

Probiotics *Lactobacillus plantarum* and *Bifidobacterium* B94: cognitive function in demyelinated model

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Abstract

Background: Multiple Sclerosis (MS) is a disease of the immune system that creates damage of Learning and memory in that. Using probiotic supplements is recommended for preventing MS disease and improving memory. This study aimed to investigate the effect of *Lactobacillus Plantarum* (LP) and *bifidobacterium* B94 (BB94), on acquisition phase of spatial memory in the local demyelination of rats' hippocampus.

Methods: In this study, 32 male Wistar rats were divided into control, damage group and treatment groups. Treatment groups were including (LP) and (BB94). After the induction of demyelination by 3 µl of EB into the right dentate gyrus of the hippocampus in treatment groups, 1.5×10^8 probiotic bacteria were administered by gavage for 28 days. Data was analyzed using one-way ANOVA and Tukey post-hoc tests ($p \leq 0.05$).

Results: Findings demonstrated that injection of EB caused a significant increase in traveled distance ($p < 0.01$) and also escape latency ($p < 0.05$) compared with control group. Also, effect administrations of (LP) and (BB94) on traveled distance and escape latency were reviewed, and it was determined that administration of them do not cause significant reduction in the traveled distance compared with the lesion group. Also mentioned probiotics has no significant effect on swimming speed compared with lesion and saline groups.

Conclusion: According to some studies, probiotics have a positive impact on improving the performance of spatial memory and learning, although the results of the current study could not indicate finality of this assumption. It seems that more researches is needed on this subject.

Keywords: probiotic, demyelination, spatial memory, hippocampus.

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Introduction

Multiple Sclerosis (MS) is an inflammatory disease of the immune system that causes damage to the central nervous system (CNS) myelinated axons (1). Demyelination caused by chemicals such as direct injection of ethidium bromide (EB) is a simple and repeatable processes that can be investigated demyelination, remyelination and repair nerve. Hippocampus can be effective in the activity of the learning, memory, and sensory-motor activity. Hence, many studies examine the factors

that associated with the hippocampus function (2,3). Learning and memory are the basic processes in the brain that acquisition, consolidation and readout (retrieve) of information, are the various stages of them (4). Learning is the change in behavior as a result of the acquisition of new experiences and memory is the ability of readout of learned experiences (5-8).

Using probiotic supplements alone in combination with vitamins and mineral material is recommended for preventing MS disease. Nutrients have a significant role in

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the prevention and treatment of MS. Probiotics, as a live and non-pathogenic microorganism's nutrient, there are in certain foods. Treatment aspect of them had been reported in certain diseases. Some of these microorganisms are selected bacterial strains, *Lactobacillus*, and *bifidobacterium* (9-13). There are little findings probiotic bacteria effects on the central nervous system (14). Given that probiotics can be effective in reducing the damage from inflammation in MS as an inflammatory disease, so with regard to learning and memory impairment in MS patients and the fact that probiotics can play an important role in improving memory, probiotics can be useful as a treatment of choice (15). Some of the studies have verified the role of probiotics in improving spatial memory, and some of them have not confirmed that.

This study aimed to investigate the effect or lack of effect of probiotics, *Lactobacillus Plantarum* and *bifidobacterium* B94, on acquisition phase of spatial memory in the local demyelination of rats' hippocampus.

Methods

Animals

In this experimental study, experiments were performed on 32 adult Wistar male rats weighing 200-250g (8-10 weeks aged). Food and water were available for animals in sufficient quantities.

The place that animals were kept in was a room with the dark-light cycle of 12 hours, and the temperature was $23 \pm 2^\circ\text{C}$. The animals were randomly divided into four groups, and there were eight rats in each group.

Glutotoxin- induced demyelination

Animals were anesthetized by intraperitoneal (LP) injection of chloral hydrate (80 mg/kg) after weighing. Then, the animals were placed in a stereotaxic apparatus. The coordination of injection site ($\text{AP} = -2.8$; $\text{ML} = +1.8$; $\text{DV} = +2.5$) was determined.

