

Bidirectional effect of Triphala on modulating Gut-brain axis to improve cognition in the Murine Model of Alzheimer's disease

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Abstract

Background

The emerging role of gut microbiota and their metabolites in the modulation of the gut-brain axis has received much attention as a new hope for the treatment of hard-to-treat chronic neurodegenerative diseases like Alzheimer's Disease. The naturally occurring polyphenols can restore the gut-brain axis by modulating gut microbiota and brain neurotransmitters. However, the mechanism of action remained unclear. The Indian traditional medicine Triphala, a rich source of polyphenols, has been used on humans based on Prakriti or disease conditions for many years.

Methods:

In this study, the dual mode (morning and evening) action of Triphala was used to provide scientific evidence of its superior preventive and therapeutic efficacy in C57BL/6 and 5xFAD, APP/PS1 transgenic mouse model of Alzheimer's disease. For behavior analysis, used the Morris water model and Y maze model to assess spatial memory and exploratory behavior. The blood serum and brain lysate were used to evaluate the inflammatory activity and oxidative parameters in the mice. The gut microbiome analysis was done by 16srRNA analysis from mice feces after 60 days of treatment.

Results

We observed that Triphala treatment has significantly improved cognitive function, by modulating the APP pathway, reducing inflammation, oxidative stress, and restoring the gut-brain axis by increasing the gut microbiota phyla of *Bacteroides*, *Proteobacteria*, *Actinobacteria*, etc., involved in maintaining the gut homeostasis.

Conclusions

Our study paved a new path for using dual modes of Triphala one or in combination to treat incurable AD.

Background

Alzheimer's disease (AD) is an irreversible and degenerative brain disease characterized by the accumulation of amyloid beta ($A\beta$) and tangles of hyperphosphorylated tau protein. Additionally, the brains of AD patients exhibit excessive neuro-inflammation and reduced hippocampus neurogenesis, leading to neuronal death, which results in a progressive decline in cognitive function. AD pathogenesis remains elusive despite extensive research worldwide for more than a century. This left AD the only disease without any preventive and therapeutic treatment options. The currently available drugs provide only symptomatic relief and do not prevent disease progression. This signals that we need to understand different aspects of pathogenesis, including role of inter-organ cross-talk in the progression of AD [1].

The hypothalamic-pituitary-adrenal (HPA) axis and the autonomic, gastrointestinal, and central nervous systems comprise the gut-brain axis, a bidirectional communication network. As a member of the microbiota-gut-brain axis, the gut microbes, which include bacteria, viruses, fungi, and archaea, as well as their metabolites and by-products, play a key role in this two-way communication. The Vagus nerve, the immune and neuroendocrine systems, the neurotransmitters and metabolites, and the gut microbiota are the key routes of interest in research on the microbiota-gut-brain axis[2].

Precision medicine presents a novel understanding of hope in therapy based on the diagnostic, genetic marker, or identifying factor for human chronic diseases. Personalized medicine is increasingly thought to include the microbiome. One hundred trillion different bacteria live inside each person's microbiome. While "microbiota" refers to the complete variety of microbial organisms found in the human body, "microbiome" refers to their genomes and collective functions[3].

Ayurveda, an ancient natural system of medicine, has gained worldwide attention for its holistic healing effect. Recently, the World Health Organization (WHO) recommended Ayurveda as a trustworthy and affordable medical system that provides efficient healthcare. However, Ayurveda has never been considered as frontline therapy due to lack of scientific evidence. To address this and establish the therapeutic efficacy of ayurvedic preparation, we provide scientific evidence for Triphala as a neuroprotective agent [4]. Ayurveda strives to prevent and treat disease by emphasizing the host rather than the condition. According to Ayurveda the body has three distinct doshas—Vata, Pitta, and Kapha—that determine a person's health and illness based on their balance or imbalance. Based on the type of doshas the ayurvedic formulation is prepared which indicate personalized precision therapy. One Ayurvedic medicine, Triphala, has demonstrated tri-dosha Rasayana effects on people as Rechak (laxative) and Poshak (food supplement). The three plants *Embllica Officinalis*, *Terminalia chebula*, and *Terminalia bellerica* fruits are mixed together in a ratio to make triphala. It consists of secondary metabolites of polyphenols viz vitamin A, gallic acid, chebulinic acid, nicotinic acid, linolenic acid, beta-sitosterol, epicatechin, and others. The antiviral, antibacterial, antifungal, and antiallergic qualities, cardiogenic, blood pressure-controlling, blood circulation-improving, cholesterol-lowering, and immunomodulatory actions of Triphala have been reported [5]. Triphala has been used since long for the treatment of gastro-intestinal disorders. Thus, in the presence of several metabolites, we hypothesize that Triphala might help in decoding the gut-brain axis.

In the present study, we used the murine model of Alzheimer's disease to study the preventive and therapeutic effect of standardized Triphala extract via modulation of the gut-brain axis. We used latest analytical tools for qualitative analysis of secondary metabolites of Triphala formulation prepared using ingredients collected from the different geographical regions of India.

Methods

Collection of Triphala from different geographical regions and Preparation of Formulation

The fruits of *Terminalia bellirica* (Gaertn.) Roxb., *Terminalia chebula* Retz., and *Embllica Officinalis* L. were procured from three different regions of India (Uttarakhand, Madhya Pradesh, Himachal Pradesh & Maharashtra). All powdered fruit samples were mixed by weight (w/w)—in equal portions and passed through sieve No 80. All dried fruit materials were purchased from the GMP-approved company based in Gujrat (M/s Phyto Lifesciences Pvt Ltd) and Noida (M/s Ambe Phytoextracts)

For The Preparation Of Triphala Extract

The mixed powdered material of weight 35 g was macerated with 70:30 ethanol and water of 200 ml for 2–3 days. The extract was then filtered and evaporated to dryness at 45°C with a rotary evaporator (Buchi R-210 Advanced, Switzerland).

Chemical Characterization By Ultra-performance Liquid Chromatography Quadrupole-time-of-flight Mass Spectrometry Analysis (Uplc-q-tof-ms)

Three different geographical plant material sources were used for chemical characterization through the ACQUITY UPLC-Q-TOF-MS/MS system (Waters Corp., Milford, MA, USA) to identify the secondary metabolites. The chromatography was carried out with an Acquity BEH C18 column (dimensions: 100 mm × 2.1 mm, 1.7 µm; temperature: 25°C). The mobile phase consisted of 0.1% formic acid (a), acetonitrile (b), and methanol (c), and its flow rate was 300 µl/min. The sample injection volume was 5 µl. The gradient elution program was optimized as follows: initial 90:10% B: C and increased to 80:20% in 2 min, 50–60% B: C for 1–3 min, 30–70% B: C for 3–6 min, then 10:90% B: C for 1 min, and finally increased quickly to 90–10% in 7–10 min. MS analysis was performed in positive and negative ion modes. Centroid mode data were collected over

the m/z range 100–1000 Da of 1.0 sec over an analysis time of 22 min. LC-MS/MS analyses were performed with a collision energy of 25 eV. The Software ReSpect for Phytochemicals (<http://spectra.psc.riken.jp/>) was used for data acquisition.

Animals

APP/PS1 mice were purchased from The Jackson Laboratory (stock no. 004462) and bred at the Small Animal Facility of the National Institute of Immunology (New Delhi, India). All mice were housed and maintained in accordance with the guidelines, with the prior approval of the Institutional Animal Ethics Committee (IAEC) of the National Institute of Immunology (Approval No. 491/18). Animals were used as per the national guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). All the animal experiments were reported according to the ARRIVE guidelines. The experiments' method and protocol were performed per relevant guidelines and regulations. In the present study, we used APP/PS1 (3xFAD) mice containing three mutations associated with familial Alzheimer's disease (APP Swedish, MAPT P301L, and PSEN1 M146V) and APP/PS1 (5xFAD) mice express human APP and PSEN1 transgenes with a total of five AD-linked mutations: the Swedish (K670N/M671L), Florida (I716V), and London (V717I) mutations in APP, and the M146L and L286V mutations in PSEN1 in C57BL6 background mice. The 5xFAD model rapidly develops severe amyloid pathology, accumulating high intraneuronal A β 42, beginning around 1.5 months of age. However, the APP/PS1 mice display both plaque and tangle pathology. A β deposition is progressive, with intracellular immunoreactivity detected in some brain regions as early as three to four months of age. These mice develop age-related, progressive neuropathology, including plaques and tangles which were used as the study's transgenic (TG) group. The C57BL6 mouse was used as a wild-type (WT) group in the study, young mice age of two months old and aged mice more than 10 months old. Each group contains n = 15 mice in the studies.

Experimental Design:

To validate Triphala in the gut-brain axis, the experimental studies have been divided into two parts: The preventive and therapeutic study. In this study, we used both the modern and traditional treatment methods with Triphala. In the contemporary practice of herbal formulation treatment, we used the Triphala extract. For the conventional system, we used the powder formulation and the treatment methodology as given in table no 2. We observed the body weight and food intake during the treatment for 60 days. The glucose tolerance test was performed on day 61 after 16 hr fasting; 2mg/kg of glucose was given intraperitoneally, and the blood glucose was measured at 0, 15, 30-, 45-, 90- and 120-min using a Glucometer (Roche) and blood from the tail vein. We performed spatial memory tests in the Morris water maze model and exploratory tests in the Y maze apparatus for the behavioral study to assess cognitive function as per the given protocol in Khandelwal et al. [6]. Blood was withdrawn on day 62 through the retro-orbital plexus of the mouse in a non-anti-coagulant tube. The collected blood samples were centrifuged at 12000 rpm for 15 min at 37°C, and serum was separated from the blood, aliquoted, and stored at – 20°C. The measurement of

Biochemical parameters (Tulip diagnostic Pvt Ltd auto-analyzer) Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), Alkaline phosphatase (ALP), Urea, Creatinine Lipase and Amylase analysis were performed as per the manual of the Coral clinical system.

ELISA

The serum was used to measure, Acetylcholine, Serotonin, Lipid peroxidation (LPO), and Lipopolysaccharides (LPS) levels as per the manufacturer's instruction (Immunotag™ G-Biosciences, Missouri, USA) using the serum of all groups of mice.

Proinflammatory and anti-inflammatory cytokines

The Serum sample used for were analyzed by using the Bio-Plex Pro™ Cytokine Standard kit (Bio-Rad, California, USA). The plate was prepared following the instruction manual provided by the manufacturer and measured using Bio-Plex 200

Systems (Bio-Rad, California, USA). The data was analyzed on Bio-Plex Manager (acquisition and Analysis) software at low PMT and RP1 instrument settings. Fecal samples were collected from animals after 60 days of treatment. The samples were prepared as per the given method by Sampson et al. 2017 [7].

