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Beetroot (*Beta vulgaris*) - Fortified Yogurt as a Natural Source of Levodopa Enhances in Rotenone – induced Parkinson's Disease in Mice

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Abstract

Background: Parkinson’s disease (PD) is a common neurodegenerative disorder; however there is still a lacks of effective and safe treatments for it. Beetroot is a unique plant containing rich in betalains. It has shown protecting dopamine cells effects.

Aim: To evaluate the effect of beetroot fortified yogurt on PD symptoms in mice compare to levodopa drug.

Methods: PD was induced in adult male Swiss albino mice by rotenone through 9 subcutaneous injections 1.5 mg/kg; 3 times / week. Locomotor activity was assessed by behavioral tests, and some biochemical analyses were conducted.

Results: Beetroot fortified yogurt improved locomotor activity and reduced PD markers such as, oxidative stress biomarkers, Neurotransmitters, Apoptotic Indices, Inflammatory Indices, liver and kidney functions and Constipation.

Conclusions: Red beet fortified yogurt and inhibited the development of PD in mice better than levodopa drug, which may be partly responsible for decreased oxidative damage and enhances dopamine production in brain.

1. Introduction

Neurodegenerative disorders pose a major global health burden, affecting millions of people worldwide and showing a notable rise in prevalence as life expectancy increases. Among the most prevalent of these conditions are Alzheimer’s disease (AD), Parkinson’s disease (PD), amyotrophic lateral sclerosis, multiple sclerosis, and Huntington’s disease [1].Parkinson's disease (PD) is a neurological disorder that worsens gradually over time, often affecting a person’s ability to move. Common symptoms are muscle rigidity, tremors, bradykinesia, and reduced motor balance [2].In recent years, the influence of intestinal microorganisms has become a growing focus of scientific investigation. An increasing number of studies have identified a link between gut microbiota$_7$ and constipation associated with PD[3, 4].As a result, conventional therapeutic approaches are increasingly being replaced by microecological strategies [5, 6]. Constipation may also hinder the absorption of anti-Parkinson’s medications, including levodopa [7].

Since ancient times, humans have depended on natural products as a fundamental source of therapeutic agents to manage various illnesses, disorders, and physical debilities. Experimental research has shown that these natural compounds possess multiple biological activities, including antioxidant, anti-inflammatory, and anti-apoptotic effects. Findings from both in vitro and in vivo studies have further confirmed their promising role in numerous preclinical models of neurodegenerative diseases[8, 9]. Beta vulgaris, widely recognized as beetroot, has been cited in traditional medicine for its neuroprotective potential in managing various CNS-related disorders. Reviews of the literature reveal that B. vulgaris

exhibits several pharmacological properties, including antidepressant, antioxidant, anticonvulsant, cerebroprotective, and hepatoprotective effects[10].Betanin, the primary constituent of red beetroot, highlights the therapeutic promise of beetroot and its bioactive compounds (betalains) as potential complementary treatments for multiple health conditions [11].Structurally, betalains share a core betalamic acid unit linked to cyclo-dopa, which originates from L-dopa metabolism [12].In addition to, the studies indicate that levodopa is naturally present in beetroot as an intermediate in the metabolic pathway for the formation of betalain pigments and has been identified using HPLC/MS analysis [13].

Yogurt contains live bacterial cultures, referred to as probiotics, which are well known for their positive effects on gastrointestinal function. These probiotics support intestinal microbial balance by stimulating the proliferation of beneficial bacteria and suppressing the growth of harmful species [14, 15].Preserving balance within the gut microbiota has been linked to better digestive function, increased nutrient uptake, and enhanced immune system efficiency [16, 17].

2. Materials and Methods

2.1 Animals

Adult male Swiss albino mice (25 ± 5 g) were used in this study, obtained from the National Research Centre (Giza, Egypt). They were housed in plastic cages under controlled conditions (23–25 °C, 40–60% humidity, 12-h light/dark cycle) with free access to food and water.

2.2 Drugs and Chemicals

Rotenone and the vehicle (dimethyl sulfoxide; DMSO) were purchased from Sigma-Aldrich (St Louis, MO, USA).

Levodopa- karpidopa (Sinemet drug) obtained from the Glopal Napi Pharmaceuticals company, Cairo, Egypt.

2.3 Food Items

Beetroot and yogurt were obtained from a local market in Cairo, Egypt.

2.4 Experimental Design

Animals were randomly allocated into 5 groups (n = 7).

Group1. Recieved the vehicle (DMSO1ml / mice, s.c 3 times / week for consecutive 3 weeks) and served as the negative control group.

In the other 4 groups, mice received rotenone (1.5 mg/kg 3 times / week on days 1,3,5) for consecutive 3 weeks, s.c + basel diet [18].

Group2. (ROT) Received ROT (1.5 mg/kg 3 times / week for consecutive 3 weeks, s.c) and served as the positive control group.

Group3. (ROT- Sinemet) Received ROT + Sinemet (100mg/kg b.wt./day p.o) daily for consecutive 3 we,eks**[19],** as standard drug and served as standard treated group.

Group4. (ROT - Beetroot) Received ROT + Beetroot extract 300 mg/kg b.wt./day p.o daily for consecutive 3 weeks [20]**,** and served as treated group.

Group5. (ROT – Beetroot- Yogurt) Received ROT+ beetroot extract + Yogurt (10 ml / kg b.wt daily for consecutive 3 weeks [21] and served as treated group.

Foods and drug treatments were administered one hour after the daily rotenone injections. Each mouse was weighed at the start of the study and then weekly until the experiment ended. Daily food intake was recorded, and treatments continued throughout the study. In the third week, behavioral tests were conducted, during which treatments were discontinued from the start of testing [22].

2.5 Black Fecal Variables

Feces excreted by mice during 2 h were collected after 7, 14, and 17 d of administration. Fecal number, weight, and water content were measured[21].

2.6 Behavioral Tests

***2.6.1 Rotarod test (RT)*:** the test was carried out in accordance with [23, 24].

***2.6.2 Balance beam walking (BBW)*:** the test was performed to[25, 26].

2.6.3 Activity cage: the test was performed based on [27].

2.6.4 Cylinder test: the test was performed as reported by **[**28].

2.6.5 ***Forced swimming test***: the test was performed according to[29].

***2.6.6 Catalepsy test* :** the test was performed based on[30, 31].

2.6.7 ***Tail suspension test:*** the test was performed as reported by[32].

2.6.8 Hot plate: the test was carried out in accordance with, [33, 34].

2.7 Biochemical Evaluation on Mice Serum:

2.7.1 Determination of Liver Functions of Mice Serum:

2.7.1.1 Determination of Alanine aminotransferase (ALT)was according to FineTest CO, Catalogue No. EM0829.

2.7.1.2 Determination of Aspartate aminotransferase (AST) was according to FineTest CO , Catalogue No. EM0857.

2.7.2 Determination of Kidney Functions of Mice Serum:

2.7.2.1 Determination of Uric Acid was according to elabscience CO , Catalogue No. E-BC-K016-S.

2.7.2.2 Determination of Creatinine was according to BioVision's CO , Catalog No. # K625-100.

2.7.2.3 Determination of Urea nitrogen was according to SunLong Biotech Co, LTD, Catalog No. SL0892Mo

2.8 Biochemical Evaluation of Mice Brain Tissues:

2.8.1 Oxidative Stress Biomarkers:

2.8.1.1 Determination of Malondialdehyde (MDA) ELISA Kit was according to life span bio sciences, inc CO, Catalog No. LS-F28474.

2.8.1.2 Determination of Glutathione (GSH) Assay Kit was according to biovision CO, Catalog No. # K464-100; 100.

2.8.1.3Determination of Nitric Oxide (NO) Assay Kit was according to Elabscience CO, Catalog No.E-BC-K035-M.

2.8.2 Neuroinflammation Biomarkers:

2.8.2.1 Determination of Tumor Necrosis Factor Alpha, TNF-A ELISA Kit was according to Bioassay Technology laboratory. CO, Catalog No.E0117Mo.

2.8.2.2 Determination of Interleukin 6 IL-6 ELISA MAX™ was according to BioLegend's. CO, Catalog. No. 431301.

2.8.2.3 Determination of Inducible nitric oxide synthase INOS ELISA Kit was according to Novus Biologicals a Biotechne Brand. CO, NBP2-80256.

2.8.3 Apoptotic Indices of Mice Brain Tissus

2.8.3.1 Determination of Caspase 3 (CASP 3) ELISA Kit was according to Novus Biologicals a Elabscience®. CO, Catalog. No. E-EL-M0238.

2.8.3.2 Determination of Alpha-synuclein (α-syn) ELISA Kit was according to Novus Biologicals a Biotechne Brand. CO, NBP3-08174.

2.8.3.3 Determination of Deoxyribonucleic acid DNA fragmentation was according to Zymoresearch Quick-gDNA™ MiniPrep kit . Catalog No. D3024.

2.8.4 Neurotransmitters of Mice Brain Tissus

2.8.4.1 Determination of Acetyl cholinesterase (ACHE) ELISA Kit was according to CUSABIO Catalog No. CSB-E17521m.

2.8.4.2 Determination of Tyrosine Hydroxylase (TH) ELISA Kit was according to Novus Biologicals a Biotechne Brand. CO, NBP3-06921

2.8.4.3 Determination of Dopamine ELISA Kit was according to CUSABIO Catalog No. CSB -E08661m.

2.8.5 Evaluation of α-syn on Mice Intestines Tissues

Determination of Alpha-synuclein (α-syn) ELISA Kit was according to Novus Biologicals a Biotechne Brand. CO, NBP3-08174.

2.9 Fecal Variables Evaluation

The moisture content of feces was evaluated by measuring the weight of freshly collected pellets, which were then dried at room temperature until a stable weight was obtained. The percentage of water content was calculated as: [(fresh weight − dry weight) / fresh weight] × 100 [35].

3. Analysis of Statistical Data

Data are presented as mean ± standard error of the mean (SEM). Differences between groups were evaluated using one-way ANOVA, followed by Tukey's post hoc test for multiple comparisons [36]. Statistical analyses were conducted using GraphPad Prism (version 6, USA). Values of $p < 0.05$ were considered statistically significant.