Demyelination was induced by direct injection of $1\mu\text{l}/\text{min}$ of 0.01% EB in sterile 0.9% saline at the rate of $1\mu\text{l}/\text{min}$ into the

right dentate gyrus of the hippocampus. In the saline group, an equal volume of normal saline was injected.

Preparation of Probiotics

Lactobacillus plantarum and *Bifidobacterium* B94 strains of (industrial enzymes company, representative of DSM Company) were selected for testing. The bacteria were cultured and after confirm the purity of bacteria were grown anaerobically in MRS broth at 37°C for 48 h in a volume of 500 ml. After 48 hours, the bacteria were isolated from growth media by centrifuging and then washed with PBS and centrifuged again for precipitation of bacteria. The bacteria were dissolved in normal saline. Using McFarland standard, bacteria were diluted to a number of $1.5 \times 10^8 \text{cfu}/\text{ml}$.

The day before the start of the experiment, the bacteria were prepared for a period of seven days which were appropriate for oral consumption of twenty samples. Seven-day preparation protocol was repeated for four times.

Behavioral testing

Morris water maze task (MWM): MWM was round and black tank with 136cm diameter and 60cm height, which was filled with water $20 \pm 1^\circ\text{C}$ to 25cm high. Maze was located in a room around the outside of symptoms of the maze (e.g. time, windows, posters, shelf, and light) that geographically are divided hypothetically into four quadrants of a circle with equal distances.

A round Plexiglas platform is placed in the center of a quarter of a circle with diameter of 10 cm and one cm below the water surface. An infrared light emitting diode and a video camera was mounted at the top of the pond to detect infrared light. This signal is then transmitted to the computer and was analyzed by the software system.

Animal training: In hidden platform test, animals for four days and a block per day, each block 4 times, was trained. Animals in each block four times and only once of each direction (North, South, East, and

West) that is determined randomly by computer, were released. Each time, 90 seconds was given to rats in order to find the platform. Otherwise, they would be guided to the platform and in any case, the animal was allowed to remain on the platform and investigate surrounding area for 30 seconds.

Evaluation of health of sensory-motor system: On the fifth day of the experiment, the platform was covered with aluminum paper and approximately one centimeter above the water level, in order to be fully visible. By this test, called the visible platform, if the animal was able to find the platform, health of visual-motor animal was confirmed. Every day after the end of each experiment, the animals was dried and transferred to a cage. Parameters of the distance traveled by the animals to reach the podium and the escape latency reaching the podium are indicators of spatial learning and memory. The swimming speed is also an indicator of the health of the animal's sensory-motor system.

The parameters of the distance traveled and the escape latency by rats reaching the

platform are indicators of spatial learning and memory. Swimming speed is also an indicator of the health of the animal's sensory-motor system. These indicators, after animal exposure to EB and treated with probiotics were studied and the following results were obtained.

Statistical analysis

In this study, Data was analyzed using one-way ANOVA and Tukey post-hoc tests ($p < 0.05$). Results have presented as mean+SEM.

Results

Gliotoxin EB caused substantial demyelination

Demyelinating damage due to EB (as a model of demyelination) on traveled distance, escape latency and swimming speed, 28 days after injury on the rat's hippocampus was investigated. It was found that injection of EB caused a significant increase in traveled distance ($p < 0.01$) and also escape latency ($p < 0.05$) compared with saline group (Figs. 1 and 2; respectively).