Short-chain fatty acids (SCFAs): The feces were used to analyzed by using HPLC (LC-20AT, Shimadzu) equipped with a carbohydrate column (Aminex HPX-87H column, Biorad) and a photodiode array detector (PDA, Shimadzu). The eluent was five mM H₂SO₄, fed at a flow rate of 0.6 mL/min, and the column temperature was 50°C. The run time was 30 min. Standard curves were generated by diluting ten mM volatile fatty acid standard solution (acetic acid, propionic acid, and butyric acid) from 50 nM to 5000 nM.

Microbiome Analysis

The fecal DNA was extracted using a kit of MACHEREY NAGEL USA, and the V3- V4 region of the **16S rRNA** gene was amplified using barcoded primers. Sequencing was performed using an Illumina MiSeq. Operational Taxonomic Units (OTUs) were picked closed reference using Sort Merna 2.0 against the August 2013 release of Greengenes in QIIME 1.9. Differential abundance calculations were performed using genus-level taxa and relative abundance of all counts offset by one done by the Clevergene Biocorp Pvt. Ltd Bangalore India and Bionivid Technology Pvt Ltd Bangalore India.

RNA (mRNA) analysis

The relative expression of specific messenger was quantified by real-time polymerase chain reaction (PCR) using SYBR Green I (Roche Diagnostics, Mannheim, Germany). The sense and antisense primers used are listed in the supplementary Table 3. Further brain and caecum were collected on day 60 after perfusion following dissections, preserved for 48 hours at room temperature with 10% neutral-buffered formalin, and then embedded in paraffin. The tissue samples were cut into 5 m thick sections, deparaffinized, and rehydrated in a specific order using xylene, 100% alcohol, and 50% alcohol.

Hematoxylin and eosin (H&E)

Brain and caecum were used to stain the rehydrated sections so that morphological alterations could be seen. Stained areas were seen and photographed using a USB 2.0 Camera Viewer (Leadzoptics Microscope, England, UK).

Statistical analysis

All experiments were repeated at least three times. The Data were presented as mean ± standard error for the indicated numbers of independently performed experiments. Statistical significance was assessed by One way or Two-way ANOVA analysis of variance coupled with a Bonferroni t-test. All statistical analyses were performed using Graph Pad Prism 9.0 for Windows (GraphPad Software, La Jolla, CA, USA).

Result

The metabolites present in Triphala are impactful for decoding the gut-brain axis.

Ethanollic extract of Triphala and powder showed several secondary metabolites through UPLCQ-TOFMS/MS (Fig S1A-H). The Riken Tandem Mass Spectral Database library confirmed the identification of compounds. The identified compounds belong to phenylpropanoid (4coumaric acid and chlorogenic acid hemihydrate), flavonoid (chalcone), and an alkaloid (melatonin) group. Table 1 summarizes the mass spectra and nature of identified compounds and their structures and biological activities.

Triphala Is Safe For Long-term Use:

Long-term Triphala administration in mice hasn't shown any significant changes (during and after the treatment) in body weight, food weight, and water intake in both the preventive (Young WT & 5XFAD) (Fig S4A & Fig S2A) and therapeutic study (Aged WT & 3XFAD) (Fig S4B & Fig S2B). For further confirmation, we performed the liver function (SGOT, SGPT ALP) and kidney function (Urea, Creatinine) tests using the serum post-treatment and found no significant changes. The results indicated that the formulation is safe for long-term use.

We reported recently that the transgenic AD mouse show disturbed glucose homeostasis [6]. We performed the glucose tolerance test in mice to get a better physiological overview of Triphala administration. In the preventive study, at 30 min and 60 min, the V5, V4, and V3 groups showed significantly improved glucose tolerance compared to the V1 group (Fig S2I). Similarly, in wild-type groups of mice, the WV5, WV4 & WV3 groups showed enhanced glucose tolerance activity at 30, 60 & 90 min compared to the WV1 group (Fig S4I).

Similarly, the WT and TG mice in the therapeutic group showed better glucose tolerance against the non-treated group, however, it was non-significant. After 30 mins, groups A2 and WA1 showed significant ($p < 0.001$) improvement against the A1 group but the rest of the groups (A3, A4 & A5) showed non-significant change (Fig S2J). However, in wild-type, aged groups of mice, the WA2, WA3, WA4 & WA5 showed significant ($p < 0.01$) tolerance activity after 15 min, 30 min, and 180 min compared to the WA1 group (Fig S4J). Thus, we found that formulation given twice daily in young WT, 5XFAD, aged WT & APP/PS1 mice have improved glucose homeostasis.

Triphala Prevents And Helps In Reversing Cognitive Deficit In Ad Mice

We studied the effect of different treatment modalities on cognition by using the Morris water maze for spatial memory. We monitored the parameters like the swim speed, distance traveled by the mice, and escape latency time using a video tracking system and Any-maze software. Interestingly, in both preventive (Fig. 2) and therapeutic studies (Fig. 3), the Disease control mice showed a significantly low swim speed, more distance traveled, and more time to find the platform after the probe trial on day five compared to other groups. However, mice treated with Triphala twice (V5 & A5) showed significant ($p < 0.001$) improvement in cognitive function by taking less time to find the platform in the target quadrant. In the probe trial, the time taken by the mice to reach the platform from Day 1 (57.33 ± 1.15) to Day 4 (15.17 ± 2.78) in the V5 group and A5 group, Day 1 (59.67 ± 0.30) to Day 4 (26 ± 2.8). Even among all Triphala-treated group, the twice daily treatment showed more significant ($p < 0.001$) effect in improvement of cognitive parameters in spatial memory test. Simultaneously with same parameters in the young mice (Fig S5A-E), the speed showed a significant ($p < 0.001$) improvement, while the aged mice (Fig S10A-E) showed the significant improvement in speed and escape latency time in twice daily Triphala (WV5&WA5) group. This was further corroborated well with other parameters like swimming pattern, and speed and was comparable to the wild type.

Next, we evaluated the exploratory behavior of mice using Y-maze. We observed that TG mice (V5 & A5) treated with Triphala showed a significantly ($p < 0.001$) high percentage of alteration in Y mazes performance and explored more Novel-arm compared to the disease control mice (V1 & WA1) (Fig. 2F-G & Fig. 3F-G). The aged (WA5) (Fig S10 F-G) and young mice (WV5) (Fig S5 F-G) also showed significant improvement after treatment compared to the control group (WA1&WV5). Our behavioral studies data showed that two times (Morning & Evening) Triphala treated group compared to once daily treatment, showed more significant improvement in cognitive parameters in spatial memory and exploratory behavior in both preventive & therapeutic studies of TG & WT mice.

To correlate behavioral data, we quantitated the brain biogenic amines, which have a direct role in cognition, in serum. The level of serotonin (Fig. 2H & Fig. 3H) and acetylcholine (Fig. 2I & Fig. 3I) were found to be significant ($p < 0.001$) high in the treatment groups (V5 & A5) indicating the protective and therapeutic effect of the brain. Concurrently, the young and aged mice showed (Fig S5I & Fig S10I) a significant ($p < 0.001$) low level (WV5 & WA5) compared to treated groups. Further, understanding the mechanistic pathway of Triphala we checked the mRNA expression of genes pertinent to AD in the brain lysate. The significant ($p < 0.001$) decrease in the mRNA level of Beta-secretase (BACE) (Fig. 2 & Fig. 3J) in the treated groups, indicates decreased AB42 production. Concomitantly, the mRNA level of Amyloid precursor protein level (APP) was found

significant ($p < 0.001$) (Fig. 2L & Fig. 2L) reduced in the brain of 5XFAD & APP/PS1 Triphala treated mice. The significantly ($p < 0.001$) reduced Acetylcholinesterase (AChE) mRNA level (Fig. 2K & Fig. 3K) in treated groups, corroborated with the level of Acetylcholine in serum. The twice daily treated group (V5 & A5) showed more significant effect among others treated groups. We extended our study at the tissue level and found more neurons in the dentate gyrus region of treated TG & WT mice in both studies (Fig. 2M Fig. 3M, Fig S5J & Fig S10J).

Thus, our results revealed the beneficial preventive and therapeutic effect of Triphala against cognitive impairment through increased spatial memory, exploratory behavior by modulating the neurotransmitter, and reduced APP processing, in the mouse model of AD.

Triphala reduces inflammation and oxidative stress.

The role of neuroinflammation and oxidative stress in the initial stage of AD pathogenesis is well established. So next we checked whether secondary metabolites in Triphala have any noticeable effect on neuro-inflammation and oxidative stress in 5XFAD and APP/PS1 mice. The anti-inflammatory and antioxidant activity in the serum and brain lysate were estimated. The pro-inflammatory TNF- α (Fig. 4A-B & 5A-B) & IFN- γ (Fig. 4C-D & 5C-D) levels in serum and brain lysate were significantly ($p < 0.001$) low in the 5XFAD & APP/PS1 treated groups. Concurrently, the serum level of TNF- α (Fig S6A & S11A) and IFN- γ (Fig S6B & S11B) showed significant reduced level in the treated group of young and old age mice. The serum level of IL-17 (Fig. 4G, 5G, S6E & S11E), IL-10 (Fig. 4H, 5H, S6F & S11F), IL-1 β (Fig. 4I, 5I, S6G & S11G), and IL-6 (Fig. 4J, 5J, S6H & S11H) were also significantly ($p < 0.001$) reduced in the treatment groups in both the studies, confirming the anti-inflammatory properties of secondary metabolites present in Triphala. Further, for oxidative stress, LPO level (Fig. 4K, 5K, S6D & S11D) was measured and found to be significantly low in the TG & WT treatment group in both studies. Next, we checked whether the gut and associated microbiota played any role in the overall reduced inflammation and oxidative stress observed in the treatment group. It is reported that the lipopolysaccharides (LPS) released by gram-negative bacteria in the gut cause inflammation by activating NF- κ B and pro-IL-1 β . So, to correlate reduced inflammatory markers in the serum to the gut, we measured serum LPS level. The LPS level was also significantly ($p < 0.001$) reduced in the animals treated with Triphala (Fig. 4E, 5E, S6C & S11C) in both therapeutic regimens. The reduced level of LPS, validated through the toll like receptor mRNA (TLR 4) expression and found significant reduced ($p < 0.001$) in treated group (Fig. 4F, 5F, S6D & S11D). Among all Triphala treated groups, the twice daily treatment in TG (V5 & A5) and WT (WV5 & WA5) showed more significant anti-inflammatory parameters in 5XFAD & APP/PS1 mice.

