD treatments, such as Sinemet, alleviate symptoms but fail to halt disease progression [10]; Sinemet is a key medication for Parkinson's disease, combining levodopa and carbidopa to increase dopamine levels in the brain. It effectively alleviates motor symptoms like tremors and rigidity but does not halt the disease's progression [13]. Targeting inflammatory pathways via dietary intervention is promising. Natural compounds activate the PI3K/AKT pathway, offering neuroprotection in diseases like PD and Alzheimer's [14]. Inulin, a prebiotic fiber derived from chicory, has been found to promote health and improve intestinal transit while also potentially influencing gut microbiota [15]. Inulin, a soluble fiber, has anti-inflammatory effects through regulating gut microbiota balance; [16] it acts as a prebiotic, selectively stimulating the growth of beneficial bacteria such as *Bifidobacteria*, which can reduce inflammation by producing short-chain fatty acids and other bioactive metabolites. These metabolites help to maintain gut barrier integrity and enhance the immune response, thereby mitigating inflammatory conditions and potentially benefiting diseases like PD where inflammation plays a key role. It regulates immune and metabolic functions, reducing inflammation and potentially reversing diseases [17]. Magnesium (Mg) is vital for brain homeostasis and nerve signaling.It also reduces inflammation. Mg deficiency is linked to systemic low-grade inflammation, common in many diseases [18]; when magnesium levels are low, immune cells may become hyperactive, releasing pro-inflammatory cytokines and reactive oxygen species, This contributes to chronic inflammation. This inflammatory state can exacerbate conditions like cardiovascular disease, diabetes, and neurodegenerative disorders, including Parkinson's disease, where inflammation is a key pathogenic mechanism [19].

This study aims to investigate the potential of inulin and magnesium in mitigating neuroinflammation, oxidative stress, and neuronal apoptosis in a PD mice model. By targeting these key pathological features, the study aims to provide new insights into the mechanisms underlying PD and explore innovative treatment strategies that could potentially alleviate disease progression and improve patient outcomes. It also for the first time highlights the competitive effects of combined supplementation with inulin and magnesium in PD mice model.

Materials and methods

Ethics

The experiment was planned and executed in accordance with the research ethics committee guidelines. The study was carried out at Biochemistry and Nutrition Department, Animal House of Faculty of Women for Arts, Science and Education at Ain Shams University, Cairo, Egypt, from March 2023 to May 2023. The experiment was designed and conducted according to the research ethics committee (Code: ASU/W/Sci-6P/23-1-5).

Chemicals

Rotenone (5″R) -4′,5′- dimethoxy-5″- (prop-1-en-2-yl)-4″,5″-dihydrofuro [2″,3″:7,8] rotenan-4-one) and magnesium sulfate ($MgSo_4$) were sourced from Sigma, St Louis, MO, USA, while sinemet tablets (100/25 levodopa/carbidopa) were from Seif pharmacy, Cairo, Egypt. Inulin was obtained from the just foods brand, Dubai, UAE.

Animals

Sixty male Swiss albino mice (10-13 weeks old, 25±5 g) from the National Research Center, Cairo, Egypt, were used. They were acclimatized before the experiment, with all procedures following ethical guidelines for animal care. All mice were randomly housed individually in stainless steel cages with constant controlled environments; temperature 25 ± 5 ºC, air humidity 55% ± 10% and 12/12 hours light /dark cycle were held. All mice were fed on a balanced diet with drinking water ad libitum for 7 days for adaptation. Mice were given an ad-libitum diet and water. During the experiment, they were fed a balanced diet based on

Reeves et al. [20], modified by Guo et al. [21] to include inulin (as cellulose substitution).

Experimental Design

Mice were randomly divided into two primary groups: group I (10 mice) served as the healthy control. Group II (50 mice) received rotenone injections (1.5 mg/kg in DMSO) every other day for 2 weeks [22] to induce Parkinson's disease. Group II was subdivided into 5 groups (10 mice/group): (1) Control PD – positive control, (2) PD + Sin – was received 25 mg/kg body weight (b.w.) sinemet by oral gavage for 6 weeks [23], (3) PD + Inu – fed an inulin diet and given 2g/kg b.w. inulin by oral gavage for 6 weeks [21], (4) PD + Mg – fed a balanced diet and administered 250 mg/kg b.w. magnesium sulfate by oral gavage for 6 weeks [24], and (5) PD + Inu + Mg – fed an inulin diet and received both 2g/kg b.w. inulin and 250 mg/kg b.w. magnesium sulfate by oral gavage for 6 weeks.