4. Results

4.1 The Loco motor and Balance of Mice

Parkinson's disease (PD) is a chronic neurological disorder marked by nigrostriatal degeneration, muscle stiffness, resting tremors, slowed motor activity (bradykinesia), and difficulties with balance and posture[37]**.**The rotarod test is widely used in rodents to assess motor coordination, balance, and learning, as well as to detect neurological impairments. Modified versions improve the accuracy of preclinical drug and therapy evaluations [38]. Furthermore, the balance beam walking test serves as a functional approach to evaluate dynamic balance in experimental models [39].

The result present in (Figure.1) that the effect beetroot fortified yogurt as a natural source of levodopa compared to drug therapy on rotarod (Running time & Number of falls) and balance beam walking tests in male mice suffering from parkinson's disease.

The result showed that the running time on the rotarod test for positive control group was much lower than negative control group. Even, there were statistical significan differences observed between them at (*P<0.05*). At the same time, the number of falls on the rotarod test for positive control group was much higher than negative control group. Even, there were statistical significan differences observed between them at (*P<0.05*). As for the time taken to cross the balance beam for positive control group was much higher than negative control group. Even, there were statistical significan differences observed between them at (*P<0.05*).

On the other hand, the treated group with sinemet drug for running time on the rotarod test was higher than positive control group and much lower than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*). While, the mean value for number of falls on the rotarod test for sinemet drug group was lower than positive control group and much higher than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*). In addition to, the mean value for time taken to cross the balance beam test for sinemet drug group was lower than positive control group and much higher than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*).

On contrast, the treated groups with beetroot as a natural source of levodopa and yogurt for running time on the rotarod test were much higher than positive control group. Even, there were statistical significant differences observed between them at (*P<0.05*).As for the number of falls on rotarod test, the treated groups with beetroot as natural source of levodopa and yogurt were much lower than positive control group. Even, there were statistical significant differences observed between them at (*P<0.05*). Remarkably, the treated groups with beetroot as a natural source of levodopa and yogurt for running time and number of falls on rotarod test were approximately similar results as compared to negative control group. Even, there were no statistical significant differences observed between them at (*P<0.05*).

As regard, the treated groups with beetroot as a natural source of levodopa and yogurt for time taken to cross the bar in the balance beam test were much lower than positive control group. Even, there were statistical significant differences observed between them at (*P<0.05*).But, the treated groups with beetroot as natural source of levodopa and yogurt for time taken to cross the bar in the balance beam test were approximately similar results as compared to negative control group. Even, there were no statistical significant differences observed between them at (*P<0.05*).

4.2 Spontaneous Locomotor Activity of Mice

The cylinder test is used to evaluate a rat’s independent use of its forelimbs for supporting its body against the interior walls of a cylindrical chamber [40].In addition, activity cages provide a practical method for monitoring spontaneous coordinated movements in both rats and mice whether tested individually or in groups as well as tracking changes in this activity over time [41].

The data in (Figures. 2) that the effect beetroot fortified yogurt as a natural source of levodopa compared to drug therapy on cylinder and activity cage tests in male mice suffering from Parkinson's

disease. The data illustrated showed that the positive control group for cylinder and activity cage tests were much lower than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*).

While, the treated group with sinemet drug for cylinder and activity cage test was higher than positive control group. But still much lower than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*).

Conversely, the treated groups with beetroot as a natural source of levodopa and yogurt for cylinder and activity cage tests were much higher than positive control group. Even, there were statistical significant differences observed between them at (*P<0.05*). While, the treated group with beetroot plus yogurt for cylinder and activity cage tests were approximately normalize as compared to negative control group. Even, there were no statistical significant differences observed between them at (*P<0.05*).But, the treated group with beetroot still lower than negative control group. The best result was the group treated with beetroot as a natural source of levodopa plus yogurt. This group outperformed their counterpart who received the same beetroot as a natural source without yogurt.

4.3 Depression, Catalepsy, and Sensation of Mice

The forced swim test relies on the observation that when rodents are placed in an unavoidable and aversive environment, they adopt different coping strategies, which can be classified as either active or passive responses [42].Similarly, the tail suspension test (TST) is employed to evaluate depressive-like behaviors in animals; in this method, mice suspended by their tails exhibit periods of immobility, interpreted as an indication of behavioral despair [32, 43].The catalepsy bar test is a common approach for assessing an animal’s inability to correct an externally imposed posture, typically resulting from muscular rigidity[44] . Furthermore, the hot plate test (HPT) in a dynamic setting is used to measure thermal nociception in rats by evaluating central mechanisms and determining the thermal nociceptive threshold [45].

The result present in (Figure.3) that the effect beetroot fortified yogurt as a natural source of levodopa compared to drug therapy on some behavior tests such as forced swim, tail suspension, catalepsy and hot plate in male mice suffering from parkinson's disease. The result showed that the positive control group for forced swim test was much lower than negative control group. Even, there were statistical significan differences observed between them at (*P<0.05*). While, the mean values for positive control group in tail suspension, catalepsy and hot plate tests were much higher than negative control group. Even, there were statistical significan differences observed between them at (*P<0.05*).

On contrast, the treated group with sinemet drug for forced swim test was higher than positive control group but still much lower than negative control groups. Even, there were statistical significant differences observed between them at (*P<0.05*). While, the means value for sinemet drug group in tail suspension, catalepsy and hot plate tests were lower than positive control group and much higher than

negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*).

As regarded, the treated groups with beetroot as a natural source of levodopa and yogurt in forced swim test were much higher than positive control group. Even, there were statistical significant differences observed between them at (*P<0.05*).While, the treated groups with beetroot as natural source of levodopa and yogurt in tail suspension, catalepsy and hot plate tests were much lower than positive control group. Even, there were statistical significant differences observed between them at (*P<0.05*). Remarkably, all treated groups with natural source of levodopa and yogurt in forced swim, tail suspension, catalepsy and hot plate tests were approximately normalize as compared negative control group. Even, there were no statistical significant differences observed between them at (*P<0.05*).

4.4 Liver Functions of Mice Serum

exposure to toxicants and impaired liver detoxification can increase susceptibility to Parkinson's disease, as liver dysfunction may lead to neurological complications including parkinsonism [46, 47].

The result illustrated in (Figure.4) that effect beetroot fortified yogurt as a natural source of levodopa compared to drug therapy on liver functions such as alanine amino transferase (ALT) and aspartate amino transferase (AST) in male mice suffering from parkinson's disease.The result illustrated showed that ALT and AST levels for positive control group were much higher than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*).

On the other hand, observed that the sinemet drug group of ALT and AST levels was lower than positive control group and still much higher than negative control group. Even, there were statistical significant differences observed between them at (P<0.05).

As regard, the treated group with beetroot as a natural source of levodopa plus yogurt and counterpart without yogurt of ALT and AST were much lower than positive control group. Even, there were statistical significant differences observed between them at (P<0.05). But, the treated group with beetroot as a natural source of levodopa plus yogurt it has approximately similar result as compared to negative control group. Even, there were no statistical significant differences observed between them at (P<0.05).While, the treated group with beetroot as a natural source of levodopa without yogurt did not same affect.

4.5 Renal Functions of Mice Serum

Evidence shows that even early renal disease is linked to cognitive decline, depression, and motor disturbances. CKD-related toxins and metabolic syndrome can impair dopaminergic neurons, while kidney-derived hormones like vitamin D provide neuroprotection [48].

The result present in (Figure.5) that the effect beetroot fortified yogurt as natural source of levodopa compared to drug therapy on renal functions such as urea nitrogen, creatinine and uric acid in male mice

suffering from parkinson's disease. The result showed that the urea nitrogen, creatinine and uric acid levels in serum male mouse for positive control group were much higher than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*).

Whereas, the data illustrated showed that the urea nitrogen, creatinine and uric acid levels for sinemet drug group were lower than positive control group. But, still much higher than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*).

On contrast, all treated groups with beetroot as a natural source of levodopa and yogurt for urea nitrogen, creatinine and uric acid levels were much lower than positive control group. Even, there were statistical significant differences observed between them at (*P<0.05*).But, the treated groups with beetroot as a natural source of levodopa plus yogurt was has approximately normal level of urea nitrogen , creatinine and uric acid as compared to negative control group. Even, there were no statistical significant differences observed between them at (*P<0.05*). While, treated group with beetroot still higher than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*).

Moreover, the result showed that the treated group with beetroot has approximately similar result as compared to sinemet drug group for uric acid level. Even, there were no statistical significant differences observed between them at (*P<0.05*).

4.6 Oxidative Stress of Mice Brain Tissue

Recent research shows that neuronal death can occur via multiple mechanisms, with oxidative stress indicated by MDA, GSH, and NO-being a major contributor [49].

The data illustrated in (Figure.6) that effect beetroot fortified yogurt as a natural source of levodopa compared to drug therapy on Oxidative Stress Biomarkers such as Malondialdehyde (MDA), Nitric oxide (NO) and Glutathione (GSH) in male mice suffering from parkinson's disease.The data illustrated showed that the oxidative stress biomarker such as MDA and NO levels in brain tissue for positive control group were much higher than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*). But**,** the level of GSH in brain tissue for positive control group was much lower than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*).

whereas, the treated group with sinemet drug for MDA and NO levels was lower than positive control group and much higher than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*). Moreover, the treated group with sinemet drug for GSH level was much higher than positive control group. But, still lower than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*).

On contrast, the treated groups with beetroot as a natural source of levodopa and yogurt for MDA and NO levels were lower than positive control group. But, the treated group with beetroot as a natural source

of levodopa without yogurt still higher than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*). While, the treated groups with beetroot plus yogurt has approximately similar result as compared to negative control group. Even, there was no statistical significant differences observed between them at (*P<0.05*).