Triphala Prevents Dysbiosis And Restored Gut Microbiota

The reduced level of LPS and inflammatory markers indicated the role and involvement of the gut and associated microbiome. We estimated metabolites produced by the gut microbiome to understand the underlying mechanism. It is hypothesized by various research groups that the bacteria present in the gut maintain the motility of the intestine; these bacteria release the metabolites which help cultivate bacteria colonies in terms of confluency. So, we measured the short-chain fatty acid (SCFA), acetic acid, propionic acid, and butyric acid from the fecal of all the groups of TG & WT mice. We observed a significantly reduced level ($p < 0.001$) of SCFA in the transgenic mouse model of AD compared to the wild type, indicating gut dysbiosis in the diseased mouse. Interestingly, treatment with the Triphala formulation has significantly improved ($p < 0.001$) levels of acetic acid (Fig. 6C & 7C), propionic acid (Fig. 6D & 7D) & butyric acid (Fig. 6E & 7E) in both preventive and therapeutic group studies, and the most significant improvement was observed in the V5 & A5 groups compared to disease control. However, the wild type young (Fig S7C-E) and aged (Fig S12C-E) mice didn't show the significant changes in comparison to the control.

To further confirm the beneficial effect of Triphala formulation on the gut, we estimated the fecal level of amylase and lipase in all the groups of animals. A significant increase ($p < 0.001$) in the level of Amylase (Fig. 6A & 7A) and lipase (Fig. 6B & 7B) was observed in both mouse models of AD compared to the disease control (V1 & A1), indicating altered/compromised gut condition in diseased mice. However, the young (Fig S7A & S11A) and aged (Fig S7B & S11B) mice didn't show the significant

improvement. The significantly reduced level of amylase and lipase in all treatment groups compared to diseased control constituted a key finding of the study and corroborated well with the alleviation of inflammation and high level of SCFA, as observed above. The SCFAs have been reported to have a bidirectional effect on the gut and brain. It also helps in the reduction of inflammation and oxidative stress. Furthermore, to validate the beneficial effect of Triphala on cognition and the role of the gut, we conducted a fecal microbiome analysis of all the groups by isolating DNA and sequencing 16s rRNA. Both studies found more diversity in bacteria genera after treatment in TG & WT mice. Some bacteria have activities directly reported for their bi-direction effect on the gut-brain axis. The reduced firmicutes in the treated group showed significant restoration of the dysbiosis condition. For confirmation on the pathological level, the significantly ($p < 0.001$) improved crypt length in the treatment TG & WT mice reveals the restoration of the tissue (Fig. 6G-H, 7G-H, S7G-H & S11G-H) morphology.

We analyzed bacterial diversity through alpha and beta diversity in a preventive study. In alpha diversity, in a sample, the diversity in treated groups of TG & WT can be seen. This diversity indicates variety in a single sample through Chao 1, ACE and Shannon, and fisher analysis. The resilient nature of gut bacteria helps in maintaining gut barrier functions. In the beta diversity analysis, the diversity between samples after the treatment in TG & WT showed more confluency in the number. We found the phylum level of bacteria *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Cyanobacteria*, *Verrucomicrobia* *Actinobacteria*, *Euryachacota*, *Deferribacteres*, *Candidatussaccharibacteria* and *Fusobacteria* in the preventive group (Fig. 8B). The percentage abundance of *Bacterdoetes*, *Verrucomicrobia*, *Actinobacteria* increased in the treatment groups. The increment of healthier bacteria abundance at genus level such as *Rumnicoccus* *Caprococcus* *Parabacteria*, *Oscillospira* etc., found in the V5 group (Fig. 8C). In the alpha diversity, Chao1 Shanon, ACE, Simpson, Inverse Simpson and Fisher showed the diversity present in the community, found more in TG control (V1) while after treatment these diversities were reduced and increased abundance (Fig. 8A). The increased abundance between the group was measured in the beta diversity and found more in the V5 group (Fig S3). The young WT mice showed the significant improvement in the treatment group (WV2-WV5) and more abundance after the treatment (Fig S8 A-C), alpha diversity changes (Fig S8A) and beta diversity (Fig S9A-D) between the group clearly stated the effect of Triphala in modulation of gut.

However, in therapeutic study, we analyzed the microbiome before and after treatment to see the improvement after 60 days in Triphala treated groups. The aged WT and APP/PS1 mice after the treatment showed the more abundance in pertinent way approximately 249 OTU. The Bacteria phyla in TG and WT found same but the diversity at genus level was more. The increased abundance of *Verrucomicrobia*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria* showed the improved gut condition after the treatment. The APP/PS1 mice showed the increased percentage abundance in Triphala treatment at genus level such as *Oscillospira*, *Rumnicoccus*, *Caprococcus*, *Lactobacillus*, *Lachnospira*, *Lactonifactor*, and others which are reported for the increased short chain fatty acids production in metabolites form. These commensal bacteria most prominently increased in the presence of polyphenols, modulate the microbiota consortium, and support in mitigation of disease. The A5 group showed the increased percentage abundance of *Bacteroidetes* 42%, *Verrucomicrobia* 5%, *Proteobacteria* 8% and *Actinobacteria* 12% among others Triphala treated group (Fig. 9). Concurrently, aged WT mice with the same treatment (WA5) also showed the increased abundance. However, the aged WT mice without treatment (WA1) showed the increased abundance of *Helicobacter* 12% and reduced level of *Bacteroidetes* from 52–30% after the 8 months, indicated the with increasing the age, diversity of microbiota also change and showed the gut disturbances (Fig S13). These results provide evidence that the Triphala has a bi-directional effect to decode the gut-brain axis.

Discussion

A growing body of research indicates that polyphenols included in diet may protect neurons from oxidative stress and inflammation induced injury that may linked to improved cognitive abilities. The active metabolites produced after the biotransformation of polyphenols through gut microbiota enters the brain may function as neurotransmitters in/directly or influence the cerebrovascular system. Thus microbiota-gut-brain axis is a neuroendocrine system that serves in both directions and is crucial for neuronal health. By acting as neurotransmitters in CNS, the metabolites can modify gut bacterial composition and brain biochemistry. Naturally occurring bioactive compounds, such as probiotics, prebiotics, and

polyphenols can affect gut microbiota composition and help in the treatment of gastrointestinal dysfunctions, which are seen in neurodegenerative disorders [8].

Additionally, several pieces of data lend credence to the notion that gut microbiota and enteric neuroimmune system changes may impact the beginning and development of these age-related diseases. The influence of polyphenols on microbiota composition supports the notion that altering food to maintain a healthy microbiome could be crucial for having a healthy brain throughout one's lifespan and they might be applied as cutting-edge therapies to stop the neurodegeneration[9].

The Ayurvedic treatment is based on multiapproach method for multifactorial diseases, by balancing the tri dosha in the body to maintain the homeostasis condition. We studied this traditional concept with a modern approach, where we procured the material from different geographical locations, to get a polyphenols-rich formulation. UPLCMS analysis confirmed presence of several secondary metabolites (Table 1) those have previously been reported for memory improvement, and gut modulation[10]. Next, we design a detailed study in murine model of AD to provide scientific evidence to our traditional knowledge by observing the impact of our formulation in reducing inflammation, antioxidant activity, and the neurogenesis process against neurodegeneration.

We used once (morning or evening) or twice (both morning and evening) treatment regimen to study preventive and therapeutic efficacy compared to triphala powder. The behavioral data of Morris and Y maze reveals that twice daily administration of our Triphala formulation has shown superior efficacy compared to other treatments. The behavioral data correlated with decreased circulating A β 42 level and increased Acetylcholine. The reduced mRNA expression of APP and BACE-1(a rate-limiting enzyme in the production of toxic amyloid- β (A β) 42 peptides) in brain suggests that Triphala treatment has significantly modulated APP pathway to decrease the amyloid burden. The dysregulation of CNS innate-immune system comprising of Microglia and Astrocytes plays critical role in the prodromal phase of AD pathogenesis. Toll-like receptors a key component of this system and association between TLR gene polymorphisms and AD risk have been reported. The significant reduction in the mRNA expression of TLR in treated mice brain suggests that Triphala has targeted initial pathway of neurodegeneration.

Neurogenesis, an important mechanism for brain development, is closely associated with hippocampus-mediated learning and memory in the adult DG. These neurons also receive abundant cholinergic innervation, and cholinergic neurotransmitters released from cholinergic neurons are known to play a key role in memory-related circuits in the brain[11]. One such neurotransmitter, Ach, is synthesized from choline and acetyl coenzyme A by ChAT and plays a critical role in learning and memory. The duration of Ach action depends on the activity of AchE, which hydrolyzes and clears Ach released from the presynaptic membrane[12]. Conversely, removal of this cholinergic innervation through depletion of forebrain Ach impairs neurogenesis in the adult DG. In our study, the data showed, low levels of AchE, and high levels of acetylcholine indicated the preservation of neurogenesis process in the brain of treated mice. The histological image of the mouse brain hippocampus showed more stained neurons in the DG area confirming the normal neurogenesis process[13].

Being the first organ to encounter treatment, it is imperative to study the role of gut and associated microbiota in observed improvement in cognitive function in AD mice. The existence of gut-brain axis and its modulation by associated microbiota and their metabolite has attracted attention worldwide as a promising target for the treatment of AD. The alterations in the microbiota's composition reported in AD, results in both in-/direct impact on the functioning of the CNS, such as the vagus nerve via short-chain fatty acids, dietary amino acids, and cytokines. Age-related changes in the gut microbiota composition, characterized by decreased diversity and stability, cause the gut barrier to break down, leading to increase in the circulatory proinflammatory cytokines and bacteria-derived products, the impairment of the blood-brain barrier, and neuroinflammation. Studies in germ-free mice have shown that the microbiome impacts the microglia's maturation[14]. Short-chain fatty acids (SCFAs), byproducts of bacterial metabolism, may mediate this. Similar to this, particular bacterial tryptophan metabolism products influence astrocyte function by binding to aryl hydrocarbon receptors[15]. Microbiota affects the cytokine profile and peripheral immune cell activation, which affect systemic and CNS inflammation and injury as well as neurodevelopment.

Peripheral lymphatic tissues are connected to the CNS by a recently discovered network of lymphatic vessels in the meningeal spaces. Importantly, intestine enterochromaffin cells, which make a variety of hormones and neurotransmitters, including serotonin, may behave differently as a result of gut microbiota signaling[16].