At the experiment's end, all mice were sacrificed by decapitation. Blood samples were collected, allowed to clot at room temperature, and then centrifuged to isolate serum. The serum was stored at -20°C for biochemical analysis. Brain and intestinal tissues were removed, washed with sterile saline, weighed, and stored for further analysis. Brain sections were fixed in 10% neutral formalin for histological analysis using hematoxylin and eosin (H & E) staining.

Methods of preparation of brain homogenate

Brain tissue was homogenized with 100 ml of Phosphate Buffered Saline (PBS) using mortar. PBS was used, with a pH typically maintained around 7.4.The homogenate was centrifuged at 1000 r.p.m for approximately 20 minutes. The supernatant was collected carefully, and used for assay.

Methods of preparation of intestine homogenate

Intestinal tissues were rinsed in ice-cold PBS (pH 7.4) to thoroughly remove excess blood and were weighed prior to homogenization. Next, the intestinal tissues were chopped and homogenized in PBS (tissue weight (g): PBS (ml) ratio of 1:9) using a glass homogenizer on ice. The homogenates were then centrifuged at 12,000 rpm for 15 minutes at 4°C to obtain the supernatant.

Biochemical measurements

The magnesium levels were determined using the magnesium assay kit (catalog number KA1650) according to the kit instructions. Also, NOD-like receptor protein 3 levels were measured using the NOD-like receptor protein 3 ELISA kit (catalog number E2283Mo). In the brain tissue, the phosphatidylinositol-3-kinase (PI3K)/Akt and the mammalian target of rapamycin (mTOR) were measured using the western blot technique with the V3 western workflow™ system (Bio-Rad®, CA, USA) [25]. The monoamine oxidase levels were determined calorimetrically [26], while caspase-3 levels were assessed using the R&D Biotechne caspase-3 assay kit (catalog number: BF3100) via the cuvette colorimetric method [27]. α-synuclein (α-syn), dopamine, cytochrome c, and ATP were measured using ELISA kits (catalog no: CSB-EL021912RA, MBS026032, MBS9304546, KT-57361) with stat fax-2100 equipment. Additionally, short-chain fatty acids (acetic and butyric acids) in intestinal tissue were measured using an ELISA kit (catalog no: abx258338) on stat fax-2100 equipment.

Statistical analyses

Data were analyzed using SPSS version 16.0 for Windows. Values are presented as mean ± standard error (S.E.). Statistical comparisons between groups were made using one-way ANOVA, with significance defined at $P<0.05$ level[28].

Results and discussion

The PD had multi-target mechanisms that need further investigation. Oxidative stress and neuroinflammation are key features of neurodegenerative diseases like PD [29]. This study evaluated how oral supplementation with inulin and magnesium, either separately or together, targeted specific neurodegenerative markers in a PD mice model induced by rotenone, a common pesticide.

Neuronal dopaminergic and serum Mg2+ alternation in Parkinson's disease mice model

To evaluate the effects of inulin or magnesium supplementation on dopaminergic neuron impairment and Mg homeostasis in mice model of PD, brain α-synlevels, dopamine and serum Mg levels were measured. The results presented in (Fig. 1a) revealed that α-syn levels in the PD model induced by rotenone injection were significantly increased ($P<0.05$) by 301% with a significant reduction in dopamine by 59.2% compared with the control (healthy) group. Also, the induction of PD by rotenone injection disrupted Mg homeostasis indicated in our results (Fig. 1b) by significant reduction ($P<0.05$) in serum Mg level in PD group by74% compared to that of healthy mice.

However, treatment with inulin or magnesium rescued these changes represented in the current results (Fig. 1a)as a significant reduction in α-synlevel (9.33 ± 1.04 and 8.90 ± 1.01 ng/g tissue) respectively, and elevation in brain dopamine in all treated groups (90.90 ± 3.04 and 90.30 ± 5.57 ng/g tissue) respectively compared to PD group.

Treatment with both inulin and magnesium supplements together showed the least significant effect compared to either individual treatment.
Similarly, the results presented in (Fig. 1b) showed thatmagnesium supplementation highly increased the magnesium level in serum (1.50 ± 0.17 mmol/l) compared with PD mice model (0.52 ± 0.16 mmol/l). On the other hand, sinemet drug (1.09 ± 0.12 mmol/l) and inulin supplementation (1.10 ± 0.20 mmol/l) to PD mice model significantly help to control Mg ions depletion induced by rotenone injection. Meanwhile, the combined supplementation of inulin with Mg (1.04 ± 0.07 mmol/l) showed limited effect on serum Mg levels compared with the individual supplementations. This might reflect that the combination of inulin and Mg intake shows an adverse effect on Mg homeostasis.

