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To Study Effect of Endophytes on Ethanol Exposed Rats

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Abstract: *Adolescent alcohol exposure poses significant risks to brain development. This study investigated the potential neuroprotective effects of a* Lactobacillus *endophyte isolated from* Azadirachta indica *(neem) against ethanol-induced neurobehavioral and histopathological alterations in adolescent Sprague-Dawley rats. Rats were exposed to ethanol (5 g/kg) for 31 days and subsequently treated with low and high doses of the* Lactobacillus *endophyte or Disulfiram (100 mg/kg) for the same duration. Behavioral assessments using the Elevated Plus Maze, Light and Dark Box, and Morris Water Maze revealed that ethanol exposure induced anxiety-like behavior and impaired learning and memory. Treatment with the* Lactobacillus *endophyte significantly attenuated these behavioral deficits. Histopathological analysis of brain tissue from ethanol-exposed rats showed neuronal shrinkage, pyknotic nuclei, cytoplasmic eosinophilia, and edema, indicative of neurodegeneration. Both the* Lactobacillus *endophyte and Disulfiram treatment groups exhibited significantly improved neuronal architecture and reduced neurodegenerative markers. These findings suggest that a* Lactobacillus *endophyte from neem possesses neuroprotective properties against ethanol-induced brain damage in adolescent rats, potentially offering a natural therapeutic strategy*

Keywords: Adolescent Alcohol Exposure, *Azadirachta indica*, Endophytes, *Lactobacillus*, Neuroprotection, Neurobehavioral Deficits, Histopathology, Rat Model

I. INTRODUCTION

Alcohol stands as one of the most widely consumed and detrimental drugs globally, with its initiation of use commonly occurring during adolescence. Early onset of alcohol consumption presents considerable risks for both immediate and long-term health outcomes, with its toxic effects amplified by heavy use during this critical developmental period. The Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) 2019 highlights the significant impact of alcohol among adolescents and young adults (aged 10–24 years), ranking alcohol-attributable burden as the second highest contributor to disability-adjusted life years within this age group.

Statistics from the National Survey on Drug Abuse and Health in the United States further underscore the prevalence of alcohol use among teenagers, indicating that approximately half of junior and senior high school students consume alcohol monthly, and 14% have experienced intoxication at least once in the past year.

Adolescence is a period of ongoing and crucial brain development, making the adolescent brain particularly vulnerable to the neurotoxic effects of alcohol, especially given that many individuals initiate alcohol use during this time. Consequently, alcohol exposure during adolescence can lead to enduring alterations in neuropsychological function and an increased susceptibility to developing alcohol use disorder (AUD) later in life.

Animal models, particularly those utilizing laboratory animals such as mice and rats, offer a valuable tool to circumvent some of the complexities inherent in human studies of adolescent alcohol exposure. Adolescent intermittent ethanol exposure (AIE) paradigms in animal models have been instrumental in elucidating the consequences of adolescent binge drinking. Research using these models has revealed that the detrimental effects of adolescent alcohol exposure extend to a wide array of lasting changes in the brain, including reduced neurogenesis, heightened proinflammatory responses, alterations in gene expression through epigenetic mechanisms, and changes in the activity of various neurotransmitter systems.

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Epidemiological data reveals that India has the largest adolescent population globally, with 253 million individuals aged 10–19 years, constituting 21% of the nation's population. Alarmingly, alcohol consumption is being initiated by adolescents as young as 13–15 years of age in India. In the United States, the 2018 National Survey on Drug Use and Health (NSDUH) reported that 14.4 million people suffered from AUD, and over 100,000 deaths were attributable to alcohol. The World Health Organization has linked alcohol consumption to over 200 health conditions, including cancer, liver cirrhosis, and neurocognitive impairment. Recent studies in the United States indicate that by the age of 14, alcohol use has become a normative behavior among youth, with significant percentages of 8th, 12th graders, and college students reporting heavy episodic binge drinking. The onset of alcohol abuse during adolescence and young adulthood is a significant risk factor for adult alcohol use disorders and other drinking problems, with a younger age of drinking onset associated with more severe outcomes.

Given the significant neurological impact of alcohol, particularly on the developing adolescent brain, there is a growing need to explore potential therapeutic strategies to mitigate these detrimental effects. Probiotics, defined as living microorganisms that confer health benefits to the host when administered in adequate amounts, and endophytes, endosymbiotic microorganisms colonizing plants and microbes and serving as reservoirs of bioactive secondary metabolites, have emerged as promising candidates due to their diverse biological activities.

This study aims to investigate the potential neuroprotective effects of a *Lactobacillus* endophyte isolated from *Azadirachta indica* (neem) against ethanol-induced neurobehavioral and histopathological alterations in adolescent rats. We hypothesize that the administration of this *Lactobacillus* endophyte will attenuate the anxiety-like behavior and cognitive impairments induced by adolescent ethanol exposure and mitigate the associated neurodegenerative changes in the brain. The findings of this research could provide valuable insights into the potential of natural, plant-derived endophytes as a therapeutic intervention for alcohol-related neurological damage during adolescence.

II. PLANT PROFILE: *Azadirachta indica* (Neem)

Neem, a natural herb derived from the neem tree, scientifically known as *Azadirachta indica* and also referred to as Indian lilac, holds a significant place in traditional medicine. The extract, primarily sourced from the seeds of the tree, has a long history of diverse applications. While renowned for its pesticidal and insecticidal properties, neem is also incorporated into various hair and dental care products. Notably, all parts of the neem tree, including the leaves, flowers, seeds, fruits, roots, and bark, have been traditionally utilized to treat a wide range of ailments, including inflammation, infection, fever, skin disorders, and dental issues. The medicinal properties of neem are particularly well-documented for its leaves. Research has demonstrated that neem leaves and their constituents exhibit a broad spectrum of pharmacological activities, including immunomodulatory, anti-inflammatory, antihyperglycemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic, and anticarcinogenic properties.

2.1. Synonyms

Azadirachta indica var. *minor* Valeton
Azadirachta indica var. *siamensis* Valeton
Azadirachta indica subsp. *vartakii* Kothari
Melia azadirachta L.
Melia indica Brandis

2.2. Common Names

Sanskrit: Nimba, Arishta, Ravipriya
Kannada: Bevina-mara, Bevu, Kahibevu, Nimba, Ollebevu
English: Neem, Margosa, Margosa tree
Telugu: Vepa
Tamil: Vembu
Malayalam: Veppu

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2.3. Taxonomical Classification

Table No. 1.: Taxonomical Classification of *Azadirachta indica*

Kingdom	Plantae
Phylum	Spermatophyta
Class	Dicotyledonae
Order	Sapindales
Family	Meliaceae
Genus	*Azadirachta*
Species	*indica*

2.4. Plant Description

Neem is a fast-growing evergreen tree that typically reaches a height of 15–20 meters (49–66 ft), although it can occasionally grow as tall as 35–40 meters (115–131 ft). While generally evergreen, it may shed most or all of its leaves during severe drought conditions. The tree features wide and spreading branches, forming a fairly dense, roundish crown that can attain a diameter of 20–25 meters (66–82 ft). The appearance of the neem tree bears a close resemblance to its relative, the Chinaberry (*Melia azedarach*).