Conversely, the data illustrated showed that GSH for the treated groups with beetroot as a natural source of levodopa and yogurt were much higher than positive control group. But, the treated group with beetroot without yogurt still lower than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*)**.** While, treated group with beetroot plus yogurt was has approximately normal level of GSH as compared to negative control group. Even, there were no statistical significant differences observed between them at (*P<0.05*)**.**Unlike the groups treated with beetroot as a natural source of levodopa without yogurt, it didn't give the same effect.

Moreover, the treated groups with beetroot as a natural source of levodopa has approximately similar result as compared to sinemet drug group in NO and GSH levels. Even, there were no statistical significant differences observed between them at (*P<0.05*).

4.7 Neurotransmitters of Mice Brain Tissu

Parkinson's disease is a progressive neurodegenerative disorder caused by the loss of dopamine-producing neurons in the substantia nigra [50].

The data illustrated in (Figure.7) that the effect beetroot fortified yogurt as a natural source of Levodopacompared to drug therapy on neurotransmitters such as Ty,rosine hydroxylase (TH), Dopamine (DOPA) and Acetylcholinesterase (ACHE) in brain tissue male mice suffering from parkinson's disease.The data illustrated showed that the neurotransmitters such as TH, DOPA and ACHE levels in brain tissue for positive control group were much lower than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*).

While, the treated group with sinemet drug for TH, DOPA and ACHE level was higher than positive control group and much lower than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*).

On contrast, all treated groups with beetroot as a natural source of levodopa and yogurt for TH, DOPA and ACHE levels were much higher than positive control group. Even, there were statistical significant differences observed between them at (*P<0.05*). As regard, the treated group with beetroot as a natural source of levodopa plus yogurt was has approximately similar result as compared to negative control groups. Even, there were no statistical significant differences observed between them at (*P<0.05*). While, the treated group with beetroot as a natural source of levodopa without yogurt still lower than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*).

4.8 Apoptotic Indices of Mice Brain Tissu

Apoptosis is a programmed cell death process, with mitochondria playing a key role; reduced mitochondrial membrane permeability is an early event in this pathway [51].

The data illustrated in (Figure.8) that the effect beetroot fortified yogurt as a natural source of Levodopacompared to drug therapy on apoptotic indices such as deoxyribonucleic acid (DNA), caspase-3 (Casp-3) and α-synuclein (α-syn) in brain tissue male mice suffering from parkinson's disease.The data illustrated showed that DNA level for positive control group was much lower than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*). While, the Casp-3 and α-syn levels in brain tissue for positive control group were much higher than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*).

On the other hand, the treated group with sinemet drug for DNA level was higher than positive control group and much lower than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*).But, the treated group with sinemet drug for Casp-3 and α-syn levels were lower than positive control group and much higher than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*).

Concerning, the treated groups with beetroot as a natural source of levodopa and yogurt for DNA level were much higher than positive control group. Even, there were statistical significant differences observed between them at (*P<0.05*). But, the treated group with beetroot as a natural source of levodopa without yogurt still lower than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*). While, surprisingly, the treated group with beetroot plus yogurt responded in a comparable way as compared to negative control group. Even, there were no statistical significant differences observed between them at (*P<0.05*). As for the other parameters, such as Casp-3 and α-syn levels, the treated groups with beetroot as a natural source of levodopa and yogurt were much lower than positive control groups. Even, there were statistical significant differences observed between them at (*P<0.05*).However, the treated group with beetroot as a natural source of levodopa without yogurt still higher than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*). While, the treated group with beetroot plus yogurt has approximately similar result as compared to negative control groups so alike, almost as if the induction with rotenone had no impact at all. Even, there were no statistical significant differences observed between them at (*P<0.05*).

Inflammatory Indices of Mice Brain Tissue4.9

Neuroinflammation, a CNS defense mechanism, involves overactivation of neurons and glial cells, releasing markers like TNF-α, IL-1β, IL-10, NO, and COX-2, which contribute to the onset, diagnosis, and treatment of neurodegenerative diseases [52]**.**

The result present in (Figure.9) the effect beetroot fortified yogurt as a natural source of Levodopa compared to drug therapy on Inflammatory indices such as tumor necrosis factor alpha (TNF-α), inducible nitric oxide synthase (Inos) and interleukin-6 (IL-6) in brain tissue male mice suffering from

parkinson's disease.The result illustrated showed that the inflammatory indices such as TNF-α, Inos and IL-6 levels in brain tissue for positive control group were much higher than negative control group. Even, there were statistical significan differences observed between them at (*P<0.05*).

In relation to, the treated group with sinemet drug for TNF-α, Inos and IL-6 levels were lower than positive control group and much higher than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*).

Whereas, the treated groups with beetroot as a natural source of levodopa and yogurt for TNF-α, Inos and IL-6 levels were much lower than positive control group. Even, there were statistical significant differences observed between them at (*P<0.05*). But, the treated group with beetroot as a natural source of levodopa without yogurt still higher than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*). While, it turns out the treated group with beetroot plus yogurt has approximately similar result as compared to negative control groups. Even, there were no statistical significant differences observed between them at (*P<0.05*), which is interesting. As also the results showed, the treated group with beetroot has approximately similar result as compared to treated group with sinemet drug .Even, there were no statistical significant differences observed between them at (*P<0.05*).

Apoptotic Indices α- Synuclein of Mice Intestines4.10

Importantly, α-Synuclein accumulation in the substantia nigra underlies motor impairments in PD, while non-motor symptoms, often affecting the gastrointestinal tract, can appear years earlier and may be linked to α-Syn aggregates in the GIT [53].

The data illustrated in (Figure.10) the effect beetroot fortified yogurt as a natural source of Levodopacompared to drug therapy yogurt and drug therapy on apoptotic indices α- Synuclein (α-syn) in intestines tissue male mice suffering from parkinson's disease. The data illustrated showed that the positive control for α-syn level in intestines tissues group was much higher than negative control group. Even, there were statistical significan differences observed between them at (*P<0.05*). on contrast, the treated group with sinemet drug for intestines α-syn level was lower than positive control group. But, still much higher than negative control groups. Even, there were statistical significant differences observed between them at (*P<0.05*).

On contrast, the treated groups with beetroot as a natural source of levodopa and yogurt for intestines α-syn level were much lower than positive control group. Even, there were statistical significant differences observed between them at (*P<0.05*). But, the treated group with beetroot as a natural source of levodopa without yogurt still higher than negative control group. Even, there were statistical significant differences observed between them at (*P<0.05*). While, the treated group with beetroot plus yogurt has approximately similar result as compared to negative control group. Even, there were no statistical significant differences observed between them at (*P<0.05*).

4.11 Constipation of Mice

Recent studies suggest that intestinal microbial dysbiosis contributes to constipation by altering gut flora[15] Such dysbiosis can also impair the absorption of Parkinson's disease medications, including levodopa [54].

The data illustrated in Table (2) that the effect beetroot fortified Yogurt as a natural source of levodopacompared to drug therapy on Number, weight (g) and water content (%) in fecal male mice suffering from parkinson's disease. The data illustrated showed that the fecal number, weight and water content of the positive control group were significantly lower than negative control group. These values began to gradually decline when measured after 7, 14, and 21 days, reflecting the severity of constipation in this group. However, these differences were not statistically significant at $p < 0.05$.

On contrast, the treated group with sinemet drug for fecal number was lower than positive control group and negative control group when measured after 7, 14, and 21 days. Although the number of fecal pellets decreased compared to the positive control group. But, don't have statistically significant differences observed at $p < 0.05$.

As regard, the treated groups with beetroot as a natural source of levodopa and yogurt for fecal number, The results were striking when measured at 7, 14 and 21 days, as some groups had fewer stools than the negative control group, and some recorded the same level of fecal count. However, it was interesting that there were groups that recorded a higher number, but the treated groups were still lower than the negative control group. But, don't have statistically significant differences observed at $p < 0.05$.

Table (1): Effect Beetroot Fortified Yogurt as a Natural Source of Levodopa on Number, Weight (g) and Water content (%) in Fecal Male Mouse Enhance in Rotenone Induced Parkinson's Disease

parameters / Groups	Number			weight (g)			water content (%)		
	7 days	14 day	21 day	7 days	14 day	21 day	7 days	14 day	21 day
Negative (-ve)	10	14	14	1.595	1.624	1.638	71.4	76.5	78.8
Positive (+ve)	8	10	8	0.645	0.582	0.417	48.4	43.7	38.2
Sinemet	6	6	7	0.815	1.124	1.207	51.8	60.5	63.6
Beetroot	8	6	8	0.894	1.194	1.326	62.5	70.2	72.3
Beetroot + Yogurt	9	5	5	1.235	1.452	1.575	69.4	76.4	79.5

Values are expressed as mean ± SE (n=6).

5. Discussion

Nutritional interventions are increasingly utilized in managing neurodegenerative disorders and may help alleviate symptoms associated with Parkinson's disease and enhance general health. Beetroot, as a

natural source of levodopa, represents a promising dietary component that could support dopaminergic function and contribute to better disease management. Compared with conventional drug therapy, such an approach may offer symptomatic relief with fewer side effects.

In the current study, the increasing in number of falls and decreasing in running time for rotarod and time taken to cross the balance beam reduction for positive control group as compared with other control group. It may be due to injection with rotenone, which led to impaired coordination and balance, which are common symptoms of Parkinson's disease. The longer time it took them to cross the balance beam indicates difficulty moving and poor muscle control, reflecting the motor dysfunction associated with Parkinson's disease.To further support this explanation, it is very important to clarify these results in the light of previous findings. Several risk factors, including age, head injury, genetic predisposition, and exposure to toxins, contribute to the progression of Parkinson's disease (PD) [55].Dopamine depletion results in motor impairments, including resting tremor, bradykinesia, postural instability, and muscle rigidity[56]. These results agreed with **Makav & Eroğlu** [57] study whom reported that the rotarod test showed significant differences in mean running times among the groups ($P < 0.05$). Daily oral administration of Sinemet improved movement and balance.This result agreed with **Perez-Pardo et al.** [58],study whom reported that rotenone injection significantly reduced rotarod performance and grip strength in mice, while levodopa improved these outcomes. In contrast, beetroot extract with yogurt produced greater benefits, enhancing dopamine levels and leading to superior improvements in rotarod and balance beam tests.These results agreed with **Leroy et al.** [59], study whom reported that this review and preliminary meta-analysis demonstrated that oral levodopa markedly enhanced performance in clinical balance assessments, such as the Berg Balance Scale (BBS) and the Postural Reaction Test (PRT), with non-significant positive trends in other tests.Also, **Kwankaew *et al.*** [60], study whom reported that betanin from red beet extract restored the impaired motor performance caused by CCI surgery in mice, as confirmed by rotarod testing compared to controls.In addition to, **Zhao et al.** [61], study whom reported that FMT-treated mice showed significant improvements in motor function, as reflected by better performance in the rotarod test ($P < 0.001$), adhesive removal test ($P < 0.001$), grip strength test ($P < 0.01$), and pole test ($P < 0.001$) compared to the rotenone-treated group (Fig.1).

