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To Study Changes in Huntington Disease by Manipulating Gut Microbiota in Animal Model

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Abstract: *Huntington's Disease (HD) is a progressive neurodegenerative disorder characterized by motor, cognitive, and psychiatric disturbances. Emerging evidence suggests a role for the gut-brain axis in neurodegenerative diseases, including HD. This study investigated the potential of manipulating gut microbiota using probiotic endophytes isolated from Citrus aurantifolia to alleviate Huntington's-like symptoms in a 3-nitropropionic acid (3-NP) induced rat model. Rats were divided into four groups: vehicle control, 3-NP-induced HD (negative control), 3-NP-induced HD treated with Lactobacillus endophytes, and 3-NP-induced HD treated with Tetrabenazine (standard). Behavioral assessments using the Elevated Plus Maze, Morris Water Maze, and Rota-Rod apparatus were conducted at baseline, day 21, and day 42 post-3-NP induction. Histopathological analysis of brain tissue was performed at the end of the study. The 3-NP group exhibited significant anxiety-like behavior, impaired learning and memory, and motor dysfunction, along with neuronal degeneration in the brain. Treatment with Lactobacillus endophytes significantly improved these behavioral deficits and reduced neuronal damage, showing comparable or, in some aspects, superior effects to Tetrabenazine. These findings suggest that manipulating gut microbiota with probiotic endophytes holds promise as a therapeutic strategy for managing Huntington's Disease.*

Keywords: Huntington's Disease, Gut Microbiota, Probiotic Endophytes, *Citrus aurantifolia*, Animal Model, 3-Nitropropionic Acid, Behavioral Studies, Histopathology

I. INTRODUCTION

Huntington's Disease (HD) stands as a devastating, inherited neurodegenerative disorder primarily resulting from an expanded cytosine-adenine-guanine (CAG) repeat within the huntingtin (HTT) gene. This genetic anomaly leads to the production of a mutant huntingtin protein with an elongated polyglutamine tract, triggering a cascade of cellular dysfunctions. The pathological hallmarks of HD include progressive neuronal loss, particularly within the basal ganglia (striatum) and cerebral cortex, culminating in a characteristic triad of motor impairments (chorea, dystonia), cognitive decline, and psychiatric disturbances (mood changes, irritability, psychosis). The onset of these debilitating symptoms typically occurs between 30 and 50 years of age, with disease progression leading to significant disability and ultimately, premature death.

Epidemiological studies reveal a global prevalence of HD, with higher rates reported in Western populations (10.6–13.7 per 100,000) compared to East Asia and Africa, a disparity potentially attributed to variations in the baseline CAG repeat lengths across different ethnicities. Currently, there is no cure for HD, and available treatments primarily focus on symptomatic management. Drugs like tetrabenazine are used to alleviate chorea, while antipsychotics such as olanzapine and risperidone help manage behavioral symptoms. However, these treatments do not halt or significantly slow down the underlying neurodegenerative process, highlighting the urgent need for novel therapeutic strategies.

In recent years, the intricate bidirectional communication between the gastrointestinal tract and the central nervous system, known as the gut-brain axis, has emerged as a critical player in both health and disease. This axis involves neural, hormonal, and immunological pathways, with the gut microbiota – the diverse community of microorganisms residing in the intestine – playing a significant modulatory role. Accumulating evidence suggests that alterations in the composition and function of the gut microbiota (dysbiosis) are implicated in the pathogenesis and progression of

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various neurodegenerative disorders, including Parkinson's disease, Alzheimer's disease, 1 and multiple sclerosis. These alterations can influence neuroinflammation, oxidative stress, and the production of neuroactive metabolites, potentially contributing to neuronal dysfunction.

II. PLANT PROFILE: CITRUS AURANTIFOLIA (LIME)

Citrus aurantifolia, commonly known as lime, holds a significant place in traditional medicine and is recognized for its diverse array of beneficial properties, largely attributed to its rich phytochemical composition [Section 1.10.1]. This section provides a detailed profile of the plant, highlighting its taxonomy, botanical characteristics, distribution, and key chemical constituents, with a particular focus on aspects relevant to its potential as a source of therapeutic agents, including probiotic endophytes.

2.1. Synonyms:

Limonia aurantifolia Christre. & Panzer, Citrus javanica Blume, Citrus notissima Blanco, Citrus acida, Citrus hystrix ssp. acida Engl, Citrus Lima Lunan .

2.2. Common Names:

The plant is known by various names across different languages and regions, reflecting its widespread cultivation and use. Some common names include:

- Sanskrit: Matulunga or Nimbuka
- Kannada: Nimbe, Limbe
- English: Lime, Sour lime, Key lime
- Telugu: Pulusunimma
- Tamil: Elumichai
- Malayalam: Vatukapuli Narakam

2.3. Taxonomical Classification:

The taxonomic hierarchy of Citrus aurantifolia places it within the plant kingdom, specifically:

Kingdom	Plantae
Phylum	Spermatophyta
Class	Magnoliopsida
Order	Sapindales
Family	Rutaceae
Genus	Citrus
Species	Citrus aurantifolia

Table 1. Taxonomical Classification of Citrus aurantifolia

2.4. Plant Description:

Citrus aurantifolia is characterized as a densely and irregularly branched, evergreen tree, typically growing up to 5 meters in height. Its twigs are armed with short, stiff, and sharp spines. The fruit is a globose to ovoid berry, ranging from 3 to 6 cm in diameter, sometimes featuring apical papillae. The fruit's peel is very thin and densely glandular,

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enclosing segments with yellow-green pulp vesicles that are highly acidic, juicy, and fragrant. The seeds are small, plump, ovoid, pale, and smooth, containing white embryos. The leaves are small and oval-shaped, distinguished by winged petioles. Crushed leaves emit a characteristic odor and possess a strong nutty taste. The flowers, which appear on the branching leaves, are small, white, fragrant, and typically occur in clusters of four to five.