2.5. Distribution

This species is likely native to the Indian subcontinent and is globally distributed within this region. Within India, it is commonly found throughout the greater part of the country and is frequently cultivated. In the state of Karnataka, it is found in large quantities in areas such as Agumbe, Sandur, Devarayandurga, and Karpakkapalli.

2.6. Chemical Constituents

Neem can be considered a "storehouse" of numerous phytochemicals, with over 300 compounds having been extracted from various parts of the tree. The two most significant classes of phytochemicals isolated from neem are isoprenoids and non-isoprenoids. Well-known isoprenoids include diterpenoids, vilasinins, triterpenoids, limonoids, and C-secomeliacins. The non-isoprenoid constituents comprise proteins, carbohydrates (polysaccharides), sulfur compounds, tannins, and polyphenolics such as flavonoids and their glycosides, dihydrochalcone, coumarin, aliphatic compounds, and phenolic acids. The neem tree serves as a source for a variety of biologically active compounds, including triterpenoids, alkaloids, flavonoids, carotenoids, phenolic compounds, steroids, and ketones. Nimbin was the first compound to be extensively studied. Other significant phytochemicals derived from neem include nimbolide, azadirachtin, azadiradione, gedunin, and azadirone. The complex structural diversity of these phytochemicals contributes to neem's wide range of biological activities. Azadirachtin, in particular, exists as a group of seven isomeric chemical compounds (azadirachtin A-G), with azadirachtin E being the most abundant.

2.7. Uses

Neem and its constituents exhibit a wide array of uses, including:

- Antioxidant activity
- Anticancerous activity
- Anti-inflammatory activity

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- Hepatoprotective activity
- Wound healing activity
- Antidiabetic activity
- Antimicrobial activity
- Antibacterial activity
- Antiviral activity
- Antifungal activity
- Antimalarial activity
- Immunomodulatory effects
- Growth promoting

III. MATERIALS AND METHODS

3.1. Materials

3.1.1. Chemicals and Reagents

Table No.2.: List of Chemicals Used in Study

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

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3.1.2. Apparatus and Instruments

Table No. 3: List of Instruments Used in Study

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

3.2. Method

3.2.1. Isolation of Bacterial Endophytes

A. Phase 1

Collection and authentication of *Azadirachta indica* plant.

Collection of leaves of *Azadirachta indica* plant.

B. Phase 2

Surface sterilization of leaves.

Isolation of bacterial endophytes by spread plate method using serial dilution.

C. Phase 3

Identification of bacterial endophytes by standard microbiological procedures.

3.3. Procedure

3.3.1. Phase 1

A. Collection & Authentication of Plant Materials: *Azadirachta indica* plant leaves were collected from the Botanical Garden of P. Wadhwani College of Pharmacy, Yavatmal. The plant material was identified and authenticated by Mrs. A. M. Gaharwar, Assistant Professor of Vasantrao Naik College of Agriculture Biotechnology, Yavatmal (Ref No. VNCABT/Ytl/Hort/1899/2024).

3.3.2. Phase 2

B. Surface Sterilization:

Azadirachta indica plant leaves were collected from the Botanical Garden of P. Wadhwani College of Pharmacy, Yavatmal.

All plant leaves were immediately transported to the Microbiology Research Laboratory, Department of Microbiology, P. Wadhwani College of Pharmacy, Yavatmal.

Leaves were washed separately under running tap water and rinsed in sterile distilled water to remove soil particles.

The leaves were then surface sterilized to isolate microbial endophytes.

Surface sterilization was performed by sequentially immersing the leaves in 70% ethanol for 1 minute, followed by immersion in 4% sodium hypochlorite for 5 minutes, and finally immersing in 70% ethanol for 30 seconds.

After each treatment, the sample was washed separately in sterile distilled water.

The surface-sterilized leaves were then dried with sterilized filter paper.

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C. Isolation of Bacterial Endophytes by Spread Plate Method using Serial Dilution:

Surface-sterilized plant leaves were crushed separately and aseptically with 9 ml of sterile distilled water in a sterile mortar and pestle to obtain a plant homogenate.

After crushing, 1 ml of the plant homogenate was serially diluted into five test tubes, each containing 9 ml of sterile distilled water, resulting in dilutions of 10−1 to 10−5.

For each dilution (10−1 to 10−5), 0.1 ml of the diluted sample was spread onto sterile MRS agar petri plates in triplicate.

All MRS agar plates were incubated at 37°C for 24 hours.

After incubation, morphologically distinct colonies on the MRS agar plates were considered as potential lactic acid bacterial endophytes. Representative colonies were selected based on their distinct morphologies. These isolates were further purified by repeated streaking on fresh MRS agar plates and preserved on MRS agar slants at 4°C for further studies. Two distinct isolates were selected and designated as Isolate 1 and Isolate 2 for further characterization. Isolate 2, showing characteristics consistent with *Lactobacillus*, was chosen for the *in vivo* study and is referred to as *Lactobacillus* endophyte (Lb.).

3.3.3. Phase 3

D. Identification of Bacterial Endophytes:

The selected isolate (*Lactobacillus* endophyte - Lb.) was identified using the following standard microbiological procedures:

Colonial Characteristics: The isolate was characterized based on its colony morphology on MRS agar, including shape, margin, texture, color, appearance, and elevation.

Motility Procedure: Motility of the isolate was determined using the hanging drop method.

Gram Staining Procedure: Gram staining was performed to determine the Gram reaction and morphology of the bacterial cells.