In our study, the preventive & therapeutic groups of mice and showed a significant reduction in the level of pro-inflammatory cytokines after the treatment of Triphala and the level of short-chain fatty acids (Acetate, Propionate & Butyrate) increased. Due to the production of short-chain fatty acids, the level of serotonin was also found to be increased upon treatment.

It has also been reported that injection of bacterial lipopolysaccharide (LPS) into the brain's fourth ventricle replicates many of the inflammatory and degenerative symptoms seen in Alzheimer's disease. Additionally, compared to healthy individuals, AD patients have plasma concentrations of LPS that are substantially greater[17]. Nucleic acids and E. coli pili protein were two more bacterial products that were discovered in the human brain and were more common in AD patients. The innate immune system's microglial cells, which express TLRs and recognize common injuries or pathogen-associated molecular patterns, are activated by LPS. LPS triggers the TLR4 receptor, activating it and encouraging an inflammatory response through interactions with the CD14 and MD-2 proteins. Additionally, CD14-mediated TLR4 activation drives the inflammatory response to the A41 and S100A8/A9 proteins. TLR2, the second LPS-activated receptor, is similarly activated by bacterial amyloids and A. The idea of molecular mimicry of those particles is supported by these interactions[18]. After treating mice with Triphala, we discovered that the mice's blood serum had a low amount of LPS and that the number of gram-negative bacterial colonies in their guts had decreased.

An important source of amyloids is the gut microbiome. Escherichia coli's curli is the most researched bacterial amyloid. Bacterial cells can build biofilms and withstand being destroyed by outside forces. According to a recent study, people with cognitive impairment and brain amyloidosis may have peripheral inflammation due to the increased abundance of proinflammatory Escherichia/Shigella and decreased abundance of anti-inflammatory *Eubacterium rectale*. The therapeutic potential of Lactobacilli and *Bifidobacteria* is supported by a number of other data from experiments conducted on animals. It has been proposed that probiotics' ability to counteract LPS-induced neuroinflammation and memory deficits may include inhibiting acetylcholinesterase and antioxidant activities. Additionally, in clinical research, supplementing with probiotics based on Lactobacilli and *Bifidobacteria* dramatically increased Mini-Mental State Examination scores in AD patients[19].

In our preventive and therapeutic study, we found the increased abundance of bacteria phyla from 15 to 45% in treated groups TG & WT, showed the effect of Triphala. These phyla have large consortium of the bacteria, produced the several metabolites responsible for the therapeutic activities. The present phylum level of bacteria *Bacteroidetes*, *Proteobacteria*, *Verrucomicrobia* *Actinobacteria*, *Saccharibacteria* and *Fusobacteria* in the treatment group, already reported for the maintain the gut homeostasis condition, reduced the GI inflammation. Some of phyla like *Verrucomicrobia*, *Actinobacteria* and *Bacteroidetes* were reported for reducing the neuroinflammation, increased gut producing serotonin, produced short chain fatty acid and helps in neurogenesis. These bacteria phyla were also reported for the reduced the LPS level via decreased the gram-negative consortium[20]. The symbiotic bacteria produce the short-chain fatty acids or other metabolites which cross the BBB, reduce the level of inflammation through cytokines, and promote neurogenesis in the presence of polyphenols. These polyphenols act as probiotics to enrich the symbiotic bacteria in the gut.

In conclusion this would be the first scientific study on the beneficial effects of Triphala in the murine model of AD. We followed the Ayurvedic concept to deliver the Triphala at both (morning and evening) times to maintain the Prakriti through the homeostasis of the gut. The presence of several polyphenols had synergistic beneficial effect in the modulation of the gut-brain axis. We found that Triphala significantly attenuates the cognitive deficits in the TG mice and increases cognitive function in the WT mice, which could be partly explained by the reshaped microbiome and enhanced SCFA formation in the gut. The gut microbiota-SCFAs-brain axis plays a crucial role in suppressing neuroinflammation, preventing oxidative stress in the CNS, and balancing the HPA axis, which is possibly the fundamental mechanism of the neuroprotection of Triphala treatment.

Abbreviations

AD

Alzheimer's disease

TMAO

Trimethylamine N-oxide

GM

Gut microbiota

SCFA

Short chain Fatty acid

OTUs

Operational Taxonomic Units

PCR

Polymerase chain reaction

CNS

Central nervous system

ENS

Enteric nervous system

CPCSEA

Commission for the Management and Supervision of Animal Experiments

IAEC

Institutional Animal Ethics Committee

HPA

Hypothalamic-Pituitary-Adrenal

LPS

Lipopolysaccharide

DG

Dentate gyrus

GMP

Good Manufacturing Practice

Declarations

Acknowledgments

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Ethics declarations

Ethics approval and consent to participate.

Not applicable.

Consent for publication

Not applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Contributions

PU & SG, Identified the ingredients for the formulation, designed the research, analyzed the final data & wrote the manuscript. AK helped in the gut microbiome study, data analysis, and manuscript review. PU, AT & SA conducted most of the experiments, collecting the animal samples, molecular study, and analysis data. All authors agreed on the final version of the manuscript.

Data availability statement

All data generated or analyzed during this study have been mentioned in the result and its supplementary information files.

Role of the funding source

This work was supported by grants from the Department of Health Research, Ministry of Health and Family Welfare Government of India grant of young scientist fellowship under the human resource development scheme (File no.R.12014/20/2018) to the PU.

Competing interest

PU was employed by the National Institute of Immunology on the fellowship of Young Scientists funded by the Department of Health Research, Ministry of Health, and Family Welfare Government of India. AT & SA was a student at the National Institute of Immunology. SG & AK is employed by the National Institute of Immunology, Department of Biotechnology Govt of India New Delhi, India. The authors declare that the research was conducted without commercial or financial relationships that could not be construed as a potential conflict of interest.

References

1. Upadhyay P, Shukla R, Tiwari KN, Dubey GP, Mishra SK. Neuroprotective effect of *Reinwardtia indica* against scopolamine induced memory-impairment in rat by attenuating oxidative stress. *Metab Brain Dis*. Springer; 2020;35:709–25.
2. Kim MS, Kim Y, Choi H, Kim W, Park S, Lee D, et al. Transfer of a healthy microbiota reduces amyloid and tau pathology in an Alzheimer's disease animal model. *Gut* [Internet]. *Gut*; 2020 [cited 2023 Jan 8];69:283–94. Available from: <https://pubmed.ncbi.nlm.nih.gov/31471351/>
3. Yiannopoulou KG, Papageorgiou SG. Current and Future Treatments in Alzheimer Disease: An Update. *J Cent Nerv Syst Dis* [Internet]. SAGE PublicationsSage UK: London, England; 2020 [cited 2022 Apr 14];12:1179573520907397. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/32165850>
4. Chatterjee B, Pancholi J. Prakriti-based medicine: A step towards personalized medicine. *Ayu*. 2011;32:141–6.
5. Omran Z, Bader A, Porta A, Vandamme T, Anton N, Alehaideb Z, et al. Evaluation of Antimicrobial Activity of Triphala Constituents and Nanoformulation. *Evid Based Complement Alternat Med* [Internet]. Hindawi Limited; 2020 [cited 2023 Jan 8];2020:11. Available from: </pmc/articles/PMC7422007/>
6. Khandelwal M, Manglani K, Upadhyay P, Azad M, Gupta S. AdipoRon induces AMPK activation and ameliorates Alzheimer's like pathologies and associated cognitive impairment in APP/PS1 mice. *Neurobiol Dis* [Internet]. *Neurobiol Dis*; 2022 [cited 2023 Jan 8];174. Available from: <https://pubmed.ncbi.nlm.nih.gov/36162737/>
7. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, et al. Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. *Cell* [Internet]. NIH Public Access; 2016 [cited 2023 Jan 8];167:1469. Available from: </pmc/articles/PMC5718049/>

8. Connell E, Le Gall G, Pontifex MG, Sami S, Cryan JF, Clarke G, et al. Microbial-derived metabolites as a risk factor of age-related cognitive decline and dementia. *Mol Neurodegener* 2022 171 [Internet]. BioMed Central; 2022 [cited 2023 Jan 17];17:1–26. Available from: <https://molecularneurodegeneration.biomedcentral.com/articles/10.1186/s13024-022-00548-6>
9. Wiciński M, Gębalski J, Mazurek E, Podhorecka M, Śniegocki M, Szychta P, et al. The Influence of Polyphenol Compounds on Human Gastrointestinal Tract Microbiota. *Nutrients* [Internet]. Multidisciplinary Digital Publishing Institute (MDPI); 2020 [cited 2023 Jan 17];12. Available from: [/pmc/articles/PMC7071305/](https://pubmed.ncbi.nlm.nih.gov/371305/)
10. Upadhyay P, Kalra D, Gupta S, Mishra SK. Reversal of Metabolic Disorder Through the Restoration of Gut Microbiota BT - Gut Microbiome in Neurological Health and Disorders. In: Tripathi AK, Kotak M, editors. Singapore: Springer Nature Singapore; 2022. p. 179–93. Available from: https://doi.org/10.1007/978-981-19-4530-4_11
11. Endres K. Amyloidogenic peptides in human neuro-degenerative diseases and in microorganisms: A sorrow shared is a sorrow halved? *Molecules*. 2020;25:925.
12. Kashyap P, Ram H, Shukla SD, Kumar S. Scopoletin: Anti-amyloidogenic, Anticholinesterase, and Neuroprotective Potential of a Natural Compound Present in *Argyrea speciosa* Roots by In Vitro and In Silico Study. *Neurosci Insights* [Internet]. SAGE Publications Ltd; 2020 [cited 2021 May 5];15. Available from: [/pmc/articles/PMC7338734/](https://pubmed.ncbi.nlm.nih.gov/37338734/)
13. Lee TH, Ahadullah, Christie BR, Lin K, Siu PM, Zhang L, et al. Chronic Adiponectin Treatment Mimics the Effects of Physical Exercise on Restoring Hippocampal Neuroplasticity in Diabetic Mice. *Mol Neurobiol* [Internet]. Springer; 2021 [cited 2022 Apr 15];58:4666–81. Available from: <https://link.springer.com/article/10.1007/s12035-021-02441-7>
14. Silva YP, Bernardi A, Frozza RL. The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication. *Front Endocrinol (Lausanne)*. Frontiers Media S.A.; 2020;11:25.
15. Sahu N, Upadhyay P, Mishra SK. Role of Short-Chain Fatty Acids from Gut Microbiota in Neuroendocrine Pathogenesis Management BT - Gut Microbiome in Neurological Health and Disorders. In: Tripathi AK, Kotak M, editors. Singapore: Springer Nature Singapore; 2022. p. 139–51. Available from: https://doi.org/10.1007/978-981-19-4530-4_9
16. Ambrosini YM, Borchering D, Kanthasamy A, Kim HJ, Willette AA, Jergens A, et al. The gut-brain axis in neurodegenerative diseases and relevance of the canine model: A review. *Front. Aging Neurosci*. 2019.
17. Lee FHF, Zhang H, Jiang A, Zai CC, Liu F. Specific Alterations in Astrocyte Properties via the GluA2-GAPDH Complex Associated with Multiple Sclerosis. *Sci Reports* 2018 81 [Internet]. Nature Publishing Group; 2018 [cited 2022 Apr 15];8:1–17. Available from: <https://www.nature.com/articles/s41598-018-31318-4>
18. Semar S, Klotz M, Letiembre M, Van Ginneken C, Braun A, Jost V, et al. Changes of the enteric nervous system in amyloid- β protein precursor transgenic mice correlate with disease progression. *J Alzheimer's Dis*. IOS Press; 2013;36:7–20.
19. Wu S, Liu X, Jiang R, Yan X, Ling Z. Roles and Mechanisms of Gut Microbiota in Patients With Alzheimer's Disease. *Front Aging Neurosci* [Internet]. Frontiers Media SA; 2021 [cited 2023 Jan 8];13. Available from: [/pmc/articles/PMC8193064/](https://pubmed.ncbi.nlm.nih.gov/38193064/)
20. Xiao Q, Qu Z, Zhao Y, Yang L, Gao P. Orientin Ameliorates LPS-Induced Inflammatory Responses through the Inhibition of the NF- κ B Pathway and NLRP3 Inflammasome. *Evidence-based Complement Altern Med*. Hindawi Limited; 2017;2017.