Furthermore, rotenone injection resulted in noticeable motor impairment.These results reflected in a reduced use of the forelimbs during the cylinder test and a decline in spontaneous activity cage test. These observations suggest that rotenone may negatively affect dopaminergic pathways, leading to deficits in motor coordination and exploratory behavior, reflecting the motor dysfunction associated with Parkinson's disease. These results agreed with **Avcı et al.** [62]**,** study whom reported that rotenone significantly impaired motor coordination and balance in the cylinder test, while daily Sinemet treatment improved these functions.These results agreed with **Gellhaar et al.** [63], study whom reported that long-term L-DOPA led to abnormal repetitive behaviors in MitoPark mice, while natural levodopa with yogurt showed more stable benefits, enhancing forelimb use and exploration in cylinder and activity cage tests. These results agreed with **ElSayed et al.** [64], study whom reported that the high-dose betanin improved locomotor activity and alleviated rotenone-induced incoordination, leading to better motor function in the

PD model.**Kim et al. [65],** study whom reported that the probiotic intake significantly improved motor function ($p < 0.05$), as confirmed by one-way ANOVA and independent t-test analyses (Fig.2).

It is noteworthy that the observed reduction in locomoter activity, along with increased immobility, hypo-responsiveness, apathy, Loss of sensation of heat and prolonged catalepsy duration, may strongly suggest that rotenone injection is reponsaible for these behavioral impairments. This was confirmed by the results of the forced swim, tail suspension, hot plate, and catalepsy tests.These results align with previous studies demonstrating that the neurotoxic effects of rotenone induce Parkinson's-like symptoms through mitochondrial complex inhibition and the promotion of oxidative stress.Such behavioral alterations reflect the neurodegenerative impact of rotenone on dopaminergic pathways particularly in the substantia nigra. This is one of common symptoms of parkinsons disease, known as non- motor symptoms. Consistent with a study on 109 newly diagnosed, untreated patients, non-motor symptoms such as apathy, sleep disturbances, daytime sleepiness, and constipation were reported in 60–70% of cases before diagnosis, occurring more frequently than in controls. Other early symptoms included anhedonia, memory issues, sensory loss, mood changes, sweating, fatigue, pain, and gastrointestinal problems, some of which appeared over a decade before motor onset [66]**.** Symptoms of depression and anxiety may appear well in advance of the actual diagnosis.

[67].Moreover, **Ahmed et al.**[68]**,** study whom reported that the Depressive-like behaviors in rotenone-treated rats were assessed by FST, TST, catalepsy, and hot plate tests, showing significantly longer immobility times ($p<0.001$) versus controls. Daily Sinemet treatment improved motor coordination and balance.These results agreed with**Luthra et al.** [69]**,** study whom reported that haloperidol-induced PD models showed motor and behavioral deficits measured by catalepsy, hot plate, and swim tests. While L-DOPA provided some recovery, beetroot extract with yogurt was more effective, enhancing dopamine, improving motor function, and reducing anxiety and stiffness, as confirmed by multiple behavioral tests. These results agreed with **Sulakhiya et al .**[70]**,** study whom reported that to assess the effect of BVEE on ARS-induced depressive-like behavior, immobility time in the FST was measured. ARS significantly increased immobility ($p<0.001$), confirming its depressive effect, while BVEE pretreatment markedly reduced this response ($p<0.05$; $p<0.01$ at 200 mg/kg).Moreover, **Sulakhiya et al . [70],** study whom reported that mice exposed to ARS showed a marked rise in immobility time in the TST ($p<0.001$), while BVEE pretreatment significantly reversed this effect at 100 mg/kg ($p<0.05$) and 200 mg/kg ($p<0.01$), demonstrating its protective role.In addition to, **Liu et al.** [71], study whom reported that restraint stress induced depressive-like behaviors and gut microbiota alterations in mice, while probiotic treatment restored microbial balance and alleviated these effects, leading to improved outcomes in FST, TST, catalepsy, and hot plate tests (Fig.3).

Evalution of liver function markers provides insight into systemic effects associated with Parkinson's disease in experimental models. In this context, the resuts showed that increased in ALT and AST reduction for positive control group as compared to other control groups. It may be due to, injection with rotenone which increase Parkinson's symptoms in mice. These results agreed with studies indicate that liver disease–related parkinsonism may result from mechanisms such as metal overload,

hyperammonemia, oxidative stress, disrupted glia-neuron balance, and neurotransmitter changes, which collectively contribute to dopaminergic neuron loss in PD [72].In addition to, **Wang et al.** [73]**,** study whom reported that

"Rotenone markedly elevated hepatic index and serum AST and ALT, and caused hepatocyte structural damage. Oral Sinemet provided only slight improvement in liver function.In this context, these results agreed with **Kura et al.** [74], study whom reported that serum biochemical analysis (ALT, AST, ALP, GGT) confirmed liver function. AST was slightly higher in levodopa-treated mice, though not significantly different from controls. In contrast, beetroot as a natural levodopa source combined with yogurt showed greater positive effects on liver function than Sinemet in PD mice.These results agreed with **Gashash & Wahba [75]**study whom reported that

the findings showed Rats receiving a diet enriched with beetroot juice showed a significant decrease ($P < 0.05$) in ALT and AST levels relative to the control group. Moreover, **Hasan et al.** [76, 77]**,** study whom reported that ALT and AST are key markers for diagnosing NAFLD, and their levels decreased with yogurt supplementation. This effect may relate to probiotic activity that normalizes liver enzymes, reduces intestinal and hepatic inflammation, and protects against liver injury (Fig.4).

As regard, the increasing in urea nitrogen, creatinine and uric acid reduction for positive control group as compared to control groups. It may be due to, injection with rotenone which increase Parkinson's disease. These results agreed with **Udeani et al. [78],** study whom reported that repeated intragastric rotenone administration caused kidney damage, evidenced by elevated BUN, uric acid, and creatinine levels. Moreover, **Juli et al.** [79]**,** study whom reported thatrotenone significantly elevated kidney function markers, including urea ($p<0.05$) and creatinine ($p<0.001$), compared to controls. Sinemet treatment showed only mild improvement in these parameters.

In addition to,the best result was the treated group with beetroot as a natural source of levodopa plus yogurt more than counterpart without yogurt did not give the same affect. Notably, the beetroot as a natural source of levodopa and yogurt can positively enhance renal functions better than the levodopa drug (sinemet) in the serum of mice suffering from Parkinson's disease. These results agreed with **Gashash & Wahba** [75]study whom reported dietary supplementation with beetroot juice resulted in a significant reduction ($P < 0.05$) in serum urea, creatinine, and uric acid levels in rats compared with the positive control group.In addition to, **Cheng et al.** [80]**,** study whom reported that yogurt significantly ($p<0.05$) lowered urinary protein, serum creatinine, and BUN levels in nephritic mice compared to controls (Fig.5).

The current findings on oxidative stress markers provide insight into the biochemical changes linked to Parkinson’s disease in the experimental group were evaluated.The increasing in NO and MDA and reduction in GSH for positive control group as compared with other control groups. It may be due to injection with rotenone which increases Parkinson's disease. These results agreed with studies have shown that lifestyle and environmental factors, such as exposure to pesticides and contact with heavy metals, contribute to one in five cases of Parkinson's disease, which can have a detrimental effect on

oxidative stress levels in brain tissue[81].And**, Abdel-Salam et al.** [82]**,** studywhom reported that rotenone treatment increased brain MDA and NO while depleting GSH, whereas oral Sinemet improved these oxidative stress markers in PD mice.These results agreed with **Ayaz et al.** [83]**,** study whom reported that the rotenone elevated brain MDA and NO and reduced GSH, while Sinemet partially restored these levels in PD mice. On contrast, It was observed that the group suffering from Parkinson's disease and treated with beetroot as a natural source of levodopa plus yogurt have best result for MDA and NO as compared to counterpart without yogurt. This result mean that, the beetroot as a natural source of levodopa and yogurt have beneficial therapeutic effect better than sinemet drug on oxidative stress markers in the tissue of mice with Parkinson's disease. These results agreed with **Sushama et al.[84],** study whom reported that our findings support that natural levodopa sources deliver L-DOPA to the brain more effectively and sustainably than conventional antiparkinsonian drugs.Moreover, **Thong-Asa et al. [85],** study whom reported that betanin extracted from red beet significantly reduced MDA levels and enhanced GSH activity ($p < 0.05$), demonstrating neuroprotective effects against rotenone-induced Parkinson's disease in mice through its potent antioxidant action (Fig.6).