IMViC Test: The following biochemical tests were performed as part of the IMViC series:

A. Indole Test: To assess the ability of the isolate to produce indole from tryptophan .

B. Methyl Red Test: To determine the type of acid produced during glucose metabolism.

C. Voges-Proskauer Test: To detect the production of acetoin (Vashist Hemraj et al., 2013).

D. Citrate Utilization Test: To assess the ability of the isolate to use citrate as a sole carbon source.

3.4. Experimental Animals

Healthy Sprague-Dawley rats (4-5 weeks old, weighing 70-100 gm) were used for this study. Animals were housed in polypropylene cages with wire mesh tops and husk bedding, maintained under controlled conditions of light (12h light, 12h dark), temperature (25 ± 2 °C), and humidity (60 ± 5 %), and fed with a standard pellet diet and water *ad libitum*. The rats were housed and treated according to the rules and regulations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the Institutional Animal Ethics Committee (IAEC). The protocol for all animal studies was approved by the Institutional Animal Ethics Committee (IAEC) with research project number 650/PO/Re/S/2002/2025/CCSEA/01.

3.5. Experimental Design

Animals were randomly divided into the following five groups (n = 6 per group):

Group 1 (Vehicle Control): Rats received only normal saline solution (oral gavage) for 31 days.

Group 2 (Negative Control): Rats received ethanol (5 g/kg body weight, 20% v/v in normal saline, oral gavage) for 31 days.

Group 3 (Low Dose of *Lactobacillus* endophyte - Lb.): Ethanol-exposed rats treated with *Lactobacillus* endophyte (2.7×10^{11} CFU/kg body weight, oral gavage) for 31 days, concurrent with ethanol administration.

Group 4 (High Dose of *Lactobacillus* endophyte - Lb.): Ethanol-exposed rats treated with *Lactobacillus* endophyte (5.4×10^{11} CFU/kg body weight, oral gavage) for 31 days, concurrent with ethanol administration.

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Group 5 (Standard): Ethanol-exposed rats treated with Disulfiram (100 mg/kg body weight, suspended in 0.5% carboxymethylcellulose, oral gavage) for 31 days, concurrent with ethanol administration.

The *Lactobacillus* endophyte culture was grown in MRS broth, harvested by centrifugation, washed with sterile PBS buffer, and resuspended in sterile normal saline to achieve the desired concentrations for administration. Disulfiram was freshly prepared as a suspension on test days.

3.5.1. Neurobehavioral Assessment

Neurobehavioral assessments for anxiety, learning, and memory impairment were conducted on all animals using the following behavioral models at baseline (Day 0, before ethanol administration), after 14 days of ethanol exposure (to assess the development of deficits), and after 32 days (after 31 days of treatment).

- **Elevated Plus Maze Apparatus:** Anxiety-like behavior was assessed by measuring the number of entries into and time spent on the open and closed arms, as well as transfer latency.
- **Light and Dark Box Apparatus:** Anxiety-related behavior was evaluated by measuring the latency of the first crossing, time spent in the light and dark compartments, and the number of transitions between the compartments.
- **Morris Water Maze Apparatus:** Spatial learning and memory were assessed using escape latency during training trials (Days 6-9 of treatment/ethanol exposure) and the time spent in the target quadrant during a probe trial on Day 10.

3.5.2. Histopathological Analysis

After the completion of the behavioral tests on Day 32, all animals were euthanized by sodium pentobarbital overdose. Brains were immediately removed, fixed in 10% formalin solution, processed for paraffin embedding, and sectioned at 5 µm thickness. Sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope for histopathological alterations. Photomicrographs were taken to document the observed changes.

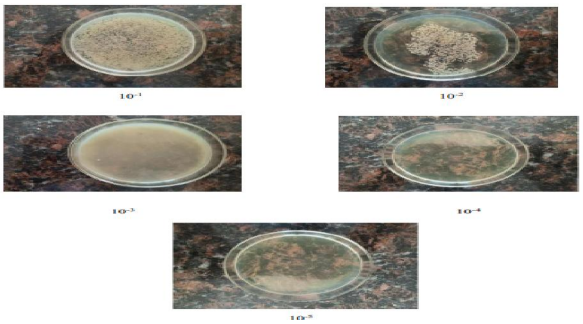
3.6. Statistical Analysis

All behavioral data were expressed as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons using GraphPad Prism software (version [Specify version]). Statistical significance was set at $p < 0.05$. Histopathological findings were assessed qualitatively.

Sources and related content

IV. RESULTS

4.1. Isolation and Identification of Bacterial Endophytes



(Figure No. 1.: Bacterial Endophytes Isolation by Spread Plate Method)

Morphologically distinct colonies were observed on MRS agar plates at different dilutions of the *Azadirachta indica* leaf homogenate. Two distinct isolates were selected for further characterization. Isolate 2, which formed white, circular, sticky colonies with a uniform margin, was chosen for the *in vivo* study and designated as *Lactobacillus* endophyte (Lb.).

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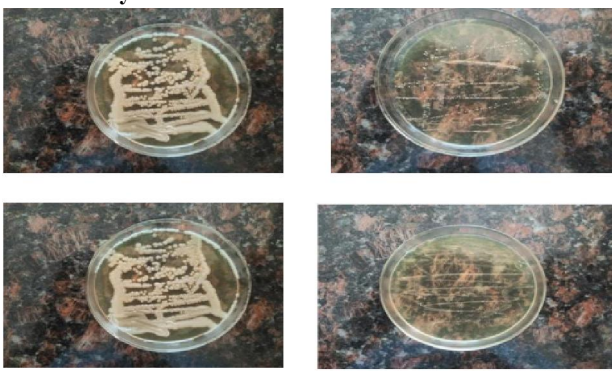
4.2. Identification of *Lactobacillus* Endophyte (Lb.)

Table No. 4.: Colonial Characteristics of *Lactobacillus* bacteria

A. Colonial Characteristics and Motility

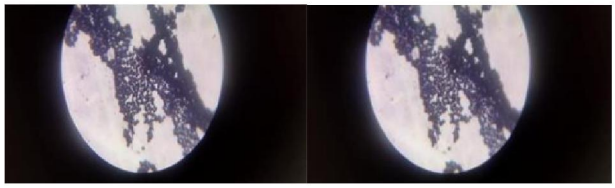


(Figure No.2.: Colonial Characteristics of *Lactobacillus* bacteria)

Table No. 4.: Colonial Characteristics of *Lactobacillus* bacteria

Morphological characteristic	***Lactobacillus* endophyte (Lb.)**
Shape	Circular
Texture	Sticky
Color	Cream color
Margine	Uniform
Motility	Non-motile

B. Gram Staining



(Figure No. 3.: Gram Staining of *Lactobacillus* bacteria)

Table No. 5.: Gram staining of *Lactobacillus* bacteria

Isolate	**Gram Reaction**	**Morphology**
Lactobacillus endophyte (Lb.)	Gram Positive	Short Rod

Gram staining revealed that the selected isolate (Lb.) was Gram-positive and appeared as short rods.