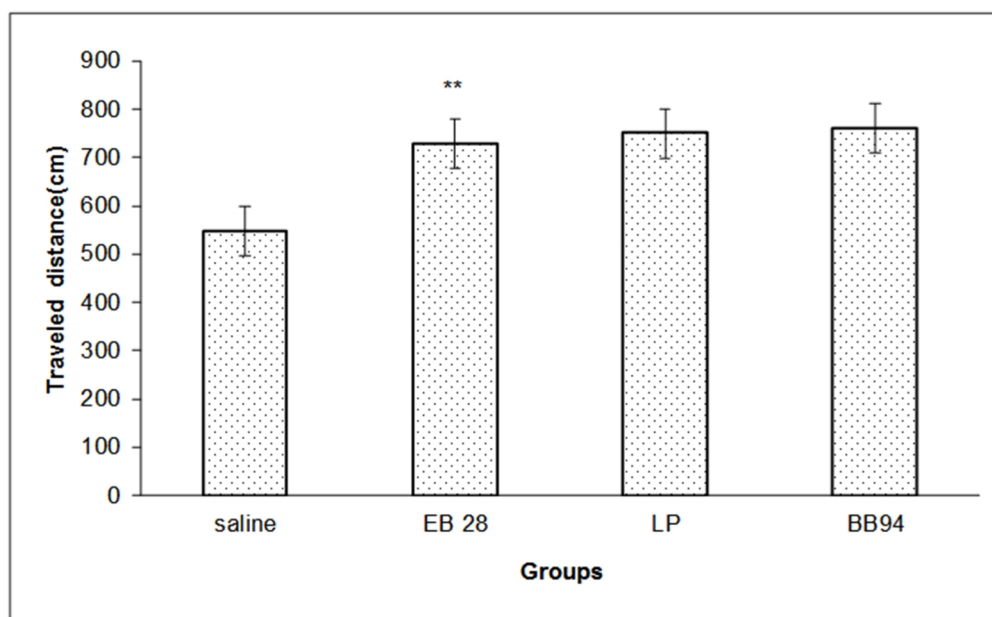


Fig. 1. Effect of ethidium bromide (EB), Lactobacillus Plantarum (LP) and Bifidobacterium B94 (BB94) on the distance traveled by the animal, 28 days after injection of the toxin. **: $p \leq 0.01$ compared with saline group. Data were presented as mean+SEM. N=8 in each group.

Probiotics Lactobacillus plantarum and Bifidobacterium B94 roles on acquisition phase of memory

Effect of 28 days administrations of *Lactobacillus plantarum* (LP) and *Bifidobacterium* B94 (BB94) on traveled distance was reviewed, and it was determined that administration of them for 28 days do not cause a significant reduction in the traveled distance compared with the lesion group

(EB group) (Fig. 1).

Effect of 28 days probiotic *Lactobacillus plantarum* (LP) and *Bifidobacterium* B94 (BB94) administrations on the escape latency was measured, and it was observed that administration of them caused no significant reduction in escape latency compared with the lesion group (Fig. 2).