2.5. Distribution:

Believed to have originated in Southeast Asia, Citrus aurantifolia is now widely distributed across tropical and subtropical regions globally. It is extensively cultivated in countries such as India, Mexico, Brazil, and throughout the Caribbean. The plant thrives in warm climates with well-drained soils and ample sunlight.

2.6. Chemical Constituents:

Citrus aurantifolia is a rich source of diverse secondary metabolites, contributing to its various biological activities. Phytochemical analysis has revealed the presence of alkaloids, coumarins, carotenoids, flavonoids (including hesperidin), triterpenoids, essential oils (predominantly D-limonene, β-linalool, and citral), phenolic acids, and limonoids. The species also contains steroids, tannins, saponins, and cardiac glycosides. These compounds, particularly flavonoids and essential oils, are known for their antioxidant, anti-inflammatory, and neuroprotective properties, making the plant a potential source of therapeutic agents.

III. MATERIALS AND METHODS

This study investigated the potential of probiotic endophytes isolated from Citrus aurantifolia to modulate Huntington's-like symptoms in a 3-nitropropionic acid (3-NP) induced rat model. The experimental procedures encompassed the isolation and identification of bacterial endophytes, the establishment of the animal model of Huntington's Disease, the administration of the isolated endophytes, and the assessment of behavioral and neuropathological changes.

3.1. Materials

3.1.1. Chemicals and Reagents:

The chemicals and reagents used in this study are listed in Table 2.

Sr. No.	Chemicals	Company
1.	Peptone water	HIMEDIA
2.	Kovac's reagent	Thermosil Fine Chem Industries
3.	Glucose phosphate broth – MR	HIMEDIA
4.	Methyl red reagent	Thermosil Fine Chem Industries
5.	α – napthol	Burgoyne Burbidges & Co.
6.	40 % KOH	Thermosil Fine Chem Industries
7.	Simmon's citrate agar	HIMEDIA
8.	Agar agar powder	HIMEDIA
9.	MRS broth	HIMEDIA
10.	Gram staining kit	HIMEDIA
11.	Ethanol	Thermosil Fine Chem Industries

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12.	Sodium hypochloride	Thermosil Fine Chem Industries
13.	Bile salts	HIMEDIA
14.	PBS buffer	HIMEDIA

Table 2. List of Chemicals Used in Study

3.1.2. Apparatus and Instruments

The apparatus and instruments used in this study are listed in Table 3.

Sr. No.	Instruments	Company
1.	Weighing balance	K - roy
2.	Magnetic stirrer	Remi
3.	Cooling centrifuge	Remi
4.	Tissue Homogenizer	Prompt
5.	Morris water maze apparatus	K - roy
6.	Elevated plus maze apparatus	K - roy
7.	Autoclave	i - therm
8.	Incubator	Bluefic

Table 3. List of Instruments Used in Study

3.2. Method:

3.2.1. Isolation and Identification of Probiotic Endophytes:

(a) Plant Material Collection and Authentication:

Fresh, healthy leaves of Citrus aurantifolia were collected from the Botanical Garden, P. Wadhwani College of Pharmacy, Yavatmal, India. The plant material was identified and authenticated by Mrs. A. M. Gaharwar, Assistant Professor, Vasantrao Naik College of Agriculture Biotechnology, Yavatmal (Ref No. VNCABT/Ytl/Hort/13121/2025). The collected leaves were immediately transported to the Microbiology Research Laboratory, Department of Microbiology, P. Wadhwani College of Pharmacy, Yavatmal.

(b) Surface Sterilization:

The leaves were washed under running tap water and rinsed with sterile distilled water to remove surface debris. Surface sterilization was performed following a modified protocol: immersion in 70% ethanol for 1 minute, followed by immersion in 4% sodium hypochlorite for 5 minutes, and finally, immersion in 70% ethanol for 30 seconds. After each step, the leaves were rinsed three times with sterile distilled water. The effectiveness of sterilization was confirmed by plating the final rinse water on MRS agar; no microbial growth indicated successful sterilization.

(c) Isolation of Probiotic Endophytes:

Surface-sterilized leaves were crushed separately and aseptically with 9 ml of sterile distilled water in a sterile mortar and pestle. The resulting plant extract was serially diluted up to 10^{-5}. One milliliter of each dilution was plated onto MRS agar plates using the spread plate method. The plates were incubated at 37°C for 24-48 hours under aerobic conditions. Morphologically distinct colonies were selected and purified by repeated streaking on fresh MRS agar plates. Pure isolates were maintained on MRS agar slants at 4°C for further studies.

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(d) Preliminary Identification of Bacterial Endophytes:

The purified isolates were subjected to preliminary identification based on standard microbiological procedures:

- Colonial Morphology: Observation and recording of colony characteristics such as shape, margin, texture, color, appearance, and elevation .
- Motility Test: Assessment of bacterial motility using the hanging drop method .
- Gram Staining: Differentiation of bacterial isolates into Gram-positive and Gram-negative based on the Gram staining procedure.
- IMViC Tests: Biochemical characterization using Indole, Methyl Red, Voges-Proskauer, and Citrate utilization tests.

(e) Selection of Lactobacillus Isolates:

Based on the preliminary identification tests (Gram-positive rods, non-motile, and growth on MRS agar), isolates presumptively identified as Lactobacillus spp. were selected for further use in the animal study. The selected isolates were cultured in MRS broth and their concentration was determined using a hemocytometer to prepare the required dosage.

3.2.2. Animal Model of Huntington's Disease:

(a) Animals and Housing:

Healthy male Sprague-Dawley rats (8-12 weeks old, weighing 220-280 gm) were used in this study. Animals were housed in polypropylene cages (n=6 per cage) with wire mesh tops and husk bedding, maintained under controlled conditions of light (12h light/12h dark cycle), temperature (25 ± 2 °C), and humidity (60 ± 5 %). They were provided with a standard pellet diet and water ad libitum. The experiments were conducted during the day (8:00 – 16:00 hrs). All animal handling and procedures were performed in accordance with the rules and regulations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and approved by the Institutional Animal Ethics Committee (IAEC) with research project number 650/PO/Re/S-2002/2025/CCSEA/12.