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C. IMViC Test

(Figure No. 4.: IMViC Test of *Lactobacillus* bacteria)

Table No. 6.: IMViC Test of *Lactobacillus* bacteria

Test	Lactobacillus endophyte (Lb.)
Indole Test	Negative
Methyl Red Test	Negative
Voges-Proskauer Test	Negative
Citrate Utilization Test	Negative

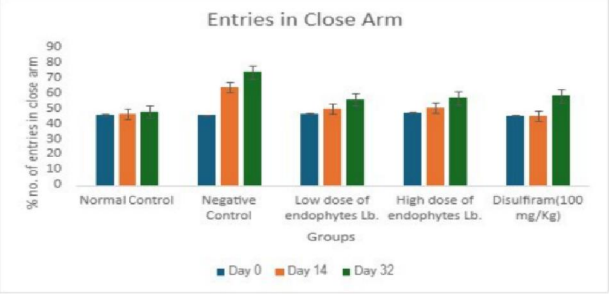
The IMViC test results for the *Lactobacillus* endophyte (Lb.) were all negative.

4.3. Estimation of Behavioral Study

4.3.1. Elevated Plus Maze Apparatus

Table no. 7. Effect of *Lactobacillus* endophyte (Lb.) on Elevated Plus Maze test [Number of entries in closed arm]

Sr. No.	Groups	Number of entries in closed arm on 0 day [%]	Number of entries in closed arm on 14 day [%]	Number of entries in closed arm on 32 day [%]
1	Normal Control	46.06 ± 0.45	46.50 ± 0.47	48.10 ± 0.50
2	Negative Control	45.90 ± 0.48#	63.90 ± 0.60@	73.90 ± 1.30@
3	Low Dose (Lb) endophyte	46.93 ± 0.37ns	49.80 ± 0.70**	56.10 ± 1.65**
4	High Dose (Lb) endophyte	47.70 ± 0.60ns	50.60 ± 0.55**	57.16 ± 1.38**
5	Disulfiram (100 mg/Kg)	45.66 ± 1.54ns	45.30 ± 1.10**	58.62 ± 0.40**



(Figure No.5.: Effect of *Lactobacillus* endophyte (Lb.) on number of entries in closed arm)

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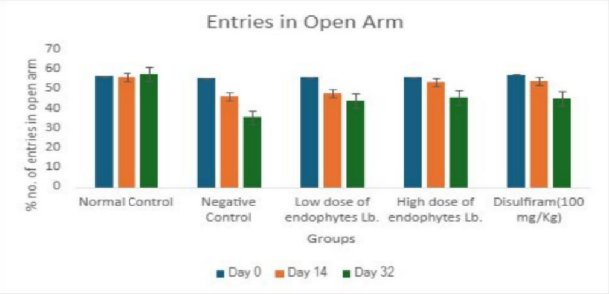
Table no. 8. Effect of *Lactobacillus* endophyte (Lb.) on Elevated Plus Maze test [open arm]

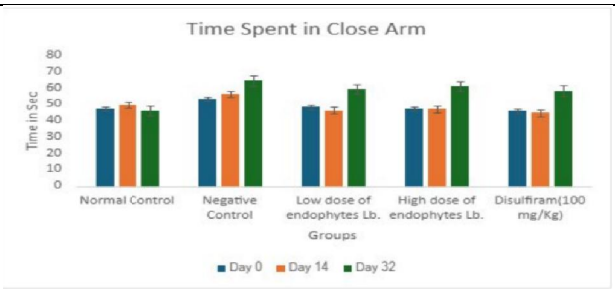
Sr. No.	Groups	Number of entries in open arm on 0 day [%]	Number of entries in open arm on 14 day [%]	Number of entries in open arm on 32 day [%]
1	Normal Control	56.60 ± 0.66	56.10 ± 0.78	57.79 ± 0.99
2	Negative Control	55.57 ± 0.37#	46.23 ± 0.65@	35.65 ± 0.55@
3	Low Dose (Lb) endophyte	56.06 ± 0.14ns	47.85 ± 1.12**	44.07 ± 1.20**
4	High Dose (Lb) endophyte	56.10 ± 0.27ns	53.47 ± 0.98**	45.63 ± 0.61**
5	Disulfiram (100 mg/Kg)	57.36 ± 1.57ns	54.12 ± 0.87**	45.30 ± 0.21**



(Figure No. 6.: Effect of *Lactobacillus* endophyte (Lb.) on number of entries in Elevated Plus Maze test [open arm])

Table No. 9. Effect of *Lactobacillus* endophyte (Lb.) on time spent on Elevated Plus Maze test [Closed arm]

Sr. No.	Groups	Time spent in closed arm on 0 day [sec]	Time spent in closed arm on 14 day [sec]	Time spent in closed arm on 32 day [sec]
1	Normal Control	47.60 ± 0.82	49.90 ± 0.75	46.14 ± 0.60
2	Negative Control	53.62 ± 0.40#	56.45 ± 2.85@	64.50 ± 3.10@
3	Low Dose (Lb) endophyte	48.40 ± 1.50ns	46.36 ± 0.91**	59.40 ± 0.42**
4	High Dose (Lb) endophyte	47.60 ± 0.21ns	47.22 ± 0.67**	61.21 ± 0.12**
5	Disulfiram (100 mg/Kg)	46.00 ± 1.35ns	44.88 ± 2.42**	58.50 ± 3.00**



(Figure No.7.: Effect of *Lactobacillus* endophyte (Lb.) on Time spent on Elevated Plus Maze test [Closed arm])

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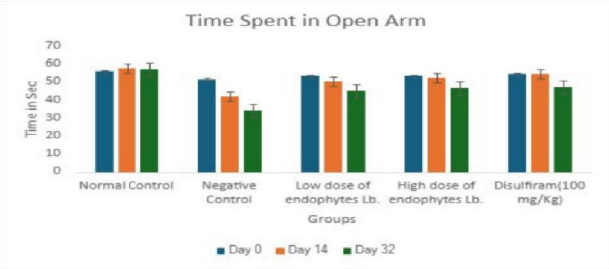
Table No. 10. Effect of *Lactobacillus* endophyte (Lb.) on time spent on Elevated Plus Maze test [open arm]