Tables

Table 1 is available in the Supplementary Files section.

Table 2: Dose regimen and Treatment chart of the study

Formulation	Transgenic (3XFAD)	Transgenic (5XFAD mice)	Aged Wild type (C57BL/6)	Young Wild type (C57BL/6 mice)	Dose, Dosage Route & Regimen	Time of Treatment	Treatment
PBS	A-1	V-1	WA-1	WV-1	0.5 ml, Oral, OD Room temp	Morning	60 Days
T Extract	A-2	V-2	WA-2	WV-2	250 mg/kg, Oral, OD Room temp	Morning	60 Days
T Powder	A-3	V-3	WA-3	WV-3	500 mg/kg, Oral, OD, Room temp	Morning	60 Days
T Powder	A-4	V-4	WA-4	WV-4	500 mg/kg, Oral, OD, Luke warm	Night	60 Days
T Powder	A-5	V-5	WA-5	WV-5	500 mg/kg, Oral, BD, Luke warm	Morning & Night	60 Days

Table 3: Primer list

S.No.	Marker	Forward	Reverse	Product length (bp)	NCBI Reference Sequence
1	APP	TCTCTGTCCCTGCTCTACAA	CAAGACATCGTCGGAGTAGTTC	102	NM_001198826.1
2	AchE	CTGTGGGCTCACGTAGATTTAT	TACAGGGTGTCCACGTACT	109	NM_001290010.1
3	TLR 4	GAGCAAACAGCAGAGGAAGA	CCAGGTGAGCTGTAGCATTTA	111	XM_036163964.1
4	BACE	ACCATCCTTCCTCAGCAATAC	ATGATGACGGCTCCCATAAC	119	NM_001410457.1
5	IFN- γ	CCTGTACCGACGAATGTTCTAA	AGGAACCCGAATACACCTTTAC	121	NM_010511.3
6	TNF- α	TTGTCTACTCCCAGGTTCTCT	GAGGTTGACTTTCTCCTGGTATG	107	NM_001278601.1
7	GAPDH	AACAGCAACTCCCCTCTTC	CCTGTTGCTGTAGCCGTATT	111	NM_001411843.1

Figures

Treatment Plan

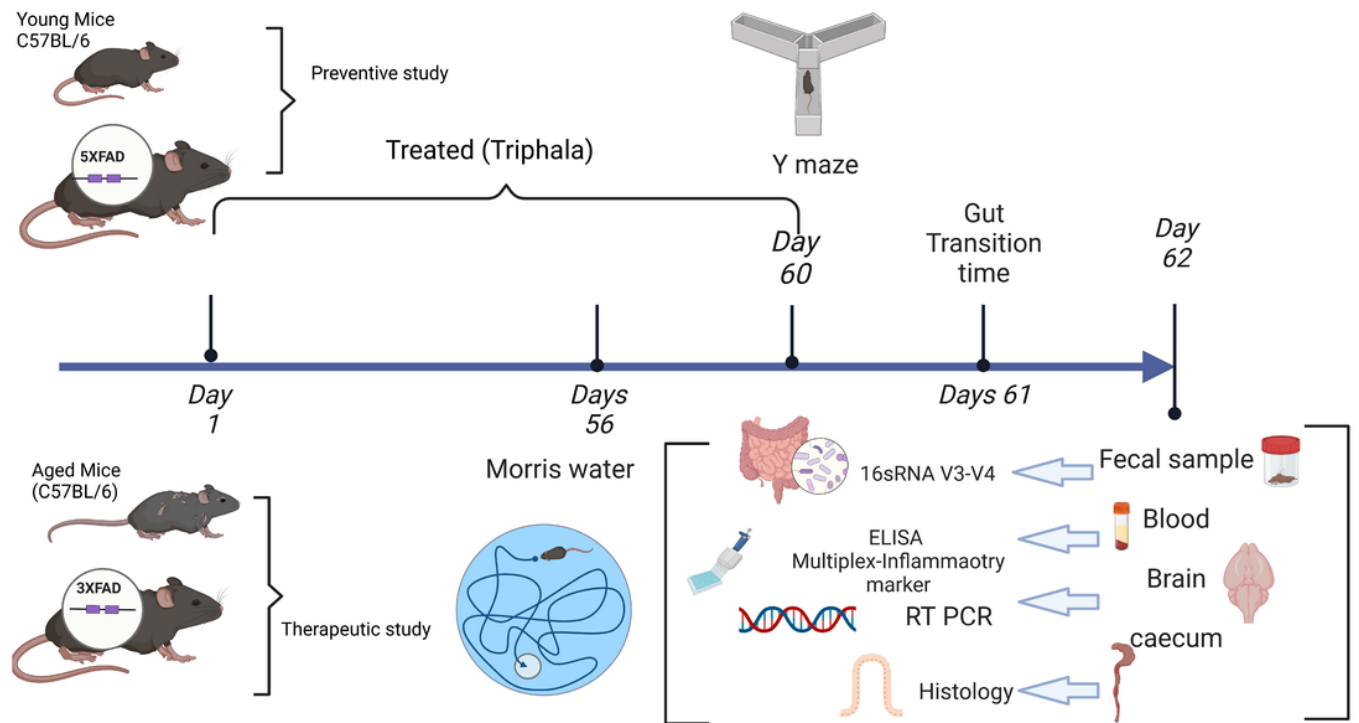


Figure 1

Illustrated the treatment plan summary of the study

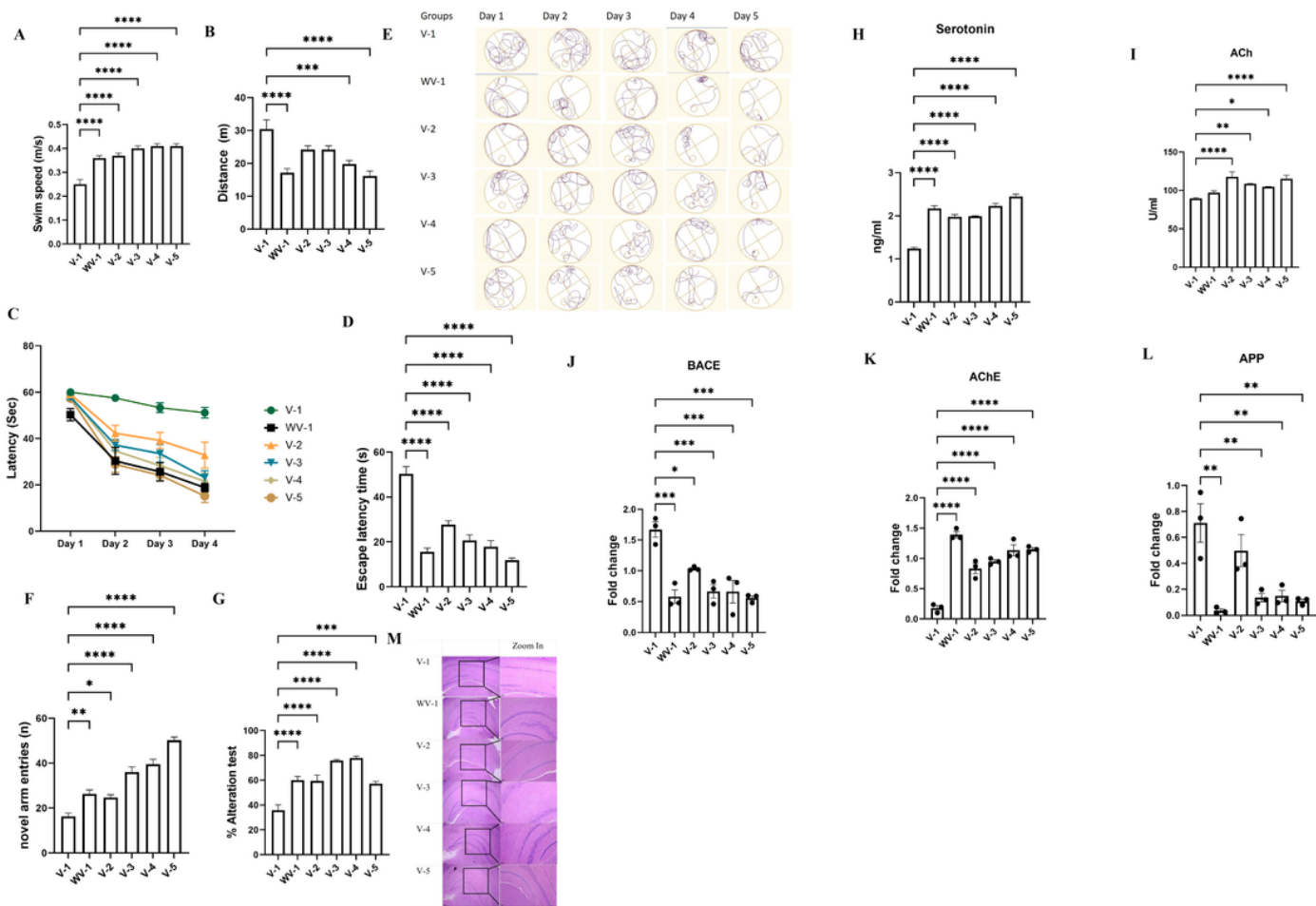


Figure 2

The preventive study of Triphala on 5xFAD mice on behavioral changes in AD. Morris water Maze (n=18) (A) Swim Speed (B) Distance travelled by the mice (C) Escape latency time during Probe trial (D) Escape latency time on Day 5 (E) Track image recorded through Any maze software Y maze (12) (F) Novel arm entries (number) (G) Percent alteration test for exploratory behavior (H) Serotonin level measured in the serum (n=8) (I) Acetylcholine (ACh) level measure in the serum (n=8). mRNA level in brain lysate of mice to see the fold change (n=3) (J) Beta secretase (BACE) (K) Acetylcholinesterase (AChE) (ML Amyloid precursor Protein (APP) (M) H& E stain of mice brain sections (n=3), Hippocampus area, 4x magnification, 100µm scale bar, Zoom area (10x) Dentate gyrus, CA1, CA2

Statistical analysis included the one- or two-way analysis of variance and Bonferroni's post hoc test. #P < 0.05 and ##P < 0.01 versus Control and *P < 0.05 versus V1 (TG control).

