



Effect of Probiotic Mixture on the Gut-Brain Axis and Immune Response in Male Wistar Rats Infected with *Salmonella typhimurium*

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Abstract

BACKGROUND: *Salmonella typhimurium* is one of the most important causative agents of gastrointestinal diseases in humans and animals. Probiotics can inhibit pathogens by producing antimicrobial compounds.

OBJECTIVES: The aim of this study was to investigate the effect of a probiotic mixture (*Lactobacillus plantarum*, *Lactobacillus paracasei*, and *Lactobacillus brevis*) on the gut-brain axis of male Sprague-Dawley rats infected with *S. typhimurium*.

METHODS: In this study, 21 adult male Wistar rats with an average weight of 220-250 g were randomly divided into three groups: the control group receiving daily water and food, the second group (infected) receiving *S. typhimurium* (10^9 CFU/ml), and the third group (treatment) receiving the probiotic mixture along with *S. typhimurium* infection.

RESULTS: The findings showed a significant increase in cortisol levels and some inflammatory factors in the infected group, while a significant decrease in these factors was observed in the treatment group. Additionally, serotonin levels increased in the treatment group. The results indicated that serotonin and dopamine levels increased, while cortisol concentration decreased in the treatment group compared to the infected group ($P < 0.05$). Moreover, significant changes were observed in the levels of malondialdehyde, total antioxidant capacity, and the expression of IL6, TNF α , NLRP genes, and inducible damages in brain and gut tissues in the treatment group compared to the infected group ($P < 0.05$).

CONCLUSIONS: These findings suggest that probiotics can have significant positive effects on reducing inflammation and improving immune response against *S. typhimurium* infection. Finally, this study demonstrates that the probiotic mixture could be an effective complementary treatment for reducing bacterial infection-related complications.

Keywords: Complementary treatment, Immune response, Inflammation, Probiotic, *Salmonella typhimurium*

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Figure Legends and Table Captions

Figure 1. From left to right: EMB culture medium, XLD culture medium, TSI biochemical test, and Simons Citrate biochemical test.

Figure 2. Comparison of different brain tissue sections in experimental groups: (A) Control, (B) Infected - infected with *Salmonella typhimurium*, (C) Treatment group - infected with *Salmonella* + probiotic mixture, stained with hematoxylin-eosin at 400x magnification.

Figure 3. Comparison of different intestinal tissue sections in experimental groups: (A) Control group, (B) Infected group (infected with *Salmonella typhimurium*), and (C) Treatment group with probiotic mixture, stained with hematoxylin-eosin at 400x magnification.

Figure 4. Comparison of cortisol (A), serotonin (B), and dopamine (C) levels in different groups of male rats. Results are presented as mean \pm standard deviation.

Figure 5. Serotonin levels in different groups of male rats. Results are presented as mean \pm standard deviation. ** $P < 0.01$: compared to the control group, ### $P < 0.001$: compared to the infected group. ST: *Salmonella typhimurium*.

Figure 6. Comparison of total antioxidant capacity (TCA) values and malondialdehyde (MDA) levels in the brain and intestine of experimental groups of male rats. Results are presented as mean \pm standard deviation.

Figure 7. Comparison of TNF α , IL-6, and NLRP gene expression in the brain (A & C) and intestinal (B & D) tissues in different groups of male rats. Results are presented as mean \pm standard deviation.