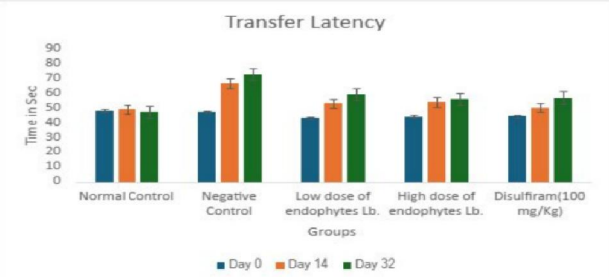
Sr. No.	Groups	Time spent in opened arm on Day 0 [sec]	Time spent in opened arm on Day 14 [sec]	Time spent in opened arm on Day 32 [sec]
1	Normal Control	56.10 ± 0.35	57.55 ± 0.48	57.12 ± 0.65
2	Negative Control	51.50 ± 1.20#	42.23 ± 1.61@	34.06 ± 1.83@
3	Low Dose (Lb) endophyte	53.30 ± 0.50ns	50.48 ± 1.15**	45.10 ± 2.50**
4	High Dose (Lb) endophyte	53.45 ± 0.35ns	52.72 ± 1.98**	46.51 ± 2.50**
5	Disulfiram (100 mg/Kg)	54.41 ± 0.45ns	54.60 ± 2.25**	47.36 ± 2.76**



(Figure No.8.: Effect of *Lactobacillus* endophyte (Lb.) on Time spent on Elevated Plus Maze test [open arm])

Table No. 11. Effect of *Lactobacillus* endophyte (Lb.) on transfer latency of rats EPM apparatus

Sr. No.	Groups	Transfer latency in seconds on Day 0	Transfer latency in seconds on Day 14	Transfer latency in seconds on Day 32
1	Normal Control	48.20 ± 2.15	49.10 ± 1.90	47.33 ± 1.27
2	Negative Control	47.33 ± 1.87#	66.90 ± 1.95@	73.00 ± 2.40@
3	Low Dose (Lb) endophyte	43.31 ± 0.89ns	53.10 ± 0.95**	59.66 ± 1.03**
4	High Dose (Lb) endophyte	44.30 ± 1.46ns	54.10 ± 1.75**	56.01 ± 2.36**
5	Disulfiram (100 mg/Kg)	44.50 ± 2.00ns	50.30 ± 1.95**	57.10 ± 1.86**



(Figure No.9.: Effect of *Lactobacillus* endophyte (Lb.) on transfer latency of rats EPM apparatus)

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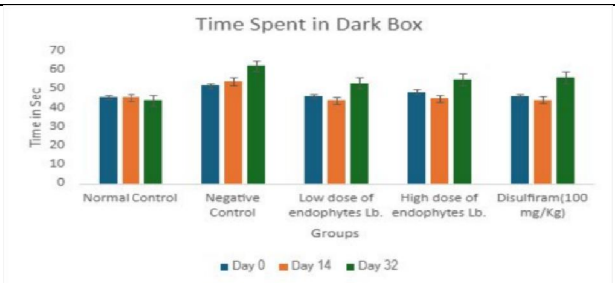
4.3.2. Light and Dark Box Apparatus

Table No. 12.: Effect of *Lactobacillus* endophyte (Lb.) on Time spent in dark box

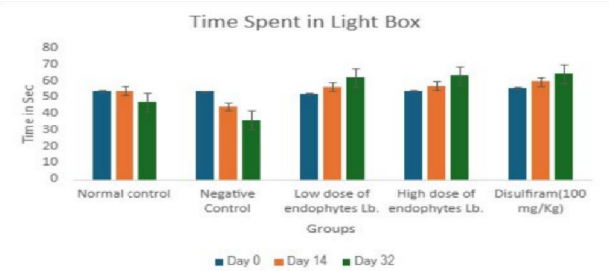
Sr. No.	Groups	Time spent in dark box on 0 day [sec]	Time spent in dark box on 14 day [sec]	Time spent in dark box on 32 day [sec]
1	Normal Control	45.56 ± 0.21	45.47 ± 1.42	44.01 ± 0.12
2	Negative Control	51.78 ± 1.90#	54.11 ± 2.50@	62.20 ± 1.50@
3	Low Dose (Lb) endophyte	46.20 ± 0.11ns	44.06 ± 1.23**	53.21 ± 0.60**
4	High Dose (Lb) endophyte	48.50 ± 2.40ns	45.07 ± 0.48**	55.15 ± 1.80**
5	Disulfiram (100 mg/Kg)	46.30 ± 1.30ns	44.27 ± 1.60**	56.18 ± 0.50**



(Figure No.10.: Effect of *Lactobacillus* endophyte (Lb.) on Time spent in dark box)

Table No.13.: Effect of *Lactobacillus* endophyte (Lb.) on Time spent in Light box

Sr. No.	Groups	Time spent in Light box on 0 day [sec]	Time spent in Light box on 14 day [sec]	Time spent in Light box on 32 day [sec]
1	Normal Control	54.00 ± 1.10	54.10 ± 0.20	47.21 ± 0.12
2	Negative Control	53.78 ± 0.40#	44.52 ± 1.16@	36.34 ± 1.20@
3	Low Dose (Lb) endophyte	52.31 ± 0.60ns	56.54 ± 1.51**	62.21 ± 1.23**
4	High Dose (Lb) endophyte	54.02 ± 1.22ns	57.15 ± 0.62**	63.43 ± 0.30**
5	Disulfiram (100 mg/Kg)	55.61 ± 0.21ns	59.73 ± 1.90**	64.65 ± 1.54**



(Figure No.11.: Effect of *Lactobacillus* endophyte (Lb.) on Time spent in Light box)

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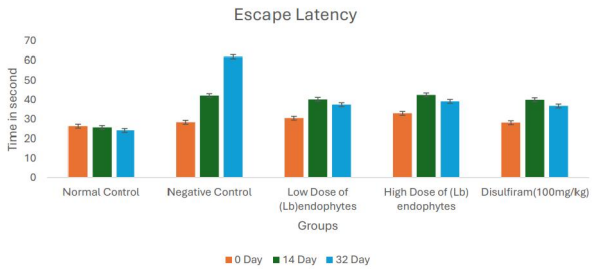
4.3.3. Morris Water Maze Apparatus

A. Escape Latency

Table no. 14.: Effect of *Lactobacillus* endophyte (Lb.) on escape latency of rats in MWM apparatus

Sr. No.	Groups	Escape latency in seconds on Day 0	Escape latency in seconds on Day 14	Escape latency in seconds on Day 32
1	Normal Control	26.20 ± 1.51	25.50 ± 1.48	24.00 ± 1.41
2	Negative Control	28.20 ± 1.41#	42.00 ± 1.80@	62.00 ± 1.32@
3	Low Dose (Lb) endophyte	30.33 ± 1.63ns	40.10 ± 1.70@	37.33 ± 1.86**
4	High Dose (Lb) endophyte	32.83 ± 1.47ns	42.40 ± 1.60**	39.04 ± 1.47**
5	Disulfiram (100 mg/Kg)	28.00 ± 1.52ns	39.90 ± 1.75**	36.66 ± 1.96**