(b) Experimental Groups and 3-NP Induction:

Rats were randomly divided into four groups (n = 6 per group):

- Group 1 (Vehicle Control): Received daily intraperitoneal injections of normal saline solution (0.9% NaCl) for 42 days.
- Group 2 (Negative Control - HD): Huntington's Disease was induced by intraperitoneal injections of 3-Nitropropionic Acid (3-NP) at a dose of 30 mg/kg body weight once daily for 42 days [Shilpa R. et al., 2007]. The 3-NP solution was prepared fresh daily in sterile saline.
- Group 3 (Endophyte Treatment - HD + Lb.): Received daily intraperitoneal injections of 3-NP (30 mg/kg) for 42 days, followed by daily oral gavage of the selected Lactobacillus endophyte isolate at a concentration of 27 $\times 10^{10}$ CFU/ml in 1 ml of sterile PBS buffer for 42 days, starting concurrently with 3-NP administration. The dosage was determined based on preliminary studies and relevant literature.
- Group 4 (Standard Treatment - HD + Tetrabenazine): Received daily intraperitoneal injections of 3-NP (30 mg/kg) for 42 days, followed by daily intraperitoneal injections of Tetrabenazine at a dose of 12.5 mg/kg body weight in sterile saline for 42 days, starting concurrently with 3-NP administration.

(c) Behavioral Testing:

Behavioral assessments were conducted at baseline (Day 0, before 3-NP induction), Day 21, and Day 42 post-3-NP induction to evaluate anxiety-like behavior, spatial learning and memory, and motor coordination. The order of testing was randomized to minimize any potential confounding effects.

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Elevated Plus Maze (EPM):
The EPM apparatus consisted of two open arms (50 x 10 cm) and two closed arms (50 x 10 x 40 cm) elevated 50 cm above the ground, arranged in a plus shape with a central platform (10 x 10 cm). Each rat was placed on the central platform facing a closed arm and allowed to explore the maze for 10 minutes. The following parameters were recorded:

- Number of entries into open and closed arms.
- Time spent in open and closed arms.
- Transfer latency (time taken to move from an open arm to a closed arm for the first time).

Morris Water Maze (MWM):
The MWM consisted of a circular pool (170 cm diameter, 45 cm height) filled with opaque water maintained at 25°C. A submerged platform (10 cm diameter) was placed in a fixed location in one of the four quadrants. Training trials were conducted for four consecutive days (Days 6-9 post-3-NP induction), with four trials per day and varying starting positions. The time taken for the rat to find the platform (escape latency) was recorded. On the probe trial day (Day 10), the platform was removed, and each rat was allowed to swim for 300 seconds. The time spent in the target quadrant (where the platform was previously located) was recorded as a measure of spatial memory.

Rota-Rod Apparatus:
Motor coordination and balance were assessed using a Rota-Rod apparatus with a rotating rod (3 cm diameter) elevated 30 cm above the base. The test involved placing each rat on the rotating rod, starting at a speed of 4 rpm with a constant acceleration to 40 rpm over 5 minutes. The latency to fall from the rotating rod was recorded, with a maximum trial duration of 300 seconds. Three trials with an inter-trial interval of 30 minutes were conducted for each rat at each time point, and the average latency to fall was calculated.

(d) Histopathological Analysis:
At the end of the 42-day treatment period, all rats were euthanized by CO_2 asphyxiation. Brains were rapidly removed and fixed in 10% neutral buffered formalin. Following fixation, the brains were processed for paraffin embedding, and coronal sections (5 µm thick) were cut at the level of the striatum. The sections were stained with hematoxylin and eosin and examined under a light microscope by a blinded observer. Qualitative analysis was performed to assess neuronal morphology (shrinkage, pyknosis, eosinophilia), gliosis, vacuolization (edema), and vascular changes.

3.3. Statistical Analysis:
All behavioral data were analyzed using two-way repeated measures analysis of variance (ANOVA) followed by Bonferroni post-hoc test to determine significant differences between groups and across time points. Histopathological findings were analyzed qualitatively and presented descriptively. Statistical analysis was performed using GraphPad Prism software. A p-value of less than 0.05 was considered statistically significant.

IV. RESULT

This study evaluated the effects of *Lactobacillus* endophyte treatment on behavioral deficits and neuropathological changes in a 3-NP-induced rat model of Huntington's Disease. The results from the behavioral tests and histopathological analysis are presented below.

4.1. Probiotic Endophyte Isolation and Identification:
Morphologically distinct colonies were observed on MRS agar plates inoculated with serial dilutions of *Citrus aurantifolia* leaf extracts. Two distinct isolates were selected for preliminary identification.

Morphological Chacarteristics	Isolate 1	Isolate 2

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Shape	Circular	Circular
Texture	Sticky	Non-sticky
Color	White	White cream color
Margine	Not uniform	Uniform
Motility	Non-motile	Non-motile

Table 4. Colonial Characteristics and Motility of Probiotic Bacteria

Isolate 1	Isolate 2
Gram Positive Long Rod	Gram Positive Short Rod

Table 5. Gram Staining of Probiotic Bacteria

Test	Isolate 1	Isolate 2
Indole Test	Negative	Negative
Methyl Red Test	Negative	Negative
Voges Proskauer Test	Negative	Negative
Citrate Utilization Test	Negative	Negative

Table 6. IMViC Test of Probiotic Bacteria

Based on these preliminary characteristics (Gram-positive rods, non-motile, negative IMViC results, and growth on MRS agar), Isolate 1, a Gram-positive long rod, was presumptively identified as a *Lactobacillus* species and selected for use in the animal study.