The chemical evaluation of neurotransmitter levels highlights the harmful effects resulting from exposure to toxins on brain neurochemistry in mice suffering with Parkinson's disease. The decreasing in TH, DOPA and ACHE reduction in positive control group as compared with other control groups. It may be due to, injection with rotenone which increase parkinson's disease. These results agreed with **Abdel-Salam et al . [86],** study whom reported that the rotenone administration resulted in a significant 57.0% reduction .in striatal tyrosine hydroxylase levels relative to the vehicle-treated control group.Moreover, **Landau et al. [87],** study whom reported that the rotenone reduced locomotor activity, lowered brain dopamine levels, and decreased vesicular sequestration indices (DOPAC/dopamine) compared to controls. In addition to, **Hasan et al. [88],** study whom reported that the rotenone markedly decreased AChE activity in the cerebellum ($p<0.05$), whereas oral Sinemet improved neurotransmitter balance by elevating DOPA, AChE, and TH levels. While, administration of the oral sinemet treatment led to an improvement in the levels of DOPA, Ache, and TH.These results agreed with **Chen et al. [89],** study whom reported that L-DOPA increased TH expression in SN and STR. On contrast, It was observed that, the groups suffering from Parkinson's disease and treated with beetroot as a natural source of levodopa plus yogurt has best result for TH, DOPA and ACHE as compared with its counterpart without yogurt.This result reflected that natural beetroot as a source of levodopa and yogurt has a positive therapeutic effect better than sinemet drug on neurotransmitters in brain tissue of mice with Parkinson's disease. These results agreed with **Ghanbari et al .[90],** study whom reported that the dopamine receptor gene expression in the hippocampus was significantly elevated ($p\leq0.001$, $p\leq0.01$) in the beetroot juice and HIIT groups versus the diabetic control, with further increases ($p\leq0.001$) observed in the combined HIIT + beetroot group. Moreover, **Olasehinde et al.[91],** study whom reported that BR improved an increase ACE activities were observed in the brain of rats compared control group. In addition to, **Hsieh et al.** [92]**,** study whom reported that the daily probiotic supplementation markedly enhanced gait, balance, and coordination compared with the sham group, while maintaining TH-positive cells in the substantia nigra.

These results suggest that prolonged probiotic intake protects dopaminergic neurons and mitigates motor impairments in MitoPark PD mice (Fig.7).

Evaluation of DNA integrity, Caspase-3, and α-synuclein levels highlights the cellular damage and apoptosis associated with Parkinson's disease in mice.The decreasing in DNA and increasing in Casp-3 and α-syn reduction in positive control group as compared with other control groups. It may be due to, injection with rotenone which increase Parkinson's disease. These results agreed with **Balakrishnan et al. [93],** study whom reported that the chronic rotenone administration induced PD-like pathology, marked by oxidative stress, elevated α-synuclein, and increased expression of apoptotic proteins caspase-3, -8, and -9.Moreover, **Khalil et al. [94],** study whom reported that the rotenone-treated mice exhibited elevated TNF-α, IL-1β, DNA damage, and caspase-3 expression, while oral Sinemet improved DNA integrity, Casp-3, and α-synuclein levels, alleviating PD symptoms.These results agreed with **Motawi et al. [95],** study whom reported thatSinemet significantly reduced caspase-3 fragmentation and α-synuclein levels ($p<0.0001$) compared to rotenone, showing approximately 70% improvement in these markers.On contrast, It was observed that the groups suffering from Parkinson's disease and treated with beetroot as a natural source of levodopa plus yogurt has best result for DNA, Casp-3 and α-syn as compared to its counterpart without yogurt.This results reflected that, the beetroot as a natural source of levodopa and yogurt have a positive therapeutic effect better than sinemet drug on apoptotic indices in brain tissue of mice with Parkinson's disease. These results agreed with **Phukan et al. [96],**studywhom reported that the red beetroot extract, rich in betanin, reduced abnormal α-synuclein accumulation in dopaminergic neurons of PD mice.Also, **Wang et al. [97],** study whom reported that our results indicated that probiotics of *L. plantarum* DP189 reduced the α-SYN accumulation in SNin brain mice in comparison with control group ($p < 0.05$) (Fig.8).

Moreover, the resuts showed that the increased in TNF-α, Inos and IL-6 levels reduction in positive control group as compared with other control groups. It may be due to, injection with rotenone which increase parkinson's disease. These results agreed with **Siracusa et al.[22],** study whom reported that iNOS expression in the SN was significantly increased 28 days after rotenone administration compared to controls.Moreover, **Zhang et al.[98],** study whom reported that the rotenone significantly increased pro-inflammatory cytokines (IL-6, IL-1β, TNF-α, IFN-γ, PGE2) and NO in the SN ($p<0.01$), while arctigenin was assessed for its effect on this neuroinflammation.On the other hand, administration of oral sinemet treatment resulted in a noticeable reduction in the levels of TNF-α, iNOS, and IL-6, indicating an attenuation of neuroinflammatory responses in Parkinson's disease.These results agreed with **Motawi et al. [95],** study whom reported that the regarding to IL-6 level showed significant decrease with sinemet (each $P < 0.0001$) treatments as compared with the rotenone group. Moreover, **Abdel-Sattar et al. [99],** study whom reported that the mice treated with L-Dopa/carbidopa showed low levels of the inflammatory markers TNF-α, NF-κB and IL-1β in the striatum compared with rotenone group.The observed results allowed us to simply conclude that groups suffering from Parkinson's disease and treated with beetroot as a natural source of levodopa plus yogurt showed the best outcomes for TNF-α, iNOS, and IL-6 compared to its counterpart without yogurt.It reflected that, natural source of levodopa and yogurt have a positive therapeutic effect better than sinemet drug on inflammatory cytokines in

brain tissue of mice with Parkinson's disease. These result agree with **Khan et al. [100],** study whom reported that the negative control group showed a significant rise ($P < 0.0001$) in brain inflammatory cytokines compared to normal controls. Conversely, betalains and chloroquine groups exhibited a marked reduction ($P < 0.0001$), with the betalains protective group restoring cytokine levels toward normal values.In addition to, **Ano et al. [101],** study whom reported that extracts from the surface of fermented dairy products suppressed microglial TNF-α production in a dose-dependent manner, enhancing anti-inflammatory activity and Aβ phagocytosis, whereas extracts from the interior or unfermented products had no effect (Fig.9).

The present observations regarding α-synuclein accumulation highlight the potential involvement of the gut–brain axis in the development of Parkinson's disease.This result supported the It may be due to, injection with rotenone leads tothe presence of pathogenic α-syn in both the gut and brain highlights the possible contribution of the enteric nervous system (ENS) to the etiology of PD. The hypothesis that α-syn pathology may propagate from the gut to the brain, leading to degeneration of the nigrostriatal dopaminergic pathway, remains highly compelling.These result agreed with **Chen et al.[102],** study whom reported that the rotenone significantly increased intestinal α-syn levels in hα-syn+/– mice, showing a 2.3-fold rise at 6 months and a 2-fold rise at 12 months compared to age-matched controls. Moreover, administration of oral sinemet treatment appeared to mitigate α-synuclein accumulation, suggesting its potential in modulating the gut–brain axis and reducing neurodegeneration in Parkinson's disease. This improvement indicates that sinemet treatment can partially counteract the effects of rotenone-induced pathogenic α-syn in both the gut and brain mice. These results agreed with **Pellegrini et al. [103],** studywhom reported that the L-DOPA/BE treatment improved colonic motor activity and normalized immunopositivity, with beetroot plus yogurt showing the greatest reduction in intestinal α-syn in PD mice compared to beetroot alone.Subsequently, it is reflected that, beetroot as a natural source of levodopa has a positive therapeutic effect better than sinemet drug on apoptotic indices such as α- synuclein in intestines tissue of mice with Parkinson's disease. These results agreed with **Ko et al. [104],** study whom reported that the red beet RP and BP altered gut microbiota composition and SCFA production differently across enterotypes, with the Bifidobacterium cluster showing significantly reduced alpha diversity and distinct microbial changes.In addition to, **Perazza et al. [105],** study whom reported that shannon index and PCoA analyses showed that dairy fermentation significantly modulated gut microbiota, with WT and LRKO mice fed fermented products exhibiting distinct α-diversity and metagenomic profiles compared to nonfermented diets(Fig.10) .

Regarding gastrointestinal function, the results showed that the positive control group showed significantly reduced fecal count, weight, and water content compared with the negative control group.Quite simply, it can be said that this occurred due to rotenone injection, which caused constipation in mice with Parkinson's disease, thereby contributing to impaired dopamine production and, consequently, reduced motor activity. These results were approximately agreed with **Chu et al.** [106]**,** study whom reported that rotenone intake led to a significant reduction in fecal pellet weight and water content compared with the control group in PD mice ($p < 0.05$). During the treatment period, a marked change in stool shape was observed, from separate pellets to more compact, sometimes

elongated, ribbon-like masses. This change in shape led to a decrease in the number of counted pellets. While, fecal weight and water content were higher than positive control group and much lower than negative control groups.These reflected that for sinemet drug group began to gradually increase when measured after 7, 14, and 21 days. This indicates that levodopa had a weak effect in improving the symptoms of constipation. However, these differences were not statistically significant at $p < 0.05$. These result was approximately agreed with **Radisavljevic et al. [107],** study whom reported that chronic oral L-DOPA plus carbidopa (LDCD) treatment reduced whole-gut transit time ($p<0.0001$), alleviating constipation-like symptoms in mice.On contrast, the number of fecal pellets decreased as compared to the positive control group, this is not necessarily indicative of worsening constipation. During the treatment period, a marked change in stool form was observed, from separate pellets to denser clumps. Sometimes longer, ribbon-like. This change in shape resulted in a decrease in the number of counted granules. This change is likely related to the effect of various natural sources of levodopa and yogurt, which improve fecal consistency and increase its viscosity, resulting in a less disintegrated and more cohesive fecal, a positive sign in the treatment of constipation. Thus, there is no exacerbation of Parkinson's disease symptoms as compared with the fecal weight and water content. But, without statistical significance. At the same time, fecal weight and water content were much higher than positive control group. And lower than negative control group. These mean،s that the treated groups with beetroot as a natural source of levodopa and yogurt began to gradually increase gradually and significantly when measured after 7, 14, and 21 days. Altogether, it was observed that, groups suffering from Parkinson's disease and treated with beetroot plus yogurt has best result for water content and fecal weight as compared to it is counterpart without yogurt. Subsequently, it was reflected that the beetroot as a natural source of levodopa and yogurt have a positive therapeutic effect better than sinemet drug for fecal weight and water content of mice with Parkinson's disease. These results were approximately agreed with **Zhao et al. [61],** study whom reported that the rotenone caused weight loss, motor deficits, and GI dysfunction in mice, while FMT treatment significantly improved fecal output and alleviated these PD-related symptoms.Also, **Mahran et al.[53],** study whom reported thatexperimental diets, especially PBM, significantly ($p\leq0.05$) normalized constipation-related parameters in rats without causing diarrhea, supporting the traditional use of PP and BP for LOP-induced constipation (Table.1).