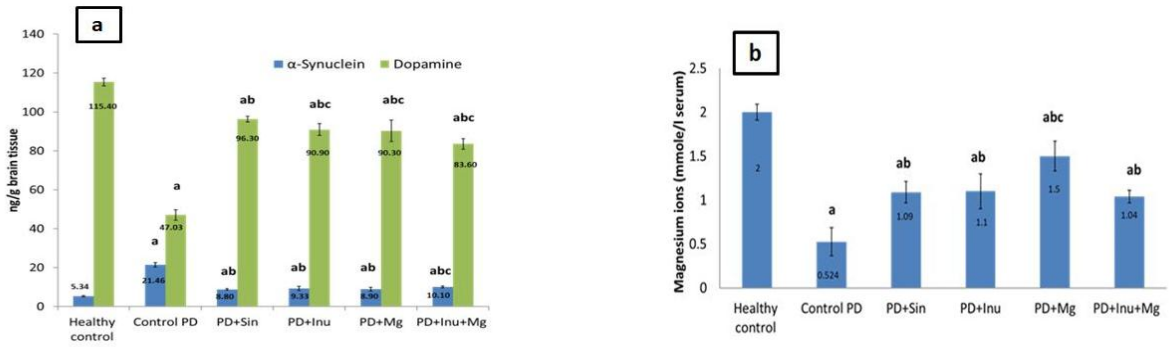

Fig. 1 Effect of inulin and/ or magnesium supplementation on brain **(a)**α- synculin, and dopamine **(b)** magnesium levels in mice model of Parkinson's disease. Values are represented as mean ±SE (n=10), significance at *(P<0.05).* a) represent a significant difference with the control group, b) represent a significant difference with Parkinson's disease group, and c) represent a significant difference with Parkinson's disease +sinmet group

Rotenone is commonly used to model Parkinson's disease (PD). This study confirmed PD mice model progression through increased α-syn and decreased dopamine levels [30]. Five days of rotenone treatment caused a rapid increase in α-synuclein levels within nigrostriatal neurons, a key brain region involved in movement control that is severely affected in Parkinson's disease (PD). α-Synuclein is a protein that misfolds and aggregates in PD, forming toxic inclusions known as lewy bodies. The observed rise in α-synuclein following rotenone exposure mirrors the pathological changes in PD brain tissue, making this experimental model valuable for studying the mechanisms underlying neurodegeneration in the disease [31]. In PD patients, dopaminergic neuron degeneration reduces dopamine levels, causing motor impairments and potentially contributing to cognitive deficits [32]. The disruptions in Mg^{2+} levels observed may play a role in neurodegenerative diseases like PD [33]. For instance, low systemic Mg^{2+} levels can lead to significant dopaminergic neuron loss in the substantianigra, a key feature of PD pathology [34]. PD is characterized by alpha-synucleinopathy, which affects the entire brain-gut axis, including the central, autonomic, and enteric nervous systems. Enteric dopaminergic neurons, which help regulate intestinal motility, are distributed along the gastrointestinal (GI) tract, with 14%-20% in the upper GI and only 1%-6% in the lower small intestine and large intestine [35]. Rotenone exposure triggers inflammation and oxidative stress in the enteric nerve plexus, leading to alpha-synuclein accumulation [36]. Oral supplementation with inulin or magnesium reduced the rotenone-induced alterations in brain tissue, with effects similar to L-dopa. Inulin improves immune function, reduces oxidative stress, and inhibits inflammatory cytokines.This helps alleviate neuronal damage and slow PD progression [37]. Magnesium supplementation restored Mg^{2+} levels, neutralized free radicals, and mitigated neuronal damage [38].

Short-chain fatty acids production changes in Parkinson's disease mice model

Based on the results of intestinal SCFAs, it was evident that both intestinal acetate and butyrate levels of PD mice were significantly (*P<0.05*) reduced in comparison to those of the healthy control group by 76.57% and 42.4% respectively (Fig. 2a, 2b). Even though, as shown in (Fig. 2a), it was observed that the administration either of inulin

or magnesium significantly increased the abundance of intestinal acetate (11.60 ± 0.61 and 9.45 ± 0.55 pg/g tissue) and in (Fig. 2b)butyrate (381.40 ± 6.24 and 349.50 ± 5.82 pg/g tissue) compared to the PD mice model. Inulin administration alone showed the highest significant elevation in intestinal SCFAs level rather than sinemet treatment, Mg supplementation, or gathering inulin with magnesium.

