



SUMMARY

This research was conducted to evaluate the immunomodulatory effects of both commercial and laboratory-isolated probiotics on Wistar rats, highlighting the role of probiotics in enhancing host immunity and gut health. Despite their widespread application, there is limited comparative research on the effectiveness of single versus combined probiotic strains, especially regarding their immune-boosting potential and gut colonization ability. About 50 probiotic samples were collected from dairy and non-dairy sources in Lahore. Approximately 40% of the probiotics from 20 samples were examined through microscopic, biochemical, and molecular tests. Molecular identification was carried out using 16S rRNA gene sequencing and visualized with PCR and agarose gel electrophoresis, employing a DNA ladder. MEGA X was utilized to explore species relationships.

A total of 5 trials were conducted, each lasting 35 days. To assess the impact of isolated probiotics on the immune system, male and female Wistar rats (n=3) were randomly grouped; including laboratory-isolated strains such as *Lactobacillus acidophilus*-14; Pro-1 (*Lactobacillus plantarum* MZ707748.1), Pro-2 (*L. plantarum* MZ729681.1), Pro-3 (*Weissella confusa* MZ735961.1), Pro-4 (*L. plantarum* MZ727611.1), Pro-5 (*Pediococcus pentosaceus* OP839483.1), Pro-6 (*Enterococcus lactis* OP831182.1), Pro-7 (*Pediococcus acidilactici* OP839485.1), and Pro-8 (*Lactocaseibacillus rhamnosus* OP831157.1). Different synergistic groups were allocated as G1, G2, G3, and G4 in the third trial, and G5, G6, G7, and G8 in the fifth trial. To evaluate the immune response, bacterial colonization, organ health, hemotological parameters (CBC and serum), fecal analysis, and histopathological examinations were performed. These assessments were more comprehensive than those