4.2. Behavioral Studies:

4.2.1. Elevated Plus Maze (EPM): The effects of 3-NP induction and *Lactobacillus* endophyte treatment on anxiety-like behavior are presented in Tables 7 and Figure 1.

Sr. No.	Groups	Number of entries in close arm on Day 0	Number of entries in close arm on Day 21	Number of entries in close arm on Day 42
1.	Normal Control	45.06 ± 0.60	45.50±0.55	47 ± 0.60
2.	Negative Control	44.90 ± 0.7$^{\#}$	62.57±0.57$^{@}$	72.30± 1.58$^{@}$
3.	Endophytes (Lb.)	45.38 ± 0.58^{ns}	47.80±0.70**	57 ±1.30**
4.	Tetrabenazine (12.5 mg/kg)	46.7 ± 0.60^{ns}	44.50±0.75**	55.40± 1.55**

Table 7. Elevated Plus Maze Test: Number of Entries in Closed Arm

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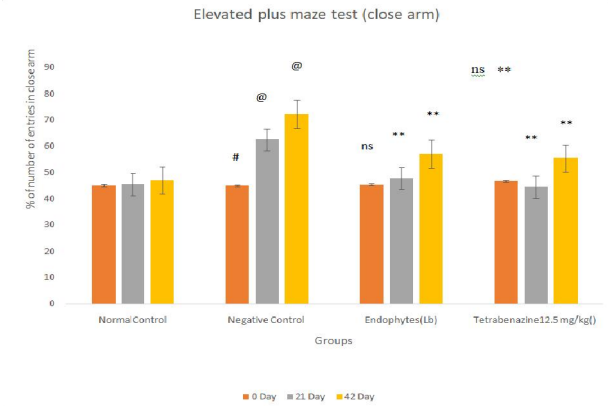

Figure 1. Number of Entries in Closed Arm (Bar graph representing data from Table 7 with appropriate error bars and statistical significance indicators).

Sr. No.	Groups	Number of entries in open arm on Day 0	Number of entries in open arm on Day 21	Number of entries in open arm on Day 42
1.	Normal Control	55.12 ± 0.47	55.80 ±0.55	56.03 ± 0.66
2.	Negative Control	54.21 ± 1.03 #	37.4 ±0.57@	27.2 ± 1.38@
3.	Endophytes(Lb.)	55.40 ± 0.49 ns	52.70±1.10**	43.20 ±1.30**
4.	Tetrabenazine (12.5 mg/kg)	56.10 ± 0.52ns	51.5± 0.75**	44.60± 1.35**

Table 8. Elevated Plus Maze Test: Number of Entries in Open Arm

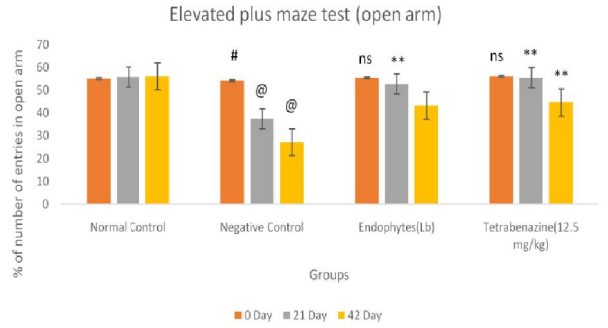

Figure 2. Number of Entries in Open Arm (Bar graph representing data from Table 8 with appropriate error bars and statistical significance indicators).

Sr. No.	Groups	Time spent in close arm on Day 0	Time spent in close arm on Day 21	Time spent in close arm on Day 42
1.	Normal Control	46.5 ±0.90	46.07 ± 0.80	45.2 ±0.60
2.	Negative Control	52.8± 3.4 #	55.96 ±2.64@	69.4± 1.10 @
3.	Endophytes(Lb.)	47.2 ± 1.10 ns	50.96 ±1.0**	54 ± 1.20**
4.	Tetrabenazine (12.5 mg/kg)	45.0± 1.25ns	49.83 ±1.18**	52.5± 1.10**

Table 9. Elevated Plus Maze Test: Time Spent in Closed Arm (seconds)

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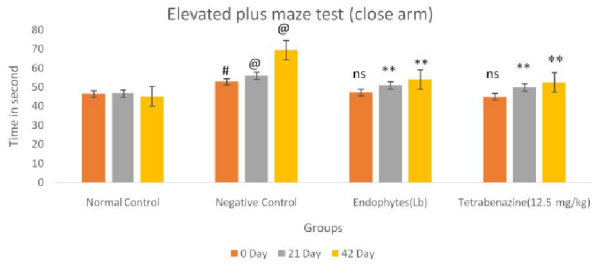

Figure 3.Time Spent in Closed Arm (Bar graph representing data from Table 9 with appropriate error bars and statistical significance indicators).

Table No.10.) Elevated Plus Maze Test: Time Spent in Open Arm (seconds

Sr. No.	Groups	Time spent in opened arm on Day 0	Time spent in opened arm on Day 21	Time spent in opened arm on Day 42
1.	Normal Control	55.20 ±0.55	56 ± 0.60	56.10 ±0.75
2.	Negative Control	50.5±1.20 $^{\#}$	41.50 ±1.50$^{@}$	33.06± 1.82$^{@}$
3.	Endophytes(Lb.)	51.50±0.60 ^{ns}	49.37 ±1.00**	44.63 ± 0.95**
4.	Tetrabenazine (12.5 mg/kg)	54.41±0.45 ^{ns}	53.20 ±0.70**	46.00 ± 2.25**

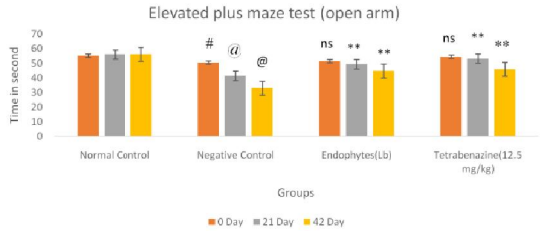

Figure 4. Time Spent in Open Arm (Bar graph representing data from Table 10 with appropriate error bars and statistical significance indicators).