6. Conclusion

Beetroot-fortified yogurt, as a natural source of levodopa, showed superior effects compared to conventional drug treatment, by enhancing brain dopaminergic neurons and improving motor symptoms in Parkinson’s-induced mice.Moreover, its antioxidant properties provided additional neuroprotection and symptom relief. These findings indicate that yogurt enriched with beetroot has the potential to be developed as a safe functional product, and cost-effective dietary strategy for Parkinson’s disease management.

Abbreviations

PD: Parkinson's disease; ROT: Rotenone; DMSO: dimethyl sulfoxide;L-DOPA:Levodopa; ALT:alanine aminotransferase; AST: aspartate aminotransferase; MDA: Malondialdehyde; GSH: Glutathione; NO: Nitric Oxide;TNF: Tumor Necrosis Factor Alpha; IL-6: Interleukin 6; INOS: Inducible nitric oxide synthase; CASP 3: Caspase 3; α-syn: Alpha-synuclein; DNA: Deoxyribonucleic acid;ACHE: Acetyl cholinesterase; TH: Tyrosine Hydroxylase; DOPA: Dopamine.

Declarations

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Availability of data and materials

The data supporting the findings of this study are available from the corresponding author upon reasonable request. All datasets and materials used in the analysis and interpret- action of results can be shared to ensure transparency and reproducibility in line with the journal's data sharing policy.

Competing Interests

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

The experimental protocol was reviewed and approved by the Ethics Committee of the Faculty of Specific Education, Ain Shams University. All animal procedures were conducted in accordance with the guidelines of the committee and international standards for the care and use of laboratory animals.

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Figures

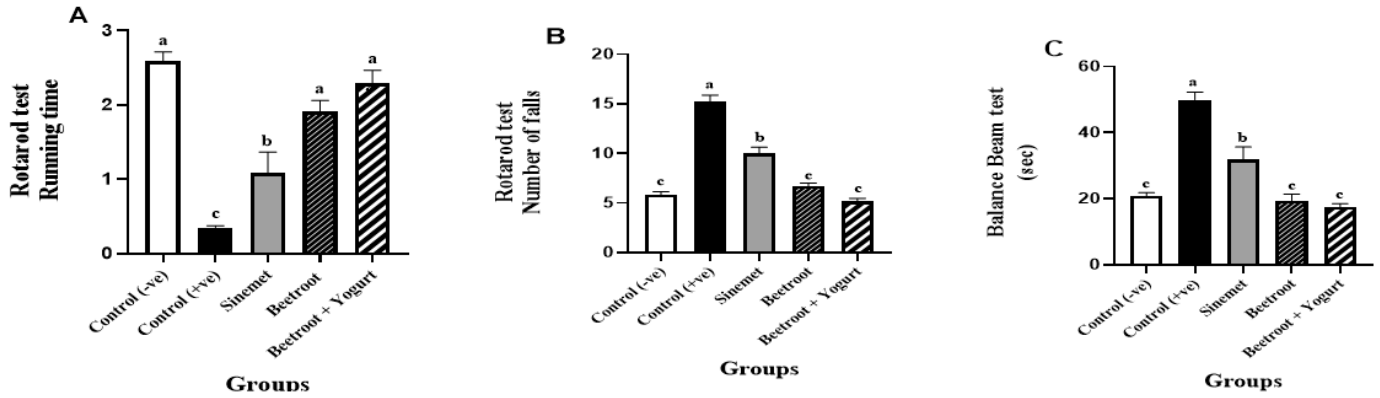

Figure 1

The effect of beetroot - fortified yogurt as a natural source of levodopa compared to drug therapy in male mice suffering from parkinson's disease on the running time (A) and number of Falls (B) on rotarod test ; balance beam test (D) ; Each bar with vertical line represents mean ± SD of 6 mice per group. Similar superscript mean insignificant difference, while different letters mean significant difference between groups at $p < 0.05$.

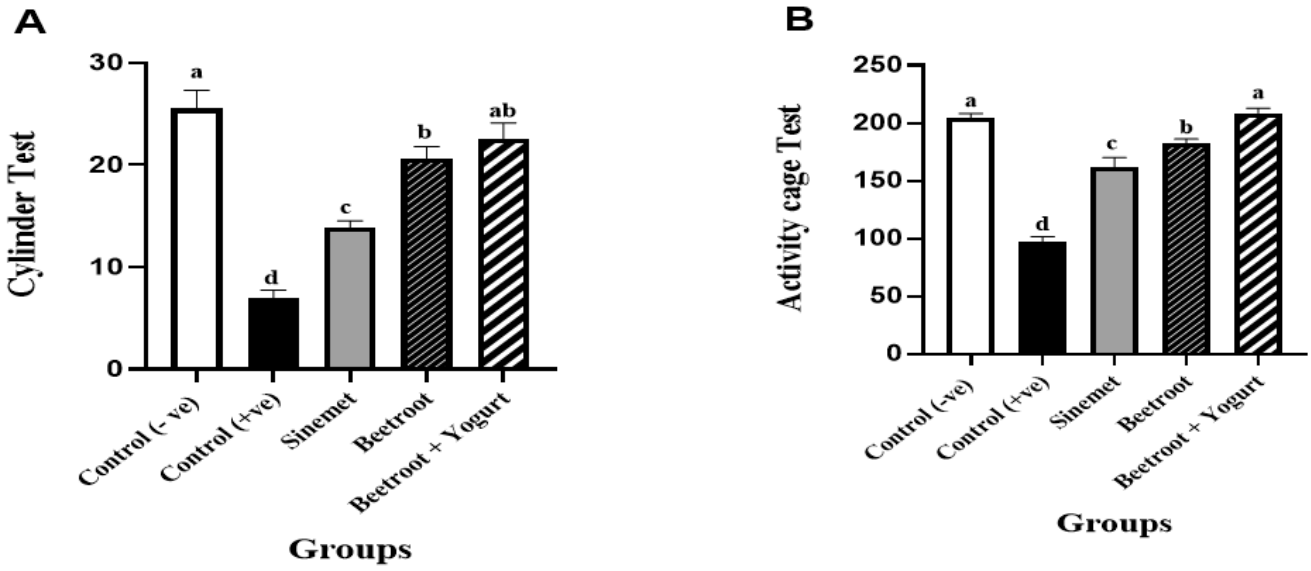

Figure 2

The effect of beetroot - fortified yogurt as a natural source of levodopa compared to drug therapy on cylinder test(A) and activity cage test (B) in male mice suffering from Parkinson's disease. Each bar with vertical line represents mean ± SD of 6 mice per group. Similar superscript mean insignificant difference, while different letters mean significant difference between groups at $p < 0.05$.

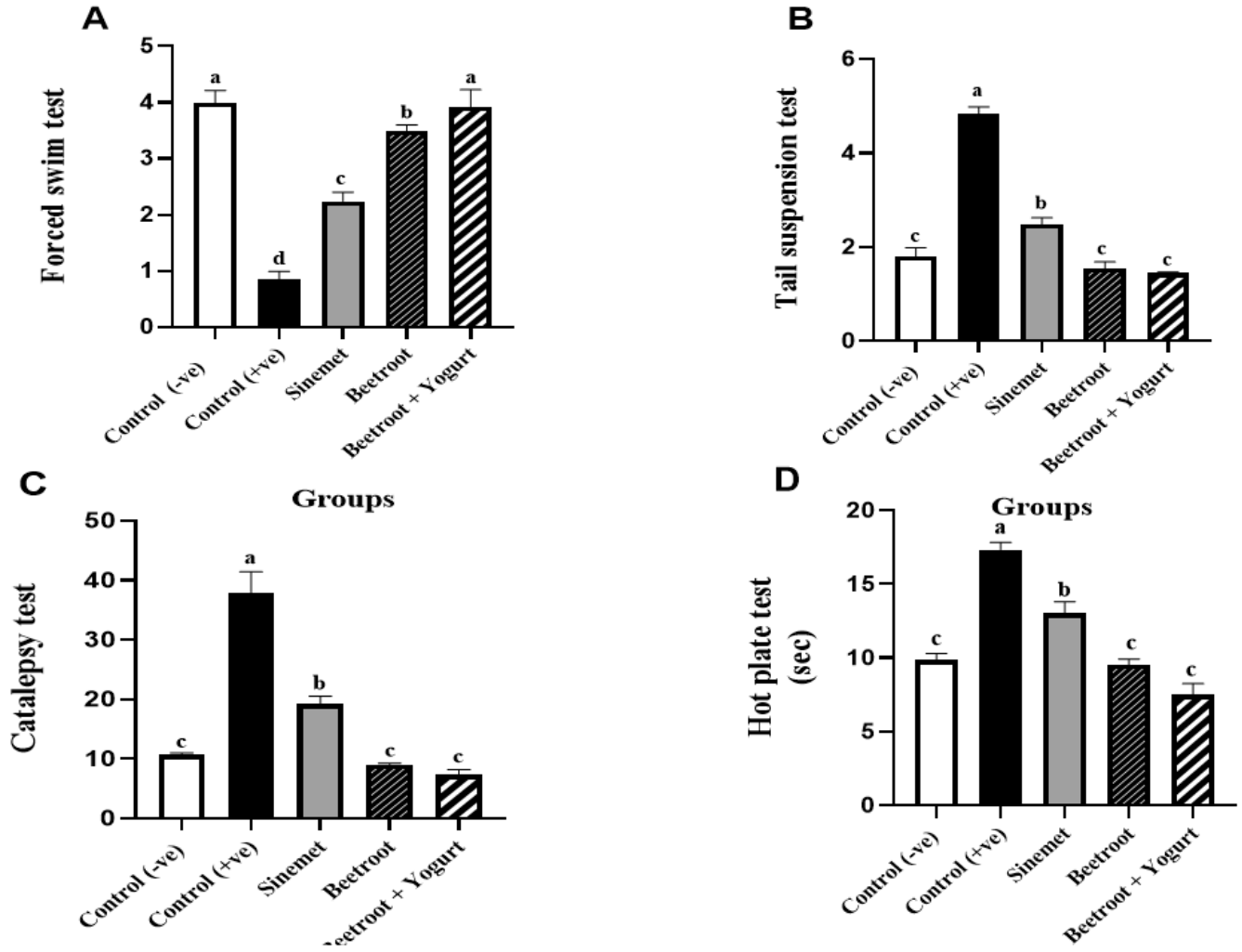

Figure 3

The effect of beetroot - fortified yogurt as a natural source of levodopa compared to drug therapy on forced swim test (A); tail suspension test (B); catalepsy test (C) and Hot Plate test (D) in male mice suffering from parkinson's disease. Each bar with vertical line represents mean ± SD of 6 mice per group. Similar superscript mean insignificant difference, while different letters mean significant difference between groups at $p < 0.05$.