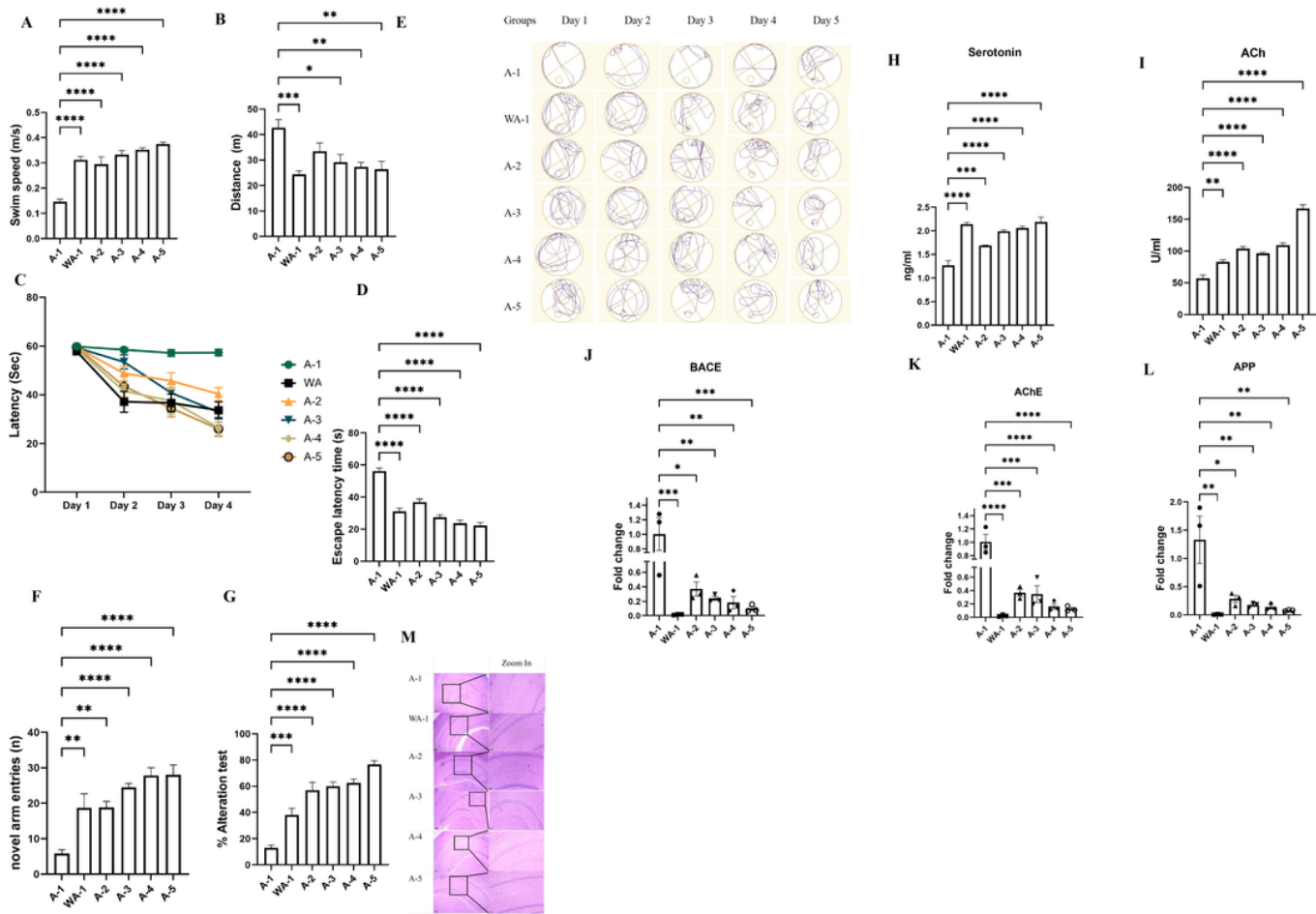


Figure 3

The therapeutic study of Triphala on 3xFAD mice on behavioral changes in AD. Morris water Maze (n=18) (A) Swim Speed (B) Distance travelled by the mice (C) Escape latency time during Probe trial (D) Escape latency time on Day 5 (E) Track image recoded through Any maze software Y maze (12) (F) Novel arm entries (number) (G) Percent alteration test for exploratory behavior (H) Serotonin level measured in the serum (n=8) (I) Acetylcholine (ACh) level measure in the serum (n=8). mRNA level in brain lysate of mice to see the fold change (n=3) (J) Beta secretase (BACE) (K) Acetylcholinesterase (AchE) (ML Amyloid precursor Protein (APP) (M) H& E stain of mice brain sections (n=3), Hippocampus area, 4x magnification, 100µm scale bar, Zoom area (10x) Dentate gyrus, CA1, CA2

Statistical analysis included the one- or two-way analysis of variance and Bonferroni's post hoc test. #P < 0.05 and ##P < 0.01 versus Control and *P < 0.05 versus V1 (TG control).

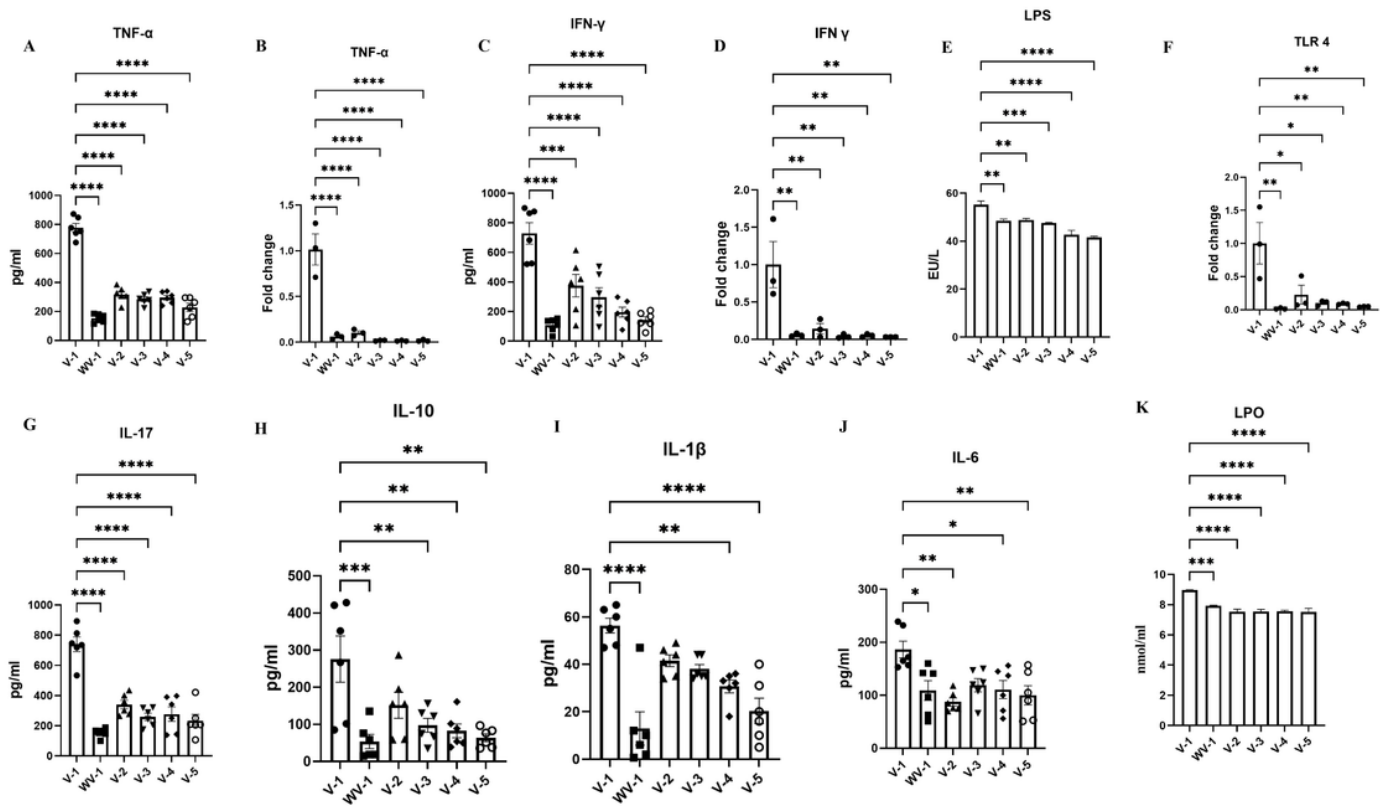


Figure 4

The Anti-inflammatory and antioxidant effect of Triphala on 5xFAD mice in preventive study. Anti-inflammatory activity (A) TNF- α level in blood serum of mice (B) TNF- α mRNA expression in brain lysate (C) IFN γ level in blood serum (D) IFN γ mRNA expression in brain lysate (E) Lipopolysaccharide level in blood serum (F) Toll like receptor 4 (TLR4) mRNA level in brain lysate of mice (n=3). Interleukins level in blood serum (G) IL-17 (H) IL-10 (I) IL-1 β (J) IL-6 (n=6) (K) Lipid peroxidation level (LPO) in blood serum

Statistical analysis included the one- or two-way analysis of variance and Bonferroni's post hoc test. #P < 0.05 and ##P < 0.01 versus Control and *P < 0.05 versus V1 (TG control).