(Figure No.12.: Effect of *Lactobacillus* endophyte (Lb.) on escape latency of rats in MWM apparatus)

B. Retention Time

Table No.15.: Effect of *Lactobacillus* endophyte (Lb.) on Retention Time of rats in MWM apparatus

Sr. No.	Groups	Retention Time in seconds on Day 0	Retention Time in seconds on Day 14]	Retention Time in seconds on Day 32
1	Normal Control	45.89±1.32	44.35±1.4	46.81 ± 1.47@
2	Negative Control	52.66±1.34#	41.08±1.35@	35.5 ± 1.41@
3	Low Dose (Lb) endophyte	58.61±2.05ns	53.56±1.96**	67.5 ± 1.87**
4	High Dose (Lb) endophyte	59.48±2.51ns	54.16±2.35**	68.83 ± 2.3**
5	Disulfiram (100 mg/Kg)	57.16 ± 1.87ns	61.68±1.63**	72.5 ± 1.47**

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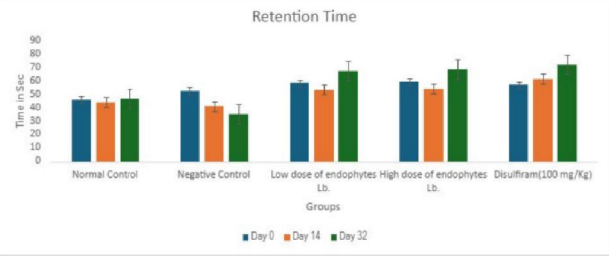

(Figure No.13.: Effect of *Lactobacillus* endophyte (Lb.) on Retention Time of rats in MWM apparatus

4.4. HISTOPATHOLOGY OF RAT BRAIN

4.4.1. Histopathology of Control Rat Brain:

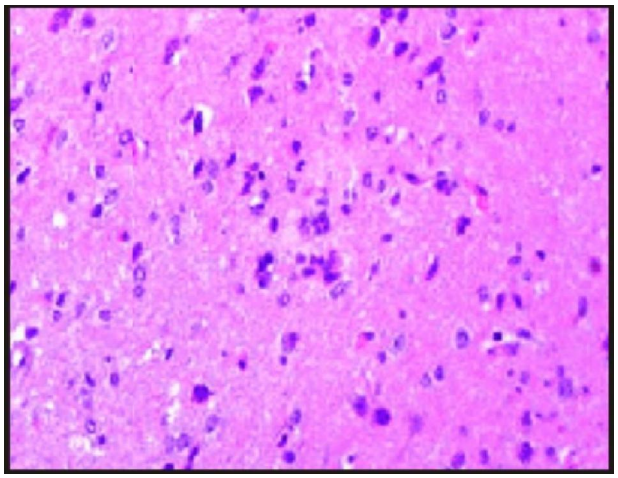

Figure-14: Photomicrograph of normal control rat brain showing normochromic neurons with well outlined cell bodies and absence of intra cellular spaces

4.4.2.Histopathology of Experimental Rat Brain (Exposed to Ethanol):

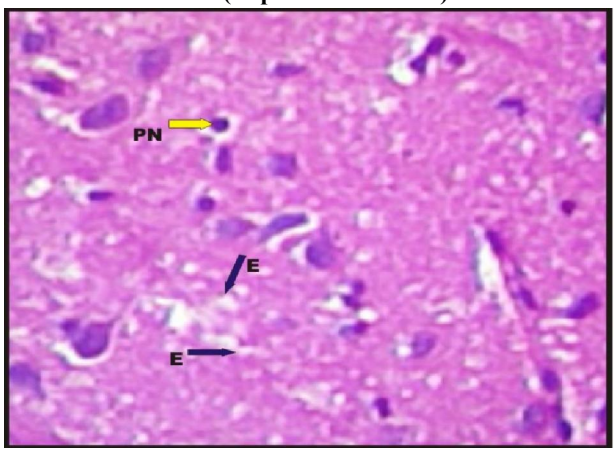

Figure-15: Photomicrograph of ethanol treated rat brain showing darkly stained pyknotic nuclei and shrunken cytoplasm (PN: yellow arrow), edema (E: black arrow).

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4.4.3.Histopathology of Endophytes (Lactobacillus) Treated Rat brain already exposed to low dose of Ethanol

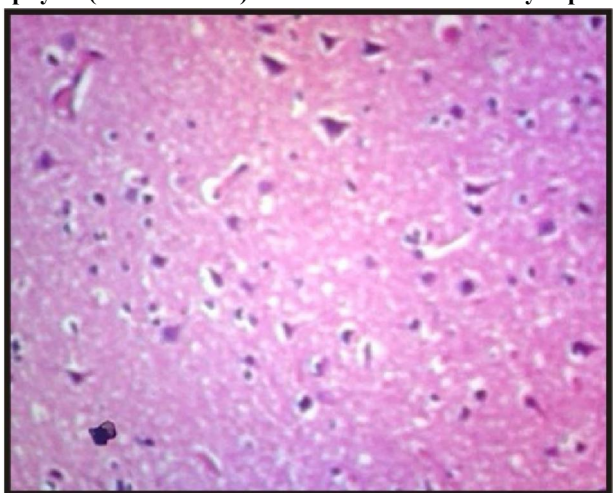

Figure-16: Photomicrograph of Endophytes (Lactobacillus) treated rat brain already exposed to low dose of Ethanol showing more prominent cells and significantly reduced incidence of pyknotic nuclei and cytoplasmic eosinophilia.

4.4.4.Histopathology of Endophytes (Lactobacillus) Treated Rat brain already exposed to high dose of Ethanol

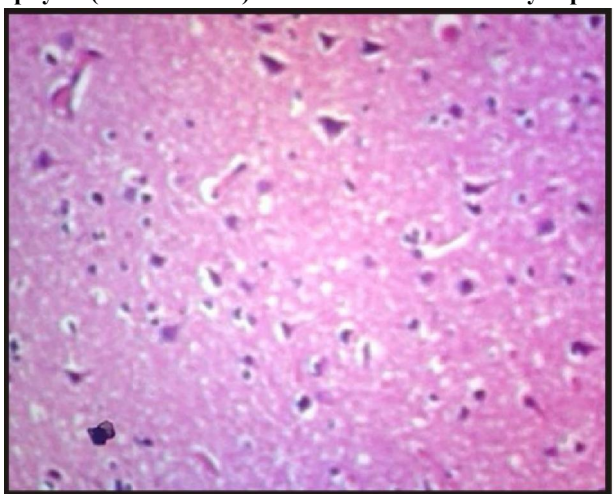

Figure-17: Photomicrograph of Endophytes (Lactobacillus) treated rat brain already exposed to high dose of Ethanol showing more prominent cells and significantly reduced incidence darkly stained pyknotic nuclei and cytoplasmic eosinophilia.