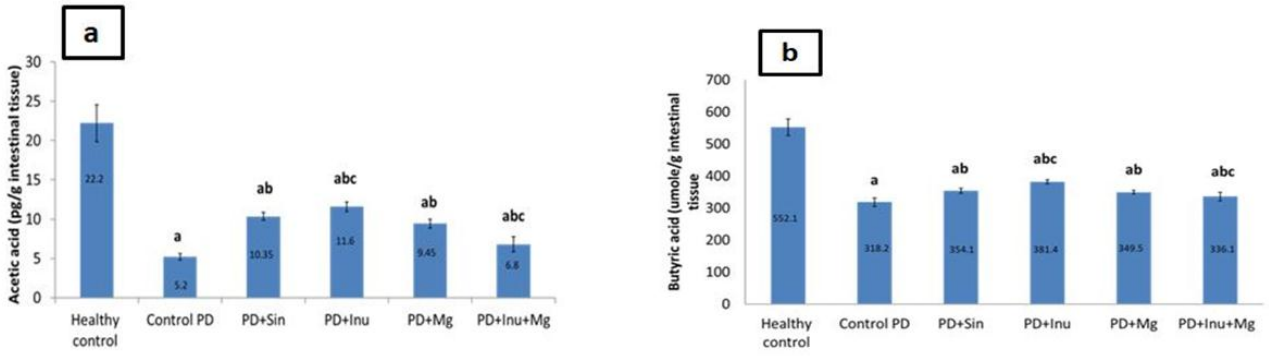

Fig. 2 Effect of inulin and/ or magnesium supplementation on intestinal short-chain fatty acid; **(a)** acetic acid **(b)** butyric acid in mice model of Parkinson's disease. Values are represented as mean ±SE (n=10), significance at *(P<0.05).* a) represent a significant difference with the control group, b) represent a significant difference with the Parkinson's disease group, and c) represent a significant difference with the Parkinson's disease +sinmet group.

The beneficial effects of inulin may be linked to its impact on gut microbiota [39], suggesting that modulating the gut microbiome could influence oxidative stress and inflammation in PD. Our results showed that inulin administration increased intestinal short-chain fatty acids (SCFAs), which were significantly reduced by rotenone injection in the PD model. SCFAs, such as acetate, butyrate, and propionate, are key metabolites produced by gut microbiota from non-digestible carbohydrates and play a crucial role in energy metabolism, as well as alleviating oxidative stress and inflammation in PD [40]. This may explain inulin's modulatory effects on PD through the gut-brain axis [41]. However, combining inulin and magnesium supplementation did not show a synergistic effect. Instead, the combination weakened the impact on SCFA levels compared to individual supplementation, reducing inulin's anti-inflammatory benefits. Previous studies have shown that magnesium oxide (MgO) can reduce the inulin effect on SCFA production [42], suggesting that this combination may worsen inflammatory conditions in PD mice.While prebiotics, such as inulin, are generally known to enhance mineral absorption, certain factors can lead to negative effects on this interaction. For instance, excessive fermentation of prebiotics in the gut can result in the overproduction of short-chain fatty acids (SCFAs), leading to an overly acidic environment that might interfere with the absorption of some minerals, particularly in individuals with sensitive gastrointestinal conditions. Additionally, the growth of specific gut bacteria stimulated by prebiotics may vary among individuals, potentially leading to imbalances that hinder mineral bioavailability. Furthermore, combining prebiotics with substances like magnesium oxide (MgO) could alter gut microbiota composition and reduce the prebiotic's effectiveness in promoting mineral absorption. Such complex interactions emphasize the importance of balanced dosing and personalized approaches to prebiotic supplementation [43].

Oxidative stress modulation, microglia-phosphatidylinositol-3-kinase (PI3K)/Akt and the mammalian target of rapamycin (mTOR) pathway and serum NOD-like receptor protein 3 inflammasome attenuation in Parkinson's disease mice model

Oxidative stress was elevated in the PD mice model as revealed by a significant (*P<0.05*) increase in MAO activity by 149.77% after rotenone injection as compared to healthy control as illustrated in (Fig. 3). Conversely, the administration of inulin or magnesium by PD mice model significantly enhanced the antioxidative mechanisms and reduced MAO activity in brain tissue (175.60 ± 2.10 and 185.10 ± 1.17 pg/g tissue respectively)(Fig. 3). This improvement was nearly approach that of sinemet drug treatment (167.50 ± 2.21 pg/g tissue)(Fig. 3). Surprisingly, the

combination of both supplements (Inulin and magnesium) for PD mice model had the lowest effect of improvement than individual treatments (189.80 ± 3.18 pg/g tissue) (Fig. 3).