Sr. No.	Groups	Transfer latency in seconds on Day 0	Transfer latency in seconds on Day 21	Transfer latency in seconds on Day 42
1.	Normal Control	47.2 ±3.1	48.5 ± 2.8	46.7± 3.6
2.	Negative Control	46 ±2.0$^{\#}$	65 ± 2.5$^{@}$	72.0 ± 2.8$^{@}$
3.	Endophytes (Lb.)	42.3 ±3.2^{ns}	52.5 ±2.4**	58.2 ± 2.2**
4.	Tetrabenazine (12.5 mg/kg)	43 ±1.9^{ns}	49.3±2.1**	55.3± 4.2**

Table 11. Elevated Plus Maze Test: Transfer Latency (seconds)

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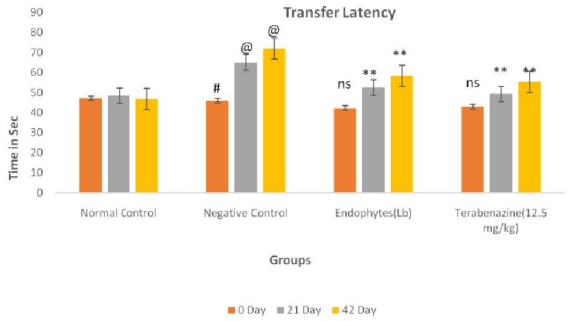

Figure 5. Transfer Latency in EPM (Bar graph representing data from Table 11. with appropriate error bars and statistical significance indicators).

4.2.2. Morris Water Maze (MWM): The effects on escape latency and retention time in the MWM are presented in Tables 12 and 13 and Figures 6 and 7.

Sr. No.	Groups	Transfer latency in seconds on Day 0	Transfer latency in seconds on Day 7	Transfer latency in seconds on Day 42
1.	Normal Control	25.2 ± 2.6	24.0 ± 2.8	23.5±2.1
2.	Negative Control	27.2 ±3.2$^{\#}$	41.3±2.8$^{@}$	61.2±5.8$^{@}$
3.	Endophytes(Lb)	29.8± 4.2^{ns}	39.6± 2.1**	37.4± 3.1^{ns}
4.	Tetrabenazine (12.5 mg/kg)	28.7 ± 2.8^{ns}	38.4±1.7**	35.7±2.8**

Table 12. Morris Water Maze Test: Escape Latency (seconds)

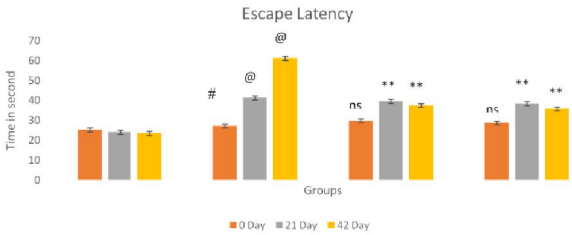

Figure 6. Escape Latency in MWM (Line graph showing escape latency over training days for each group with appropriate error bars and statistical significance indicators).

Table 13. Morris Water Maze Test: Retention Time (seconds) - Probe Trial (Day 10)

Sr. No.	Groups	Transfer latency in seconds on Day 0	Transfer latency in seconds on Day 7	Transfer latency in seconds on Day 42
1.	Normal Control	25.2 ± 2.6	24.0 ± 2.8	23.5±2.1
2.	Negative Control	27.2 ±3.2$^{\#}$	41.3±2.8$^{@}$	61.2±5.8$^{@}$
3.	Endophytes(Lb)	29.8± 4.2^{ns}	39.6± 2.1**	37.4± 3.1^{ns}
4.	Tetrabenazine (12.5 mg/kg)	28.7 ± 2.8^{ns}	38.4±1.7**	35.7±2.8**

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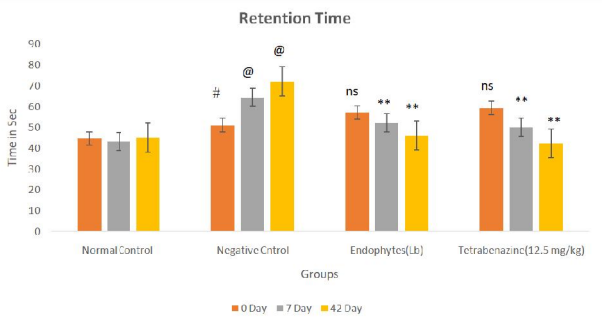

Figure 7. Retention Time in MWM (Bar graph representing time spent in the target quadrant during the probe trial for each group with appropriate error bars and statistical significance indicators).

4.2.3. Rota-Rod Apparatus: The effect on motor coordination and balance is presented in Table 14 and Figure 8.

Sr. No.	Groups	Day 0 (sec)	Day 21 (sec)	Day 42 (sec)
1.	Normal Control	93.3±6.2	95.8±6.0	96.8±5.9
2.	Negative Control	94.1±5.8$^{\#}$	55.0±6.5$^{@}$	38.4±7.1$^{@}$
3.	Endophytes(Lb)	93.5±6.4^{ns}	73.2±6.7**	88.8±6.6**
4.	Tetrabenazine (12.5 mg/kg)	94.7±5.5^{ns}	71.7±10.0**	90±5.0**

Table 14. Rota-Rod Test: Latency to Fall (seconds)

Figure 8. Latency to Fall in Rota-Rod Test (Bar graph representing latency to fall for each group at different time points with appropriate error bars and statistical significance indicators).