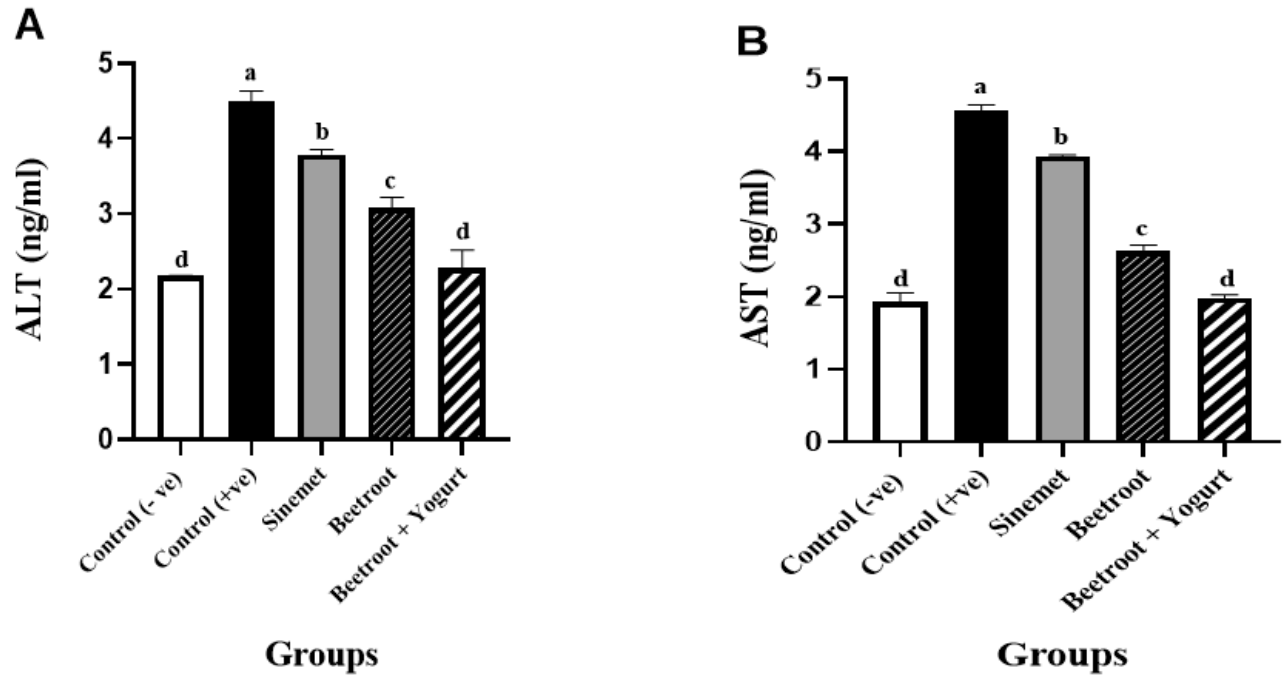

Figure 4

The effect of beetroot - fortified yogurt as a natural source of levodopa compared to drug therapy on ALT (A) and AST (B) in male mice suffering from parkinson's disease.Each bar with vertical line represents mean ± SD of 6 mice per group.Similar superscript mean insignificant difference, while different letters mean significant difference between groups at $p < 0.05$.

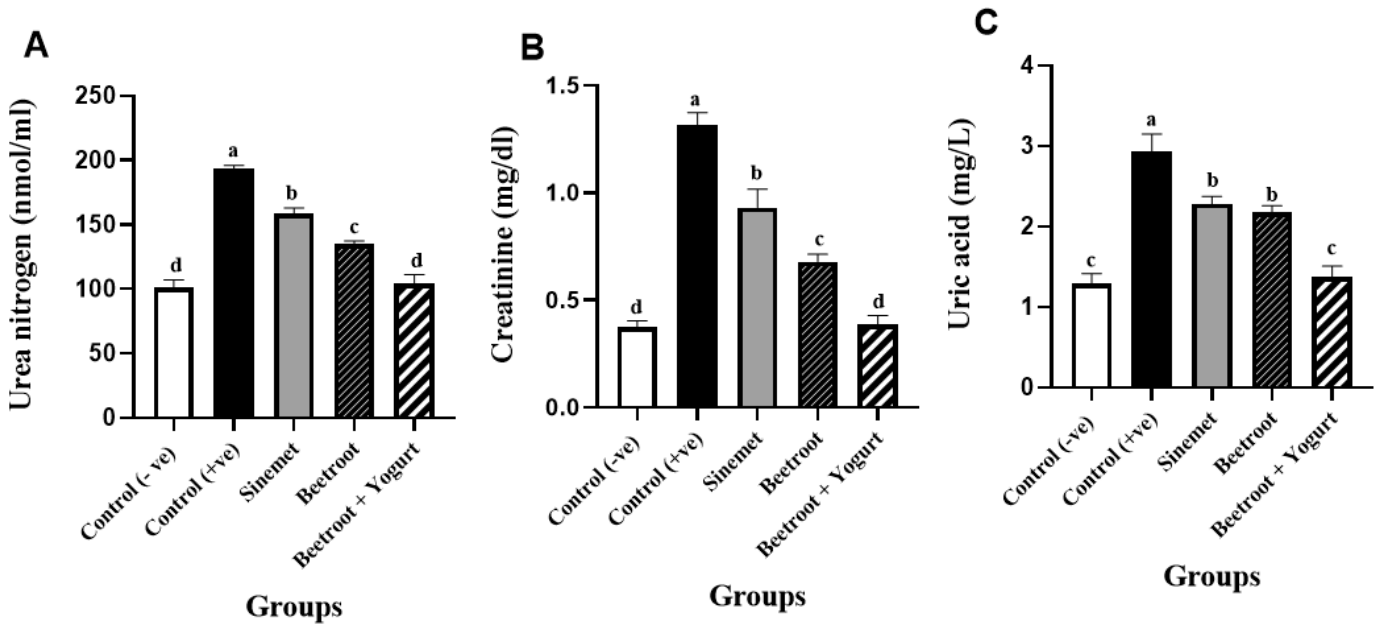

Figure 5

The effect of beetroot - fortified yogurt as a natural source of levodopa compared to drug therapy on urea nitrogen (A); creatinine (B) and uric acid (c) in male mice suffering from parkinson's disease. Each bar with vertical line represents mean ± SD of 6 mice per group. Similar superscript mean insignificant difference, while different letters mean significant difference between groups at $p < 0.05$.

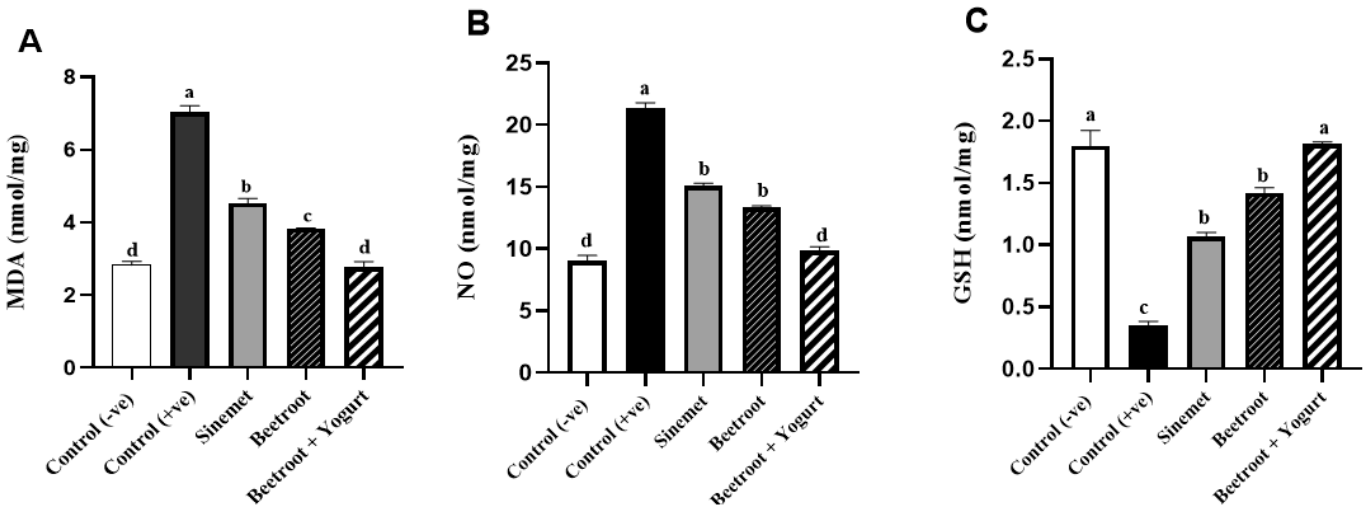

Figure 6

The effect of beetroot - fortified yogurt as a natural source of levodopa compared to Drug therapy on MDA (A); NO (B) and GSH (B) in male mice suffering from parkinson's disease.Each bar with vertical line represents mean ± SD of 6 mice per group. Similar superscript mean insignificant difference, while different letters mean significant difference between groups at $p < 0.05$.