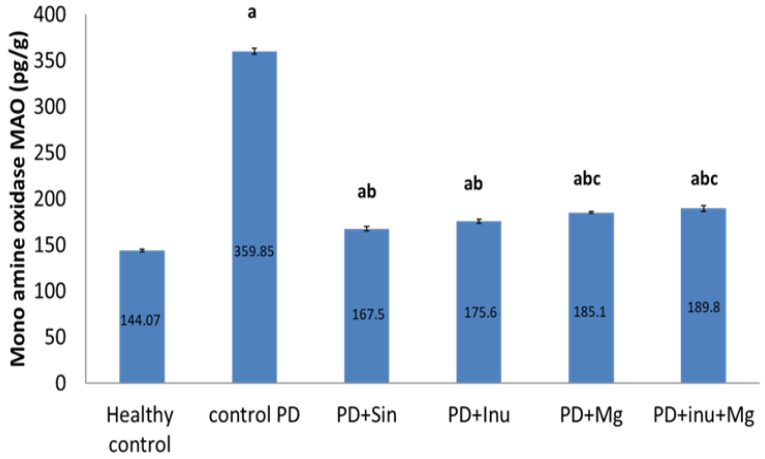

Fig. 3 Effect of inulin and/ or magnesium supplementation on monoamine oxidase activity in mice model of Parkinson's disease. Values are represented as mean ±SE (n=10), significance at *(P<0.05).* a) represent a significant difference with the control group, b) represent a significant difference with the Parkinson's disease group, and c) represent a significant difference with the Parkinson's disease +sinmet group.

Neuroinflammation is one of the most common leading causes of neurodegeneration and PD illustrated in the current result (Fig. 4) as marked reduction in PI3K/AKT/mTOR signaling by 82.17 %, and 76.23%, and 69.3% respectively in brain tissue with significant elevation of NLRP-3 level in the serum of the PD mice model by 284.04 % comparing to healthy mice (12.16 ± 0.89 ng/ml) indicating a systemic and inflammatory condition (Fig. 5). This alternation in inflammatory signals was attenuated significantly (P<0.05) by oral inulin or Mg supplementation which is comparable to sinemet drug treatment and clearly observed by significant elevation of brain signaling PI3K/AKT/mTOR (Fig. 4) with the reduction in NLRP-3 level (Fig. 5) compared to PD group almost reach that of sinemet group. In the same manner, the combined supplementation showed similar and comparable results to the other treated groups demonstrated in Fig. 4 & 5.

In the PD mice treated with rotenone, increased pro-oxidants, pro-inflammatory factors, and mitochondrial dysfunction were observed. Elevated monoamine oxidase (MAO) activity, which promotes dopamine breakdown, is linked to dopamine depletion in PD [44]. Chronic rotenone exposure activates the PI3K/Akt/mTOR pathway, leading to neuropathological and behavioral changes [45]. NLRP3 inflammasome activation, involving NF-kB and other inflammatory factors, contributes to mitochondrial dysfunction and cell death [46]. Studies, such as Ogunruku et al. [47], show that rotenone raises MAO activity and inflammatory markers like NLRP-3, supporting these findings. NLRP3 is crucial for microglial function and cytokine release. Mitochondrial dysfunction triggers NLRP3 activation, contributing to neuroinflammation in PD. Additionally, the PI3K/Akt/mTOR pathway is downregulated in rotenone-treated PD mice, leading to dopaminergic neuron loss, consistent with findings by Chen et al. [48]. Our results confirm that rotenone-induced PD increases MAO production, neuroinflammation, and α-synuclein levels, while reducing dopamine. Levodopa, a standard PD treatment, mitigates rotenone-induced oxidative stress, supported by Kasem and Hamza [49], who observed reduced lipid peroxidation, increased GSH, and enhanced antioxidant enzyme activities (SOD and catalase), suggesting that it helps alleviate neuroinflammation, mitochondrial dysfunction, and modulate dopaminergic activity.