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4.4.5.Histopathology of Disulfiram Treated Rat brain already exposed to Ethanol:

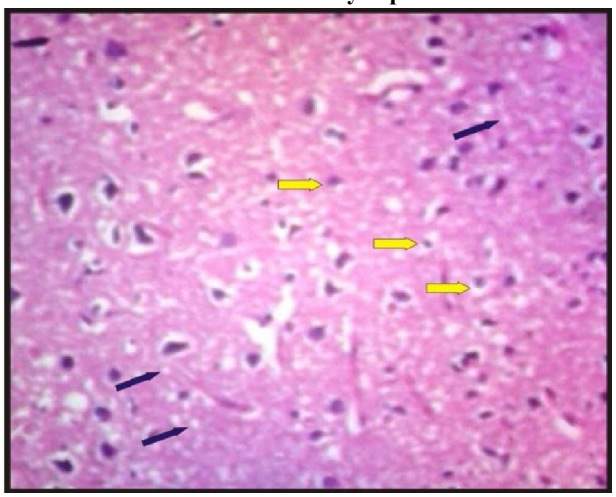

Figure-18: Photomicrograph of Disulfiram treated rat brain already exposed to Ethanol showing significantly improved histopathological alterations, highly reduced incidence of pyknotic nuclei and cytoplasmic eosinophilia (yellow arrow) and improved edema (black arrow).

V. DISCUSSION

Alcohol consumption, particularly during adolescence, poses a significant global health concern due to its widespread use and harmful consequences. Adolescence represents a critical window of brain development, making it uniquely susceptible to the neurotoxic effects of ethanol. In this study, we investigated the potential of a *Lactobacillus* endophyte, isolated from the neem plant (*Azadirachta indica*), to mitigate the neurobehavioral and histopathological alterations induced by adolescent ethanol exposure in a rat model.

Our behavioral assessments using the Elevated Plus Maze and Light/Dark Box apparatus revealed that chronic ethanol exposure in adolescent rats led to increased anxiety-like behavior, as evidenced by a decrease in open arm entries and time spent in the open arms of the EPM, and an increase in time spent in the dark compartment of the LDB. These findings are consistent with previous studies demonstrating that adolescent ethanol exposure can induce anxiety-related behavioral changes in rodents.

Furthermore, ethanol exposure impaired learning and memory functions, as demonstrated by the increased escape latency during the training trials and decreased retention time in the probe trial of the Morris Water Maze. This aligns with existing literature highlighting the detrimental effects of adolescent alcohol on hippocampal-dependent spatial learning and memory. The increased transfer latency observed in the EPM also supported the impairment of spatial memory.

Importantly, treatment with both low and high doses of the *Lactobacillus* endophyte (Lb.) significantly attenuated these ethanol-induced neurobehavioral deficits. In the EPM and LDB tests, the *Lactobacillus*-treated groups showed a significant increase in exploratory behavior in the anxiogenic open arms and light compartment, respectively, indicating a reduction in anxiety-like behavior. Similarly, in the MWM, these groups exhibited a significant decrease in escape latency and an increase in retention time, suggesting an improvement in learning and memory functions. The standard drug, Disulfiram, also demonstrated comparable beneficial effects on the behavioral parameters.

Histopathological analysis of the brain tissue from ethanol-exposed rats revealed significant neurodegenerative changes, including neuronal shrinkage, cytoplasmic eosinophilia, pyknotic nuclei, and vacuolization indicative of edema. These observations are consistent with the known neurotoxic effects of ethanol, which can lead to neuronal damage and tissue disruption.

In contrast, the brain sections from rats treated with the *Lactobacillus* endophyte showed a relatively preserved neuronal architecture, with more prominent neurons and a significant reduction in the incidence of pyknotic nuclei and

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cytoplasmic eosinophilia. Vacuolization and tissue edema were also minimal in these treatment groups, suggesting a neuroprotective effect of the *Lactobacillus* endophyte against ethanol-induced brain damage. The Disulfiram-treated group also exhibited marked improvements in the histopathological findings.

The potential mechanisms underlying the neuroprotective effects of *Lactobacillus* endophytes may involve the gut-brain axis. Ethanol is known to disrupt the gut microbiome, leading to increased intestinal permeability and systemic inflammation, which can negatively impact brain function. *Lactobacillus* species are known to modulate the gut microbiota, potentially restoring gut homeostasis and reducing the inflammatory signals reaching the brain. Furthermore, some *Lactobacillus* strains have been shown to possess antioxidant properties and can influence the production of neurotrophic factors such as Brain-Derived Neurotrophic Factor (BDNF) and modulate inflammatory cytokines like IL-6 and IL-10, which are crucial for neuronal survival and function. The observed improvement in neurobehavioral outcomes and the preservation of neuronal integrity in the *Lactobacillus*-treated groups in our study may be attributed to these multifaceted effects.

The comparison with Disulfiram, a medication used in the treatment of alcohol use disorder, showing similar beneficial effects, suggests that the *Lactobacillus* endophyte may target some of the underlying mechanisms involved in ethanol-induced neurotoxicity. However, further research is needed to elucidate the specific pathways and bioactive compounds responsible for the observed neuroprotective effects of the *Lactobacillus* endophyte isolated from neem.

This study has some limitations. We used a specific dose and duration of ethanol exposure in adolescent rats, and the findings may not directly translate to other exposure paradigms or human populations. Furthermore, while our microbiological characterization suggested the isolate belonged to the genus *Lactobacillus*, further molecular identification would be beneficial to determine the specific species. The precise bioactive compounds produced by this *Lactobacillus* endophyte and their mechanisms of action in the context of ethanol-induced neurotoxicity warrant further investigation.

VI. CONCLUSION

In conclusion, our findings provide compelling evidence that a *Lactobacillus* endophyte isolated from *Azadirachta indica* possesses significant neuroprotective effects against ethanol-induced neurobehavioral deficits and histopathological alterations in adolescent rats. This suggests the potential of neem-derived *Lactobacillus* as a natural therapeutic strategy for mitigating the harmful neurological consequences of adolescent alcohol exposure. Future research should focus on identifying the specific *Lactobacillus* species and the underlying mechanisms of action to further explore its translational potential.