4.3. Histopathological Analysis:

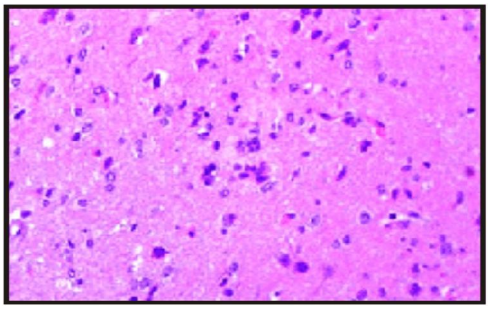

Figure 9. Photomicrograph of Normal Control Rat Brain (H&E staining, 400x) (Image showing normochromic neurons with well-outlined cell bodies and absence of intracellular spaces).

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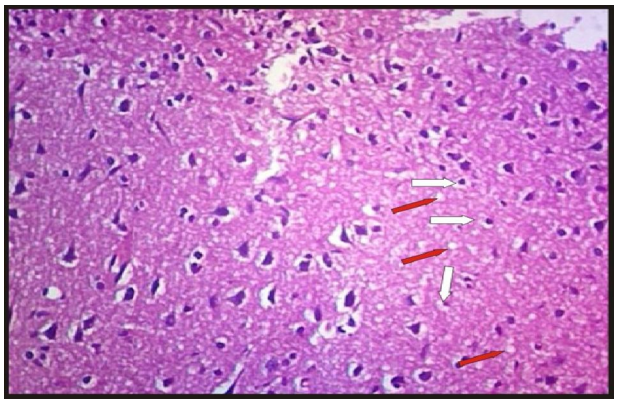

Figure 10. Photomicrograph of 3-NP Treated Rat Brain (H&E staining, 400x) (Image showing darkly stained pyknotic nuclei and shrunken cytoplasm (PN: white arrow), edema (E: red arrow)).

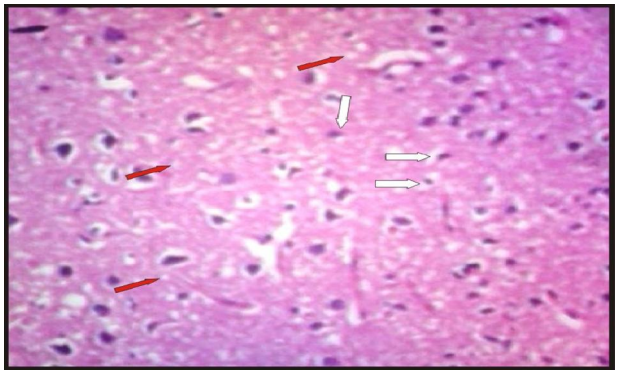

Figure 11. Photomicrograph of Tetrabenazine Treated Rat Brain (H&E staining, 400x) (Image showing reduced incidence of pyknotic nuclei and cytoplasmic eosinophilia (white arrow) and improved edema (red arrow)).

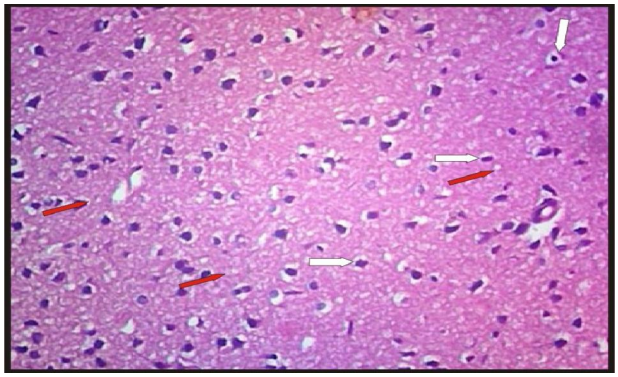

Figure 12. Photomicrograph of *Lactobacillus* Treated Rat Brain (H&E staining, 400x) (Image showing relatively preserved neuronal architecture, reduced pyknotic nuclei and cytoplasmic eosinophilia (white arrow), and minimal edema (E: red arrow)).

Histological examination of the control rat brain revealed normal neuronal architecture with distinct cell borders and intact structures. In contrast, the 3-NP treated group (Negative Control) exhibited significant neurodegenerative changes, including neuronal shrinkage, cytoplasmic eosinophilia, pyknotic nuclei, and evidence of edema and vacuolization. Brain sections from rats treated with Tetrabenazine showed a noticeable improvement in neuronal morphology, with a reduced incidence of pyknotic nuclei and edema. Similarly, the *Lactobacillus* endophyte treated group demonstrated relatively preserved neuronal architecture, with more prominent and less shrunken neurons,

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significantly reduced pyknotic nuclei and cytoplasmic eosinophilia, and minimal edema compared to the negative control group.

This study investigated the potential of Lactobacillus endophytes, isolated from Citrus aurantifolia, to mitigate Huntington's-like symptoms in a 3-NP-induced rat model. The findings demonstrate that 3-NP administration successfully induced behavioral deficits characteristic of HD, including increased anxiety-like behavior in the EPM, impaired spatial learning and memory in the MWM, and motor dysfunction in the Rota-Rod apparatus. These behavioral changes were accompanied by significant neurodegenerative alterations observed in the histopathological analysis of the brain tissue.

The negative control group exhibited a significant increase in closed arm entries and time spent in closed arms, coupled with a decrease in open arm entries and time spent in open arms in the EPM, indicating heightened anxiety-like behavior. In the MWM, these animals showed prolonged escape latencies during training and reduced time spent in the target quadrant during the probe trial, signifying impaired spatial learning and memory. Furthermore, the negative control group displayed a marked reduction in latency to fall on the Rota-Rod, indicating significant motor coordination and balance deficits [Robert M.J. et al., 2018]. Histopathological examination of their brains revealed prominent neuronal shrinkage, pyknotic nuclei, cytoplasmic eosinophilia, and edema, consistent with 3-NP-induced neurodegeneration .