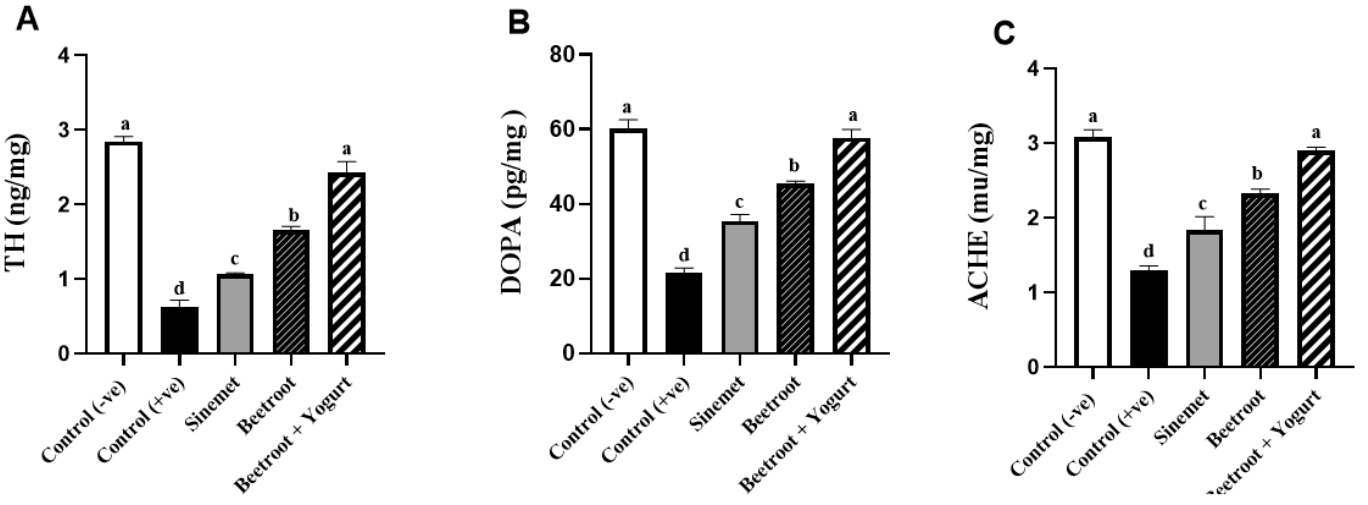

Figure 7

The effect of beetroot - fortified yogurt as a natural source of levodopa compared to drug therapy on TH (A); DOPA (B) and ACHE (C) in male mice suffering from parkinson's disease. Each bar with vertical line

represents mean ± SD of 6 mice per group. Similar superscript mean insignificant difference, while different letters mean significant difference between groups at $p < 0.05$.

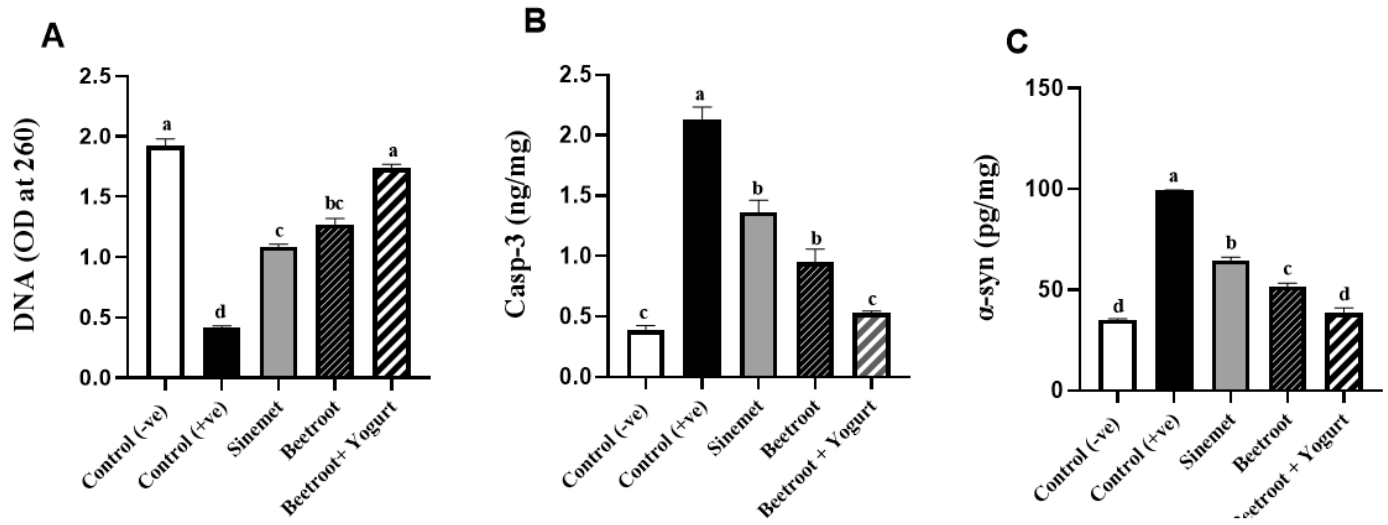

Figure 8

The effect of beetroot - fortified yogurt as a natural source of levodopa compared to drug therapy on DNA (A); Casp-3(B) and α-syn (C) in male mice suffering from parkinson's disease. Each bar with vertical line represents mean ± SD of 6 mice per group. Similar superscript mean insignificant difference, while different letters mean significant difference between groups at $p < 0.05$.

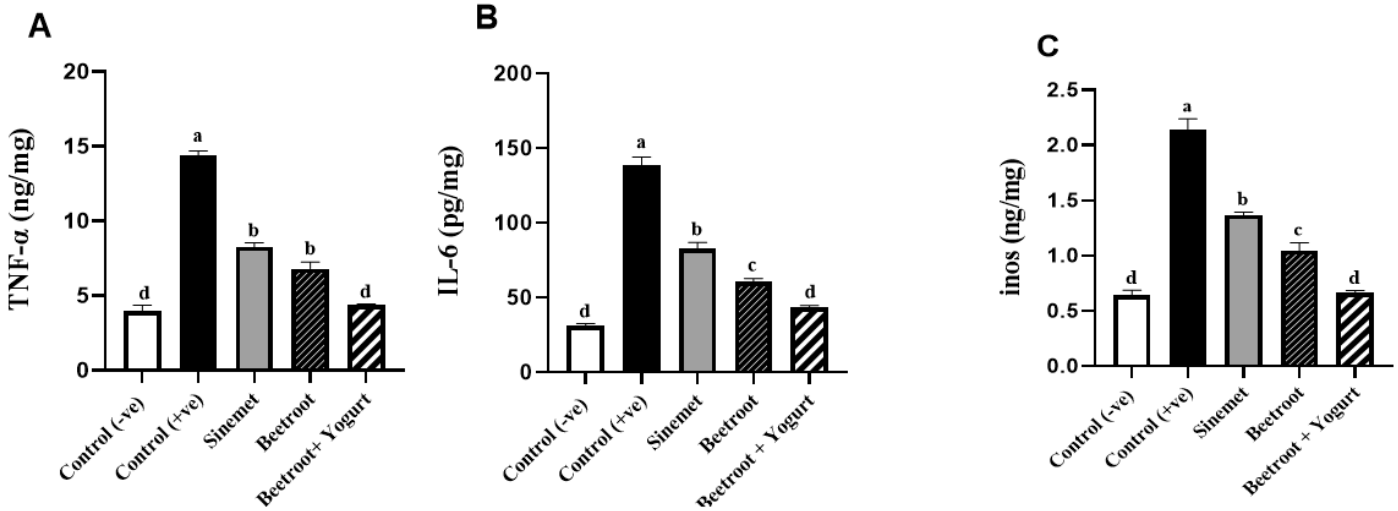

Figure 9

The effect of beetroot - fortified yogurt as a natural source of levodopa compared to drug therapy on TNF-α (A); IL-6 (B) and Inos (C) in male mice suffering from parkinson's disease.Each bar with vertical line represents mean ± SD of 6 mice per group. Similar superscript mean insignificant difference, while different letters mean significant difference between groups at $p < 0.05$.

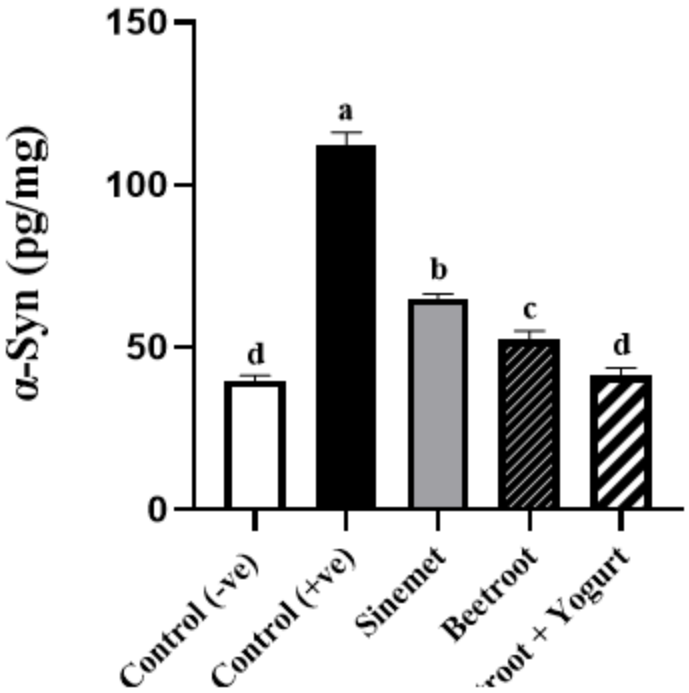

Figure 10

The effect of beetroot - fortified yogurt as a natural source of levodopa compared to drug therapy on α-Syn in intestines tissue male mice suffering from parkinson's disease. Each bar with vertical line represents mean ± SD of 6 mice per group. Similar superscript mean insignificant difference, while different letters mean significant difference between groups at $p < 0.05$.