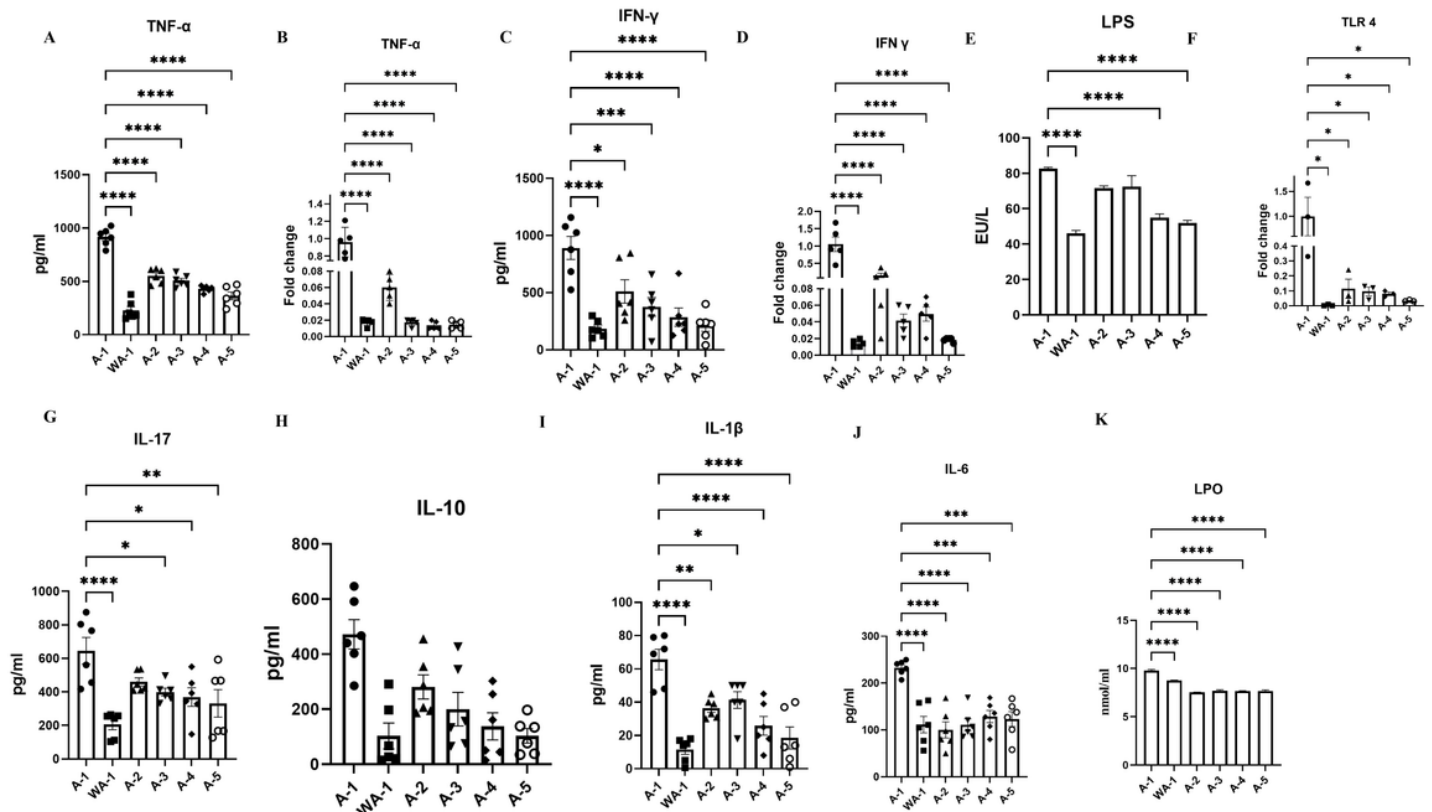


Figure 5

The Anti-inflammatory and antioxidant effect of Triphala on 3xFAD mice in therapeutic study. Anti-inflammatory activity ((A)TNF-α level in blood serum of mice (B) TNF-α mRNA expression in brain lysate (C) IFN γ level in blood serum (D) IFN γ mRNA expression in brain lysate (E) Lipopolysaccharide level in blood serum (F) Toll like receptor 4(TLR4) mRNA level in brain lysate of mice (n=3) . Interleukins level in blood serum (G) IL-17 (H) IL-10 (I) IL-1β (J) IL-6 (n=6) (K) Lipid peroxidation level (LPO) in blood serum

Statistical analysis included the one- or two-way analysis of variance and Bonferroni's post hoc test. #P < 0.05 and ##P < 0.01 versus Control and *P < 0.05 versus V1 (TG control).

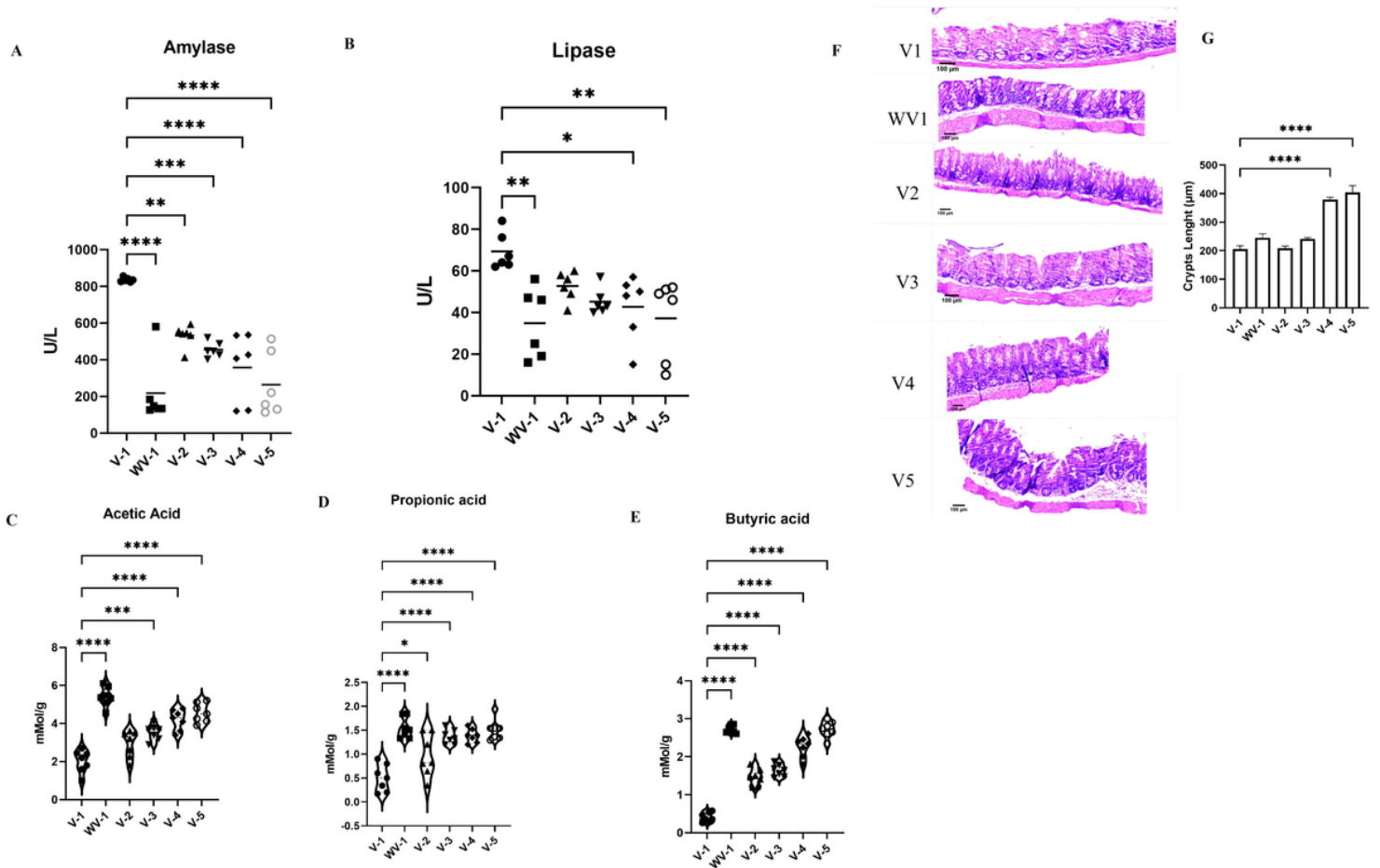


Figure 6

The effect of Triphala on 5xFAD mice on gut in preventive study. (A) Amylase level (B) Lipase level. Short chain fatty acid measured from feces of mice after 60 days treatment (C) Acetic acid (D) Propionic acid (E) Butyric acid The mice sample used for the above analysis n=6.

(F) Hematoxylin & Eosin staining of the mice caecum, at 10x, 100 µm scale bar used (n=3)

(G) Measured the crypt length from Image J software

The experiment has been conducted in two set of experiment.

Statistical analysis included the one- or two-way analysis of variance and Bonferroni's post hoc test. #P < 0.05 and ##P < 0.01 versus Control and *P < 0.05 versus V1 (TG control).