Administration of the mentioned probiotics has no significant effect on swimming

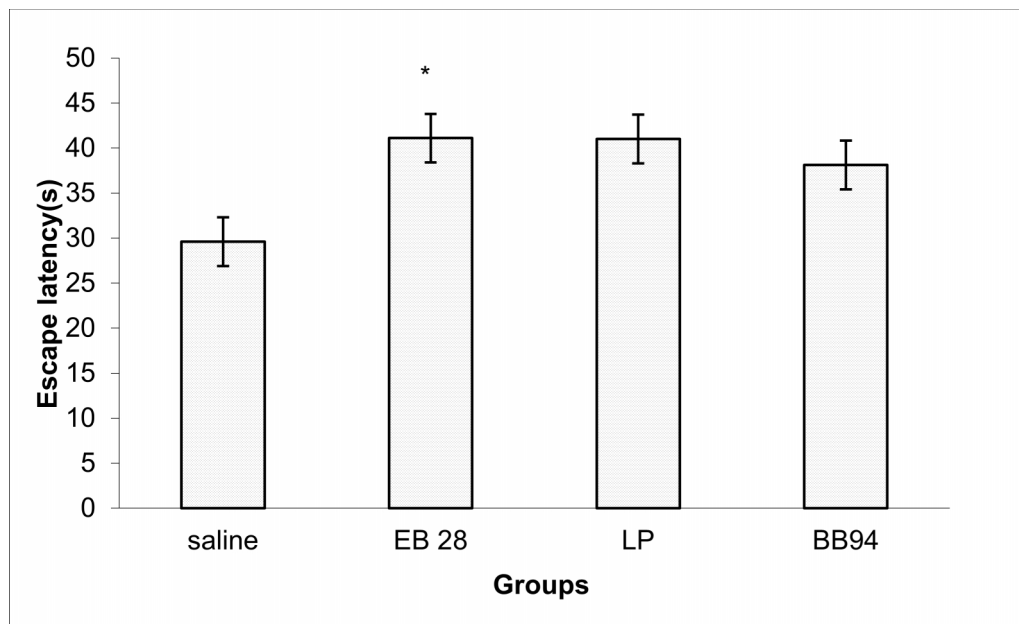


Fig. 2. Effect of ethidium bromide (EB), *Lactobacillus Plantarum* (LP) and *Bifidobacterium* B94 (BB94) on the escape latency to find the platform by the animal, 28 days after injection of the toxin. *: $p \leq 0.05$ compared with saline group. Data were presented as mean+SEM. N=8 in each group

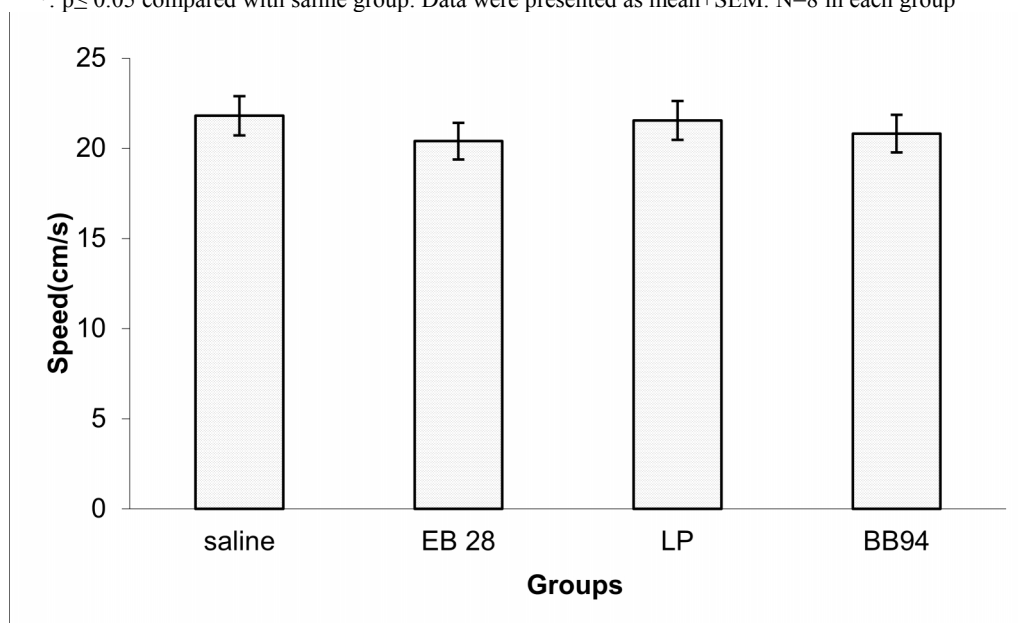


Fig. 3. Effect of ethidium bromide (EB), *Lactobacillus Plantarum* (LP) and *Bifidobacterium* B94 (BB94) on the swimming speed by the animal 28 days after injection of the toxin. Data were presented as mean+SEM. N=8 in each group.

speed compared with lesion and saline groups (Fig. 3).