Treatment with the isolated Lactobacillus endophytes demonstrated significant positive effects across the behavioral assessments. In the EPM, the endophyte-treated group showed a notable decrease in closed arm entries and time, along with an increase in open arm entries and time, suggesting a reduction in anxiety-like behavior. In the MWM, these animals exhibited improved learning, as evidenced by reduced escape latencies over the training days, and enhanced memory retention, indicated by increased time spent in the target quadrant during the probe trial. Moreover, the endophyte-treated group showed a significant increase in latency to fall on the Rota-Rod, indicating an improvement in motor coordination and balance.

The neuroprotective potential of the Lactobacillus endophyte treatment was further supported by the histopathological findings. Brain sections from these animals showed relatively preserved neuronal architecture, with a significant reduction in neuronal shrinkage, pyknotic nuclei, and edema compared to the negative control group. These findings suggest that the Lactobacillus endophytes exerted a protective effect against 3-NP-induced neurodegeneration.

Interestingly, the standard treatment with Tetrabenazine also yielded significant improvements in behavioral parameters and reduced neuropathological changes, validating its established role in managing HD symptoms. In several aspects, the efficacy of the Lactobacillus endophyte treatment appeared comparable to or even approached that of Tetrabenazine, particularly in the behavioral assessments of anxiety and motor coordination.

The observed neuroprotective and behavioral benefits of the Lactobacillus endophytes may be attributed to several potential mechanisms. Lactobacillus species are known to produce various bioactive compounds, including antioxidants and anti-inflammatory molecules. secondary metabolites present in Citrus aurantifolia, from which these endophytes were isolated, also possess neuroprotective properties. It is plausible that the administered Lactobacillus endophytes, either directly or through the modulation of the gut microbiota, contributed to a reduction in oxidative stress and neuroinflammation, both of which are critical pathological processes in HD .

Furthermore, the gut-brain axis likely plays a significant role in the observed effects. Lactobacillus species are known to influence the gut environment and can potentially modulate the production of neuroactive metabolites, such as short-chain fatty acids (SCFAs) and neurotransmitter precursors, which can impact brain function [Dinan, T.G., & Cryan, J.F., et al., 2017]. By restoring microbial balance and potentially reducing gut permeability, the Lactobacillus endophytes may have mitigated gut-derived neuroinflammation, thereby contributing to the observed neuroprotection and behavioral improvements.

The histopathological findings of reduced neuronal damage in the endophyte-treated group further support a direct or indirect neuroprotective effect. This could be mediated by the endophytes' ability to scavenge free radicals, inhibit pro-inflammatory signaling pathways, or potentially influence mitochondrial function, which is a primary target of 3-NP toxicity.

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V. DISCUSSION

This study investigated the potential of *Lactobacillus* endophytes, isolated from *Citrus aurantifolia*, to mitigate Huntington's-like symptoms in a 3-NP-induced rat model. The findings demonstrate that 3-NP administration successfully induced behavioral deficits characteristic of HD, including increased anxiety-like behavior in the EPM, impaired spatial learning and memory in the MWM, and motor dysfunction in the Rota-Rod apparatus. These behavioral changes were accompanied by significant neurodegenerative alterations observed in the histopathological analysis of the brain tissue.

The negative control group exhibited a significant increase in closed arm entries and time spent in closed arms, coupled with a decrease in open arm entries and time spent in open arms in the EPM, indicating heightened anxiety-like behavior. In the MWM, these animals showed prolonged escape latencies during training and reduced time spent in the target quadrant during the probe trial, signifying impaired spatial learning and memory. Furthermore, the negative control group displayed a marked reduction in latency to fall on the Rota-Rod, indicating significant motor coordination and balance deficits. Histopathological examination of their brains revealed prominent neuronal shrinkage, pyknotic nuclei, cytoplasmic eosinophilia, and edema, consistent with 3-NP-induced neurodegeneration .

Treatment with the isolated *Lactobacillus* endophytes demonstrated significant positive effects across the behavioral assessments. In the EPM, the endophyte-treated group showed a notable decrease in closed arm entries and time, along with an increase in open arm entries and time, suggesting a reduction in anxiety-like behavior. In the MWM, these animals exhibited improved learning, as evidenced by reduced escape latencies over the training days, and enhanced memory retention, indicated by increased time spent in the target quadrant during the probe trial. Moreover, the endophyte-treated group showed a significant increase in latency to fall on the Rota-Rod, indicating an improvement in motor coordination and balance.

The neuroprotective potential of the *Lactobacillus* endophyte treatment was further supported by the histopathological findings. Brain sections from these animals showed relatively preserved neuronal architecture, with a significant reduction in neuronal shrinkage, pyknotic nuclei, and edema compared to the negative control group. These findings suggest that the *Lactobacillus* endophytes exerted a protective effect against 3-NP-induced neurodegeneration.

Interestingly, the standard treatment with Tetrabenazine also yielded significant improvements in behavioral parameters and reduced neuropathological changes, validating its established role in managing HD symptoms. In several aspects, the efficacy of the *Lactobacillus* endophyte treatment appeared comparable to or even approached that of Tetrabenazine, particularly in the behavioral assessments of anxiety and motor coordination.

The observed neuroprotective and behavioral benefits of the *Lactobacillus* endophytes may be attributed to several potential mechanisms. *Lactobacillus* species are known to produce various bioactive compounds, including antioxidants and anti-inflammatory molecules. The secondary metabolites present in *Citrus aurantifolia*, from which these endophytes were isolated, also possess neuroprotective properties. It is plausible that the administered *Lactobacillus* endophytes, either directly or through the modulation of the gut microbiota, contributed to a reduction in oxidative stress and neuroinflammation, both of which are critical pathological processes in HD.

Furthermore, the gut-brain axis likely plays a significant role in the observed effects. *Lactobacillus* species are known to influence the gut environment and can potentially modulate the production of neuroactive metabolites, such as short-chain fatty acids (SCFAs) and neurotransmitter precursors, which can impact brain function. By restoring microbial balance and potentially reducing gut permeability, the *Lactobacillus* endophytes may have mitigated gut-derived neuroinflammation, thereby contributing to the observed neuroprotection and behavioral improvements.