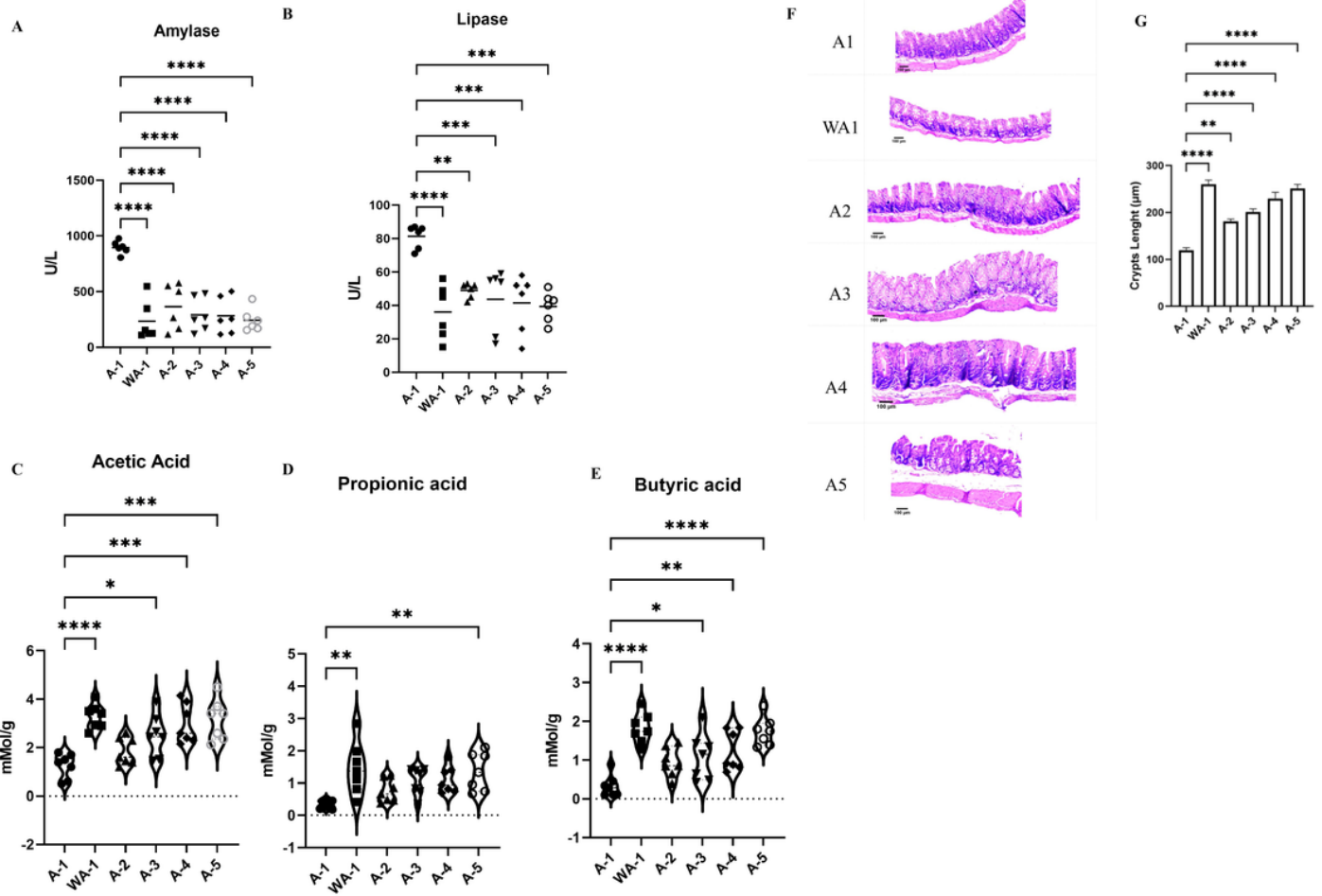


Figure 7

The effect of Triphala on 3xFAD mice on gut in therapeutic study. (A) Amylase level (B) Lipase level. Short chain fatty acid measured from feces of mice after 60 days treatment (C) Acetic acid (D) Propionic acid (E) Butyric acid The mice sample used for the above analysis n=6.

(F) Hematoxylin & Eosin staining of the mice caecum, at 10x, 100 µm scale bar used (n=3)

(G) Measured the crypt length from Image J software

The experiment has been conducted in two set of experiment.

Statistical analysis included the one- or two-way analysis of variance and Bonferroni's post hoc test. #P < 0.05 and ##P < 0.01 versus Control and *P < 0.05 versus V1 (TG control).

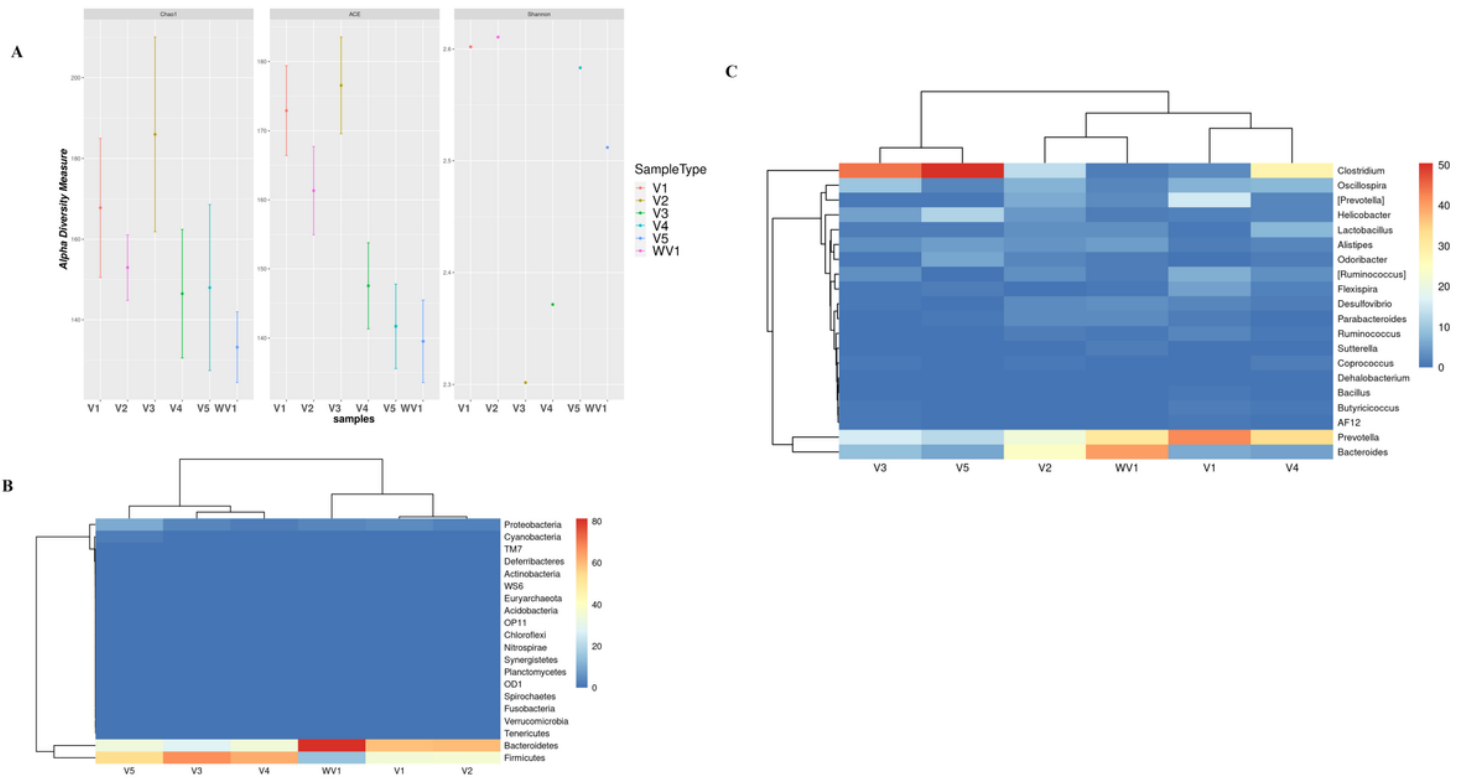


Figure 8

Effect of Triphala on modulation of the gut microbiota composition in 5XFAD Tg mice. (A) Effect on the number of sequences analyzed, operational taxonomic units (OTUs), abundance-based coverage estimator (ACE), Chao1, and Shannon. Effect on the fecal microbiota composition: (B) phylum (C) Genus

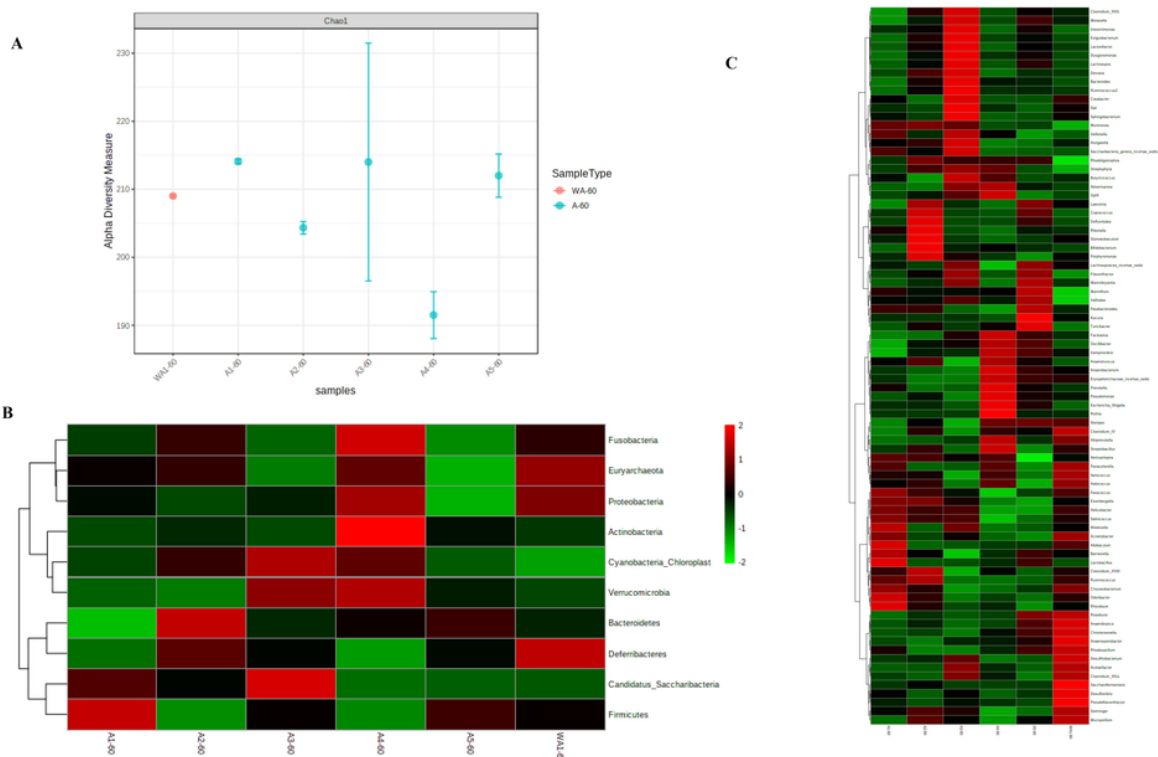


Figure 9

Effect of Triphala on modulation of the gut microbiota composition in 3XFAD Tg mice. (A) Effect on the number of sequences analyzed, operational taxonomic units (OTUs), abundance-based coverage estimator Chao1. Effect on the fecal microbiota composition: (B) phylum (C), Genus

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarydata.pdf](#)
- [Table1.docx](#)