REFERENCES

[1]. Aaberg, K. M., et al. (2017). Incidence and Prevalence of Childhood Epilepsy: A Nationwide Cohort Study. Pediatrics, 139(5), e20163908.

[2]. Ana Contreras, Eduardo PolÃ-n, Miguel MiguÃ©ns, Carmen PÃ©rez-GarcÃ-a, Vicente PÃ©rez, Mariano Ruiz-Gayo, Lidia Morales, Nuria Del Olmo, Intermittent Excessive and Chronic-Moderate Ethanol Intake during Adolescence Impair Spatial Learning, Memory and Cognitive Flexibility in the Adulthood, Neuroscience, Volume 418, 2019, Pages 205-217, ISSN03064522, https://doi.org/10.1016/j.neuroscience.2019.08.051.(https://www.sciencedirect.com/science/article/pii/S0306452219306335.

[3]. Avani K Patel, Balasanova AA. Treatment of Alcohol Use Disorder. JAMA. 2021;325(6):596. doi:10.1001/jama.2020.2012 in Chan

[4]. E, Fogler JM, Hammerness PG. Treatment of Attention-Deficit/Hyperactivity Disorder Adolescents: A Systematic Review. JAMA. 2016 May 10;315(18):19972008. doi: 10.1001/jama.2016.5453. PMID: 27163988.

[5]. Choudhary N, Dhingra N, Gacem A, Yadav VK, Verma RK, Choudhary M, Bhardwaj U, Chundawat RS, Alqahtani MS, Gaur RK, Eltayeb LB, Al Abdulmonem W, Jeon BH. Towards further understanding the applications of endophytes: enriched source of bioactive compounds and bio factories for nanoparticles. Front

Plant Sci. 2023 Jul 10;14:1193573. doi: 10.3389/fpls.2023.1193573. PMID: 37492778; PMCID: PMC10364642.

[6]. Cortese, S., et al. (2018). Prevalence of ADHD in non-Western countries: a systematic review and meta-analysis. The Lancet Psychiatry, 5(5), 379-388.

[7]. Cryan JF, O'Riordan KJ, Cowan CSM, Sandhu KV, Bastiaanssen TFS, Boehme M, Codagnone MG, Cussotto S, Fulling C, Golubeva AV, Guzzetta KE, Jaggar M, LongSmith CM, Lyte JM, Martin JA, Molinero-Perez A, Moloney G, Morelli E, Morillas E, O'Connor R, Cruz-Pereira JS, Peterson VL, Rea K, Ritz NL, Sherwin E, Spichak S, Teichman EM, van de Wouw M, Ventura-Silva AP, Wallace-Fitzsimons SE, Hyland N, Clarke G, Dinan TG. The Microbiota-Gut-Brain Axis. Physiol Rev. 2019 Oct 1;99(4):1877-2013. doi: 10.1152/physrev.00018.2018. PMID: 31460832.

[8]. Dazzi L, Sanna F, Talani G, Bassareo V, Biggio F, Follesa P, Pisu MG, Porcu P, Puliga R, Quartu M, Serra M, Serra MP, Sanna E, Acquas E. Binge-like administration of alcohol mixed to energy drinks to male adolescent rats severely impacts on mesocortical dopaminergic function in adulthood: A behavioral, neurochemical and electrophysiological study. Neuropharmacology. 2024 Feb 1;243:109786. doi:

[9]. 10.1016/j.neuropharm.2023.109786. Epub 2023 Nov 11. PMID: 37952712.

[10]. Gelf and A. A. (2016). Migraine and childhood periodic syndromes in children and adolescents. Current Opinion in Neurology, 29(3), 309–313.

[11]. Gilmour GS, Nielsen G, Teodoro T, Yogarajah M, Coebergh JA, Dilley MD, Martino D, Edwards MJ. Management of functional neurological disorder. J Neurol. 2020 Jul;267(7):2164- 2172. doi: 10.1007/s00415-020-09772-w. Epub 2020 Mar 19. PMID: 32193596; PMCID: PMC7320922.

[12]. Giulia Umbrello and Susanna Esposito Microbiota and neurologic diseases: potential effects of probiotics Umbrello and Esposito J Transl Med (2016) 14:298 DOI 10.1186/s12967- 016-1058-7(Journal ofTranslational Medicine)

[13]. Gouda S, Das G, Sen SK, Shin H-Sand Patra JK (2016) Endophytes:A Treasure House of BioactiveCompounds of Medicinal Importance.Front. Microbiol. 7:1538.doi: 10.3389micb.2016.01538

[14]. Hingson, RW (Hingson, Ralph W.); Heeren, T (Heeren, Timothy) ; Winter, MR (Winter, Michael R.) (provided by Clarivate) Volume1 60 Issue7 Page739746 DOI10.1001 /archpedi.160.7.739

[15]. Huiying Wang,1,2,3 In-Seon Lee,1,2,3 Christoph Braun,2,4 and Paul Enck1 Effect of Probiotics on Central Nervous System Functions in Animals and Humans: A Systematic Review J Neurogastroenterol Motil, Vol. 22 No. 4 October, 2016pISSN: 2093-0879 eISSN: 2093-0887 http://dx.doi.org/10.5056/jnm16018Journal of Neurogastroenterology and Motility

[16]. Keith Clay The potential role of endophytes in ecosystems Biotechnology of endophytic fungi of grasses, 73-86, 2018.

[17]. Macleod S, Appleton RE. Neurological disorders presenting mainly in adolescence. Arch Dis Child. 2007 Feb;92(2):170-5. doi: 10.1136/adc.2005.088070. PMID: 17264287; PMCID: PMC2083313.

[18]. Macleod S, Appleton RENeurological disorders presenting mainly in adolescence Archives of Disease in Childhood 2007;92:170-175

[19]. Mallikarjuna Nimgampalle, Yellamma Kuna et. al. 2017. A review on AntiAlzheime Properties of Probiotic, Lactobacillus plantarum MTCC 1325 in Alzheimer's Disease induced Albino Rats. (Journal of Clinical and Diagnostic Research. 2017 Aug.

[20]. Nadkarni A, Tu A, Garg A, et al. Alcohol use among adolescents in India: a systematic review. Global Mental Health. 2022;9:1-25. doi:10.1017/gmh.2021.48

[21]. Nathalie Boutros, Svetlana Semenova, Wen Liu, Fulton T. Crews, Athina Markou, Adolescent Intermittent Ethanol Exposure Is Associated with Increased Risky Choice and Decreased Dopaminergic and Cholinergic Neuron Markers in Adult Rats, International Journal of Neuropsychopharmacology, Volume 18, Issue 2, February 2015, pyu003, https://doi.org/10.1093/ijnp/pyu003