Inulin and magnesium administration in PD mice reversed the effects of rotenone by mitigating NLRP3 inflammasome activation through inhibition of the PI3K/Akt/mTOR signaling pathway. Oral supplementation with either inulin or magnesium restored alterations in this pathway, yielding effects similar to L-dopa treatment. Inulin has been shown to improve immune function and reduce oxidative stress by inhibiting inflammatory cytokines, which alleviates inflammation and slowing PD progression. Our study found that inulin supplementation significantly lowered pro-oxidant levels and NLRP3 in the PD model. Ruiz and Eldar-Finkelman [50] reported that inulin alleviated enterotoxicity by activating the PI3K/Akt/mTOR pathway, suppressing TNF-α, and reducing intestinal inflammation. Additionally, inulin mitigated brain oxidative stress and mitochondrial dysfunction in a rotenone-induced neurotoxicity model, suggesting its therapeutic

potential for neurodegenerative diseases [51]. Butyrate, produced by gut microbiota from inulin, reduces mitochondrial ROS, activates antioxidant enzymes, alleviates oxidative stress, and reduces inflammation, protecting against PD risk factors. Magnesium ions neutralize free radicals by accepting or donating electrons, converting reactive radicals into less damaging forms [38]. Arjunan et al. [52] showed that magnesium ions regulate Akt expression, activate mTOR via the PI3K/Akt pathway, and influence cell functions [53]. However, our results indicated antagonistic effects between inulin and magnesium in combined supplementation, lowering their anti-inflammatory and antioxidative effects. Studies show that magnesium oxide (MgO) reduces inulin-induced increases in short-chain fatty acids (SCFAs), which are essential for gut regulation and systemic physiological functions, and act as anti-inflammatory agents.

Apoptotic markers (cytochrome-c, caspase-3) and mitochondrial dysfunction improvement in Parkinson's disease mice model

The PD is characterized by mitochondrial dysfunction leading to cell death as illustrated in the current results (Fig. 6) with a significant decrement in ATP% by 67.4% and a significant increment in cytochrome-c and caspase-3 levels by 393% and 494.68% respectively compared to non-conditioned brain cells. PD mainly affects the mitochondrial ability to produce ATP resulting in a lower percentage of ATP in the brain tissue of PD mice. Meanwhile, supplementation of PD mice with inulin and/or Mg enhanced the cellular production of ATP (37.95 ± 0.79 and 41.10 ± 1.13 ng/g tissue) and its percentage levels which reflect improved mitochondrial function along with the reduction of cytochrome-c (6.10 ± 0.15 and 5.05 ± 0.28 ng/g tissue) and caspase-3 (4.80 ± 0.26 and 4.50 ± 0.29 ng/g tissue)displayed in (Fig. 6)

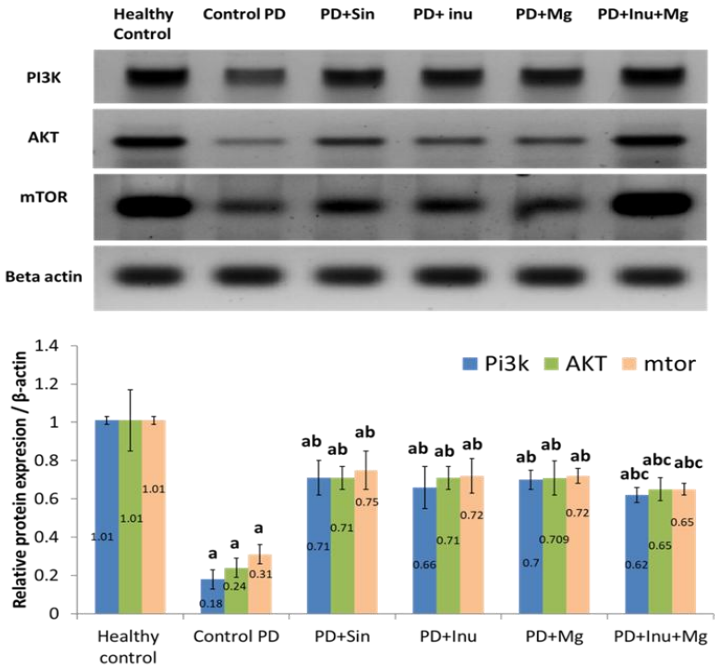

Fig. 4 Effect of inulin and/ or magnesium supplementation on microglia- phosphoinositide 3 kinase (PI3K)/Akt/mammalian (or mechanistic) target of rapamycin (mTOR) pathway in mice model of Parkinson's disease. Values are represented as mean ±SE (n=10), significance at *(P<0.05).* a) represent a significant difference with the control group, b) represent a significant difference with the Parkinson's disease group, and c) represent a significant difference with the Parkinson's disease +Sinmet group.