The histopathological findings of reduced neuronal damage in the endophyte-treated group further support a direct or indirect neuroprotective effect. This could be mediated by the endophytes' ability to scavenge free radicals, inhibit pro-inflammatory signaling pathways, or potentially influence mitochondrial function, which is a primary target of 3-NP toxicity.

VI. CONCLUSION

In conclusion, this study provides compelling evidence that the administration of Lactobacillus endophytes isolated from Citrus aurantifolia can significantly alleviate behavioral deficits and reduce neuronal damage in a 3-NP-induced

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rat model of Huntington's Disease. These findings highlight the potential of manipulating the gut microbiota with specific probiotic endophytes as a novel therapeutic strategy for managing this devastating neurodegenerative disorder.

REFERENCES

[1]. A.I. Smith-Dijak, M.D. Sepers, L.A. RaymondAlterations in synaptic function and plasticity in Huntington disease J. Neurochemical.,150(2019),pp. 346-365, 10.1111/jnc.14723.

[2]. 2.Ali Esmail Al-Snafi Nutritional value and pharmacological importance of citrus species grown in Iraq IOSR Journal Of Pharmacy www.iosrphr.org (e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219 Volume 6, Issue 8 Version. 1 (Aug 2016), PP. 76-108

[3]. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC, Sanders ME. The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11:506–14.

[4]. Bauer KC, Huus KE, Finlay BB. Microbes and the mind: emerging hallmarks of the gut microbiota-brain axis. Cell Microbiol. 2016;18:632–44.

[5]. Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, Bienenstock J, Cryan JF. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc Natl Acad Sci. 2011;108:16050–

[6]. Bodke H, Jogdand S (November 09, 2022) Role of Probiotics in Human Health. Cureus 14(11): e31313. DOI 10.7759/cureus.31313

[7]. Caron NS, Wright GEB, Hayden MR. Huntington Disease. In: Adam MP, Feldman J, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, editors. GeneReviews® [Internet]. University of Washington, Seattle; Seattle (WA): Oct 23, 1998. [PubMed]

[8]. C.J. Klein, E.E. Benarroch Epigenetic regulation: basic concepts and relevance to neurologic diseaseNeurology. 82 (2014), pp. 1833-1840, 10.1212/WNL.0440.

[9]. Cryan J.F., O'Riordan K.J., Cowan C.S.M., Sandhu K.V., Bastiaanssen T.F.S., Boehme M., Codagnone M.G., Cussotto S., Fulling C., Golubeva A.V., et al. The Microbiota-Gut-Brai Axis Physiol.Rev. 2019;99:1877–2013.doi: 10.1152/physrev.00018.2018. [PubMed] [CrossRef] [Google Scholar]

[10]. de la Cuesta-Zuluaga J, Mueller NT, Corrales-Agudelo V, Velásquez-Mejía EP, Carmona JA, Abad JM, Escobar JS. Metformin Is Associated With Higher Relative Abundance of Mucin-Degrading Akkermansia muciniphila and Several Short-Chain Fatty Acid-Producing Microbiota in the Gut. Diabetes Care. 2017 Jan;40(1):54-62. doi: 10.2337/dc16-1324. Epub 2016 Nov 14. PMID: 27999002

[11]. Emilia M. Gattoa,b,□,1, Natalia González Rojas Huntington disease: Advances in the understanding of its mechanisms Institute of Neuroscience Buenos Aires (INEBA), Argentina online 6 May 2020

[12]. F. Arenas, C. Garcia-Ruiz, J.C. Fernandez-Checa Intracellular cholesterol trafficking and impact in neurodegenerationFront. Mol. Neurosci., 10 (2017).p. 382, 10.3389/fnmol.2017.00382

[13]. Francelle, C. Lotz, T. Outeiro, E. Brouillet, K. Merienne Contribution of neuroepigenetics to Huntington's diseaseFront. Hum. Neurosci., 11 (2017), p. 17, 10.3389/fnhum.2017.00017.

[14]. Forsythe P, Bienenstock J, Kunze WA. Vagal pathways for microbiomebrain-gut axis communication. Adv Exp Med Biol. 2014;817:115–33.

[15]. Fröhlich EE, Farzi A, Mayerhofer R, Reichmann F, Jačan A, Wagner B, Zinser E, Bordag N, Magnes C, Fröhlich E, Kashofer K, Gorkiewicz G, Umbrello and Esposito J Transl Med (2016) 14:298 Page 10-11.

[16]. Fontana L, Bermudez-Brito M, Plaza-Diaz J, Muñoz-Quezada S, Gil A. Sources, isolation, characterisation and evaluation of probiotics. Br J Nutr. 2013 Jan;109 Suppl 2:S35-50. doi: 10.1017/S0007114512004011. Erratum in: Br J Nutr. 2014 Feb;111(4):760. PMID: 23360880.

[17]. Holzer P. Cognitive impairment by antibiotic-induced gut dysbiosis: analysis of gut microbiota-brain communication. Brain Behav Immun. 2016;56:140–55.

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[18]. G. Cisbani, F. Cicchetti An in vitro perspective on the molecular mechanisms underlying mutant huntingtin protein toxicity Cell Death Dis., 3 (2012), Article e382, 10.1038/cddis.2012.121

[19]. Gil, J.M.; Rego, A.C. Mechanisms of neurodegeneration in Huntington's disease. Eur. J. Neurosci. 2008, 27, 2803–2820.

[20]. Gouda S, Das G, Sen SK, Shin HS, Patra JK. Endophytes: A Treasure House of Bioactive Compounds of Medicinal Importance. Front Microbiol. 2016 Sep 29;7:1538. doi: 10.3389/fmicb.2016.01538. PMID: 27746767; PMCID: PMC5041141