Discussion

CNS regeneration in mammals due to reasons such as the presence of inhibitory molecules and low potential damaged cells will fail. The CNS will be able to rebuild, under appropriate conditions. Demyelination is a pathological event in the nervous system that may be followed by a spontaneous remyelination. Some of the studies have shown that the gray hippocampus tissue is demyelinated in MS patients. Also, 25 to 60 percent cognitive impairment in these patients was reported (16). In addition, according to some studies on the beneficial effect of probiotics, we made the decision to work on *Lactobacillus plantarum* and *Bifidobacterium* B 94 on the learning and memory improvement following by EB-induced demyelination in the hippocampus. In this study, the amount of traveled distance and escape latency to find the platform was measured as indexes of spatial learning and memory. As well, the speed of swimming of the animals, as an indicator of the health of the sensory-motor, was studied.

28 days after EB injection, the distance traveled and the escape latency by animals compared with the control group (saline) showed a significant increase ($p \leq 0.01$ and $p \leq 0.05$; respectively), however, the speed of swimming of the animals did not change.

It has been found that EB injection into the hippocampus can cause localized gray matter demyelination (17). The study that was conducted in 2011 also showed that the use of EB cause increased oxidative stress or reduced antioxidant capacity in brain tissues and the hippocampus (18). Therefore, EB as a cause of oxidative stress can result in the death of brain cells and impairment of the memory. Several studies have shown the effects of oxidative agents in the form of damage or memory impairment (19) and our results confirming spatial memory decline subsequent EB insult.

According to other studies, the most like-

ly reason for this result was apoptotic pathway activation by EB injection and hippocampal insult that caused a gradual increase in distance traveled by animals (the findings were published) (17).

Probiotics reduce the risk of diseases that caused after consumption of some food, by maintaining normal intestinal micro-flora and control of pathogenic microorganisms. Indirect findings of the relationship between the normal microbial flora and the central nervous system have been published. Effects of probiotic bacteria on brain neurotransmitters and reduced stress-induced changes have been shown. Also, some studies have pointed to the lack of effect of probiotics on the nervous system (20-23).

So, the present study investigated the therapeutic effects of probiotics on acquisition phase of spatial memory.

Probiotic *Lactobacillus plantarum* (LP) and *Bifidobacterium* B94 (BB94) for 28 days administrations did not significantly change distance traveled by animals and escape latency to find out the hidden platform compared with EB induced-group (Figs. 1 and 2). The swimming speed of animals receiving probiotics compared with the lesion group does not show significant change; that is a reason for the health of the sensory - motor system in animals of study groups.

Our findings are in agreement with other studies. However, others state contradictory. Male rats receiving bacteria *Bacillus subtilis* (Iran native probiotic) did not show any significant decrease in their escape latency in dark room (23). Some studies mentioned the role of probiotics like *Lactobacillus* in the spatial memory through reducing the escape latency in the group that received probiotic. Probiotics through secretory immunoglobulin A and increased production of cytokines stimulate the immune system. Laboratory and clinical studies show that GABA receptors can affect learning and memory while in one study, *Lactobacillus* has not any significant effect on GABA neurotransmission. Also, it has been

said that chronic treatment with *L. rhamnosus* in relation to GABA is region-dependent so that GABA_{B1b} mRNA expression reduces in the hippocampus but increases in the cortical regions of brain (21,24).

As well, the results of other researches explain that findings of probiotic studies can be dose-dependent. The dose and type of probiotics and also duration of treatment and combination of probiotics may be effective and more studies are recommended (25).

Conclusion

This study demonstrated the negative impact of EB as a catalyst for negative oxidative performance on learning and memory. According to some studies, the evidence suggests that probiotics have a positive impact on improving the performance of spatial memory and learning. Although, the results of this study could not indicate finality of this assumption. Given the scarcity of studies on the effects of probiotics on spatial memory, in the future, more researches are recommended with a different number of bacteria and duration of treatment or other strains of probiotics.

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