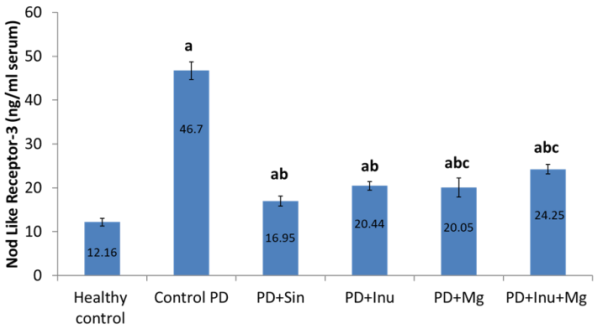

Fig. 5 Effect of inulin and/ or magnesium supplementation on serum NOD-like receptor protein 3 inflammasome in mice model of Parkinson's disease. Values are represented as mean ±SE (n=10), significance at (P<0.05). a) represent a significant difference with the control group, b) represent a significant difference with the Parkinson's disease group, and c) represent a significant difference with the Parkinson's disease +sinmet group.

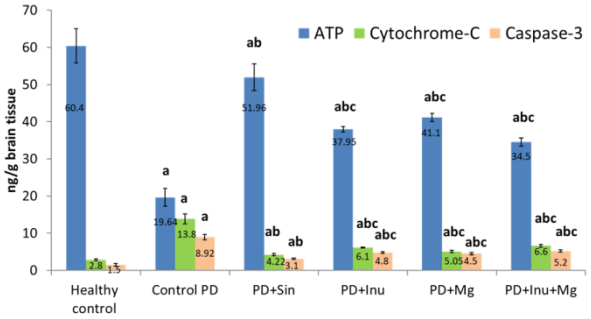

Fig. 6 Effect of inulin and/ or magnesium supplementation on neural ATP%, cytochrome -c and caspase-3 levels in mice model of Parkinson's disease. Values are represented as mean ±SE (n=10), significance at *(P<0.05).* a) represent a significant difference with the control group, b) represent a significant difference with the Parkinson's disease group, and c) represent a significant difference with the Parkinson's disease +sinmet group.

Mitochondria are vital for ATP production, and any impairment in mitochondrial function leads to decreased ATP production, bioenergetic dysfunction, apoptosis, and oxidative stress, especially in the brain with its high metabolic demands. In PD mitochondrial dysfunction is associated with neuroinflammation, neuronal death, and disease progression [54]. Rotenone inhibits mitochondrial complex I, disrupting ATP synthesis, increasing oxidative stress, and triggering neuronal death via both apoptotic and necrotic mechanisms [55]. Rotenone-induced ATP depletion impairs caspase-dependent apoptosis, causing caspase-independent neuronal cell death [56]. Chen et al. [48] showed that levodopa enhances motor function and boosts mitochondrial complex I activity in PD models. Our study aligns with these findings, showing significant improvements in ATP levels and pro-apoptotic markers in PD mice treated with sinemet tablets, while inulin and magnesium may offer similar neuroprotective effects by reducing oxidative stress and neuroinflammation. Inulin's effects on mitochondrial function may be linked to its positive impact on gut microbiota and SCFA production, which can enhance ATP production, improve mitochondrial function, and reduce neuronal cell death [39]. SCFAs, such as butyrate, serve as substrates for mitochondrial energy metabolism and play a key role in promoting mitochondrial biogenesis, reducing inflammation, and protecting cells from death, thus lowering Parkinson's disease risk [40, 50]. Additionally, Mg2+ ions are vital for neuronal development and function, with changes in cytosolic magnesium levels occurring during apoptosis, as apoptotic stimuli release Mg2+ ions from mitochondria [57]. Mg2+ ions also influence ATP production, supporting various mitochondrial functions [58].

Histological alternation of brain tissues

Microscopic examination of hematoxylin and eosin (H & E) stained brain sections (cerebral cortex(Fig. 7a), subiculum (Fig. 7b), and fascia dentate and hilus (Fig. 7c) revealed that rotenone-induced Parkinson's Disease (PD) caused nuclear pyknosis and neuronal degeneration in the cerebral cortex, subiculum, and fascia dentate.

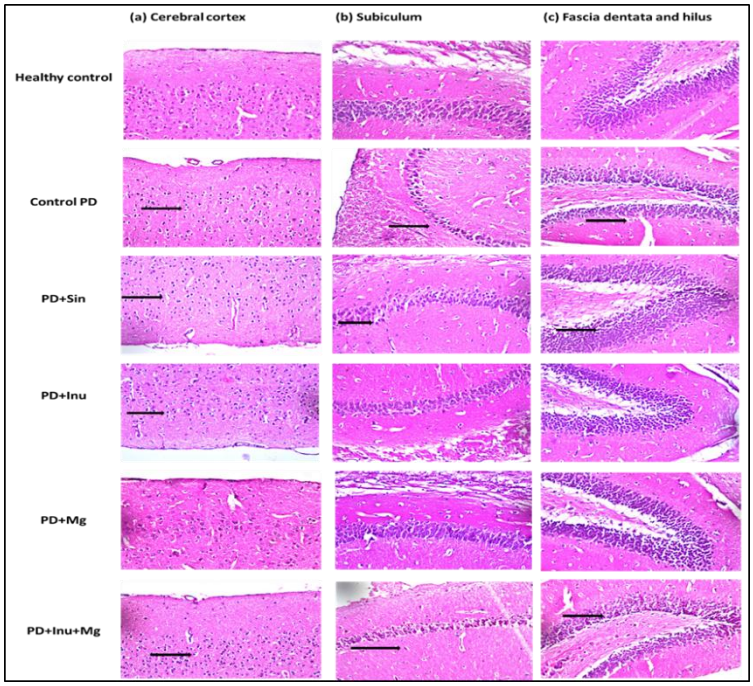

Fig. 7 Effect of inulin and/ or magnesium supplementation on histological alternation in brain tissue (**(a)** cerebral cortex, **(b)**subiculum, and **(c)** fascia dentate and hilus) of mice model of Parkinson's disease.

In PD mice treated with Sinemet tablets, nuclear pyknosis and degeneration were observed in some neurons of the cerebral cortex and hippocampus, with no changes in the cerebellum. However, in the PD+ Inu and PD+ Mg groups, which received inulin or magnesium supplementation, an improvement in brain tissue structure was noted. Inulin supplementation showed partial protection, with nuclear pyknosis and degeneration in some neurons of the cerebral cortex but no degeneration in the hippocampus or cerebellum. Magnesium supplementation provided substantial protection, showing no histopathological alterations in any brain region. These findings were supported by biochemical results, indicating that both inulin and magnesium supplementation alleviated neurodegenerative effects by improving brain tissue structure, likely due to their antioxidative, anti-inflammatory properties, and potential restoration of mitochondrial function.

Interestingly, in the combined supplementation of inulin and magnesium in PD mice, no further improvement in brain structure was observed. Nuclear pyknosis and degeneration were present in most neurons in the cerebral cortex, subiculum, and fascia dentate and hilus, with no changes in the cerebellum. This suggests that the combination of inulin and magnesium does not provide marked improvements. Similar findings were reported in previous studies by Guo et al. [21] and Serita et al. [60].

The limited improvement observed in the Parkinson's disease (PD) mice model with the combination of inulin and magnesium can be attributed to several factors. Inulin, known for promoting beneficial gut bacteria, may have its prebiotic effects counteracted by magnesium oxide (MgO), which can alter gut microbiota composition and potentially reduce the production of short-chain fatty acids (SCFAs) essential for anti-inflammatory and neuroprotective processes. Furthermore, MgO at higher doses has been reported to decrease SCFA production, overriding inulin's SCFA-enhancing properties. The combination might also result in an imbalanced inflammatory response, as magnesium, under certain conditions, can act as a mild gut irritant, counteracting inulin's anti-inflammatory effects. Additionally, the interaction between magnesium and inulin is dose-dependent, and improper dosing could lead to suboptimal or even antagonistic outcomes. Competing mechanisms, such as magnesium's antacid effect neutralizing the acidic environment required for inulin fermentation, may further reduce efficacy. These complexities underscore the need for precise dose optimization and deeper investigation into the interplay between these treatments [61].

Conclusion

The current findings highlight the potential of individual inulin or magnesium supplementation in modifying brain pathology in experimental PD induced by rotenone. The novelty of this study lies in the contrasting effects of both supplements, showing that individual supplementation with either inulin or magnesium controls PD progression similarly to Sinemet. This occurs by modulating the gut-brain axis, oxidative status, and inflammatory response via the PI3K/AKT/mTOR cell signaling pathway, which helps reduce mitochondrial dysfunction and suppress apoptotic signals.

Conflict of interest

The authors reported no potential conflicts of interest.

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Authors' contributions

All authors contributed equally to this manuscript, including the experimental design, conducting experiments, and preparing and reviewing the manuscript prior to submission.

Ethics approval

This study was approved by the Medical Research Ethics Committee (MREC), with approval Code: ASU/W/Sci-6P/23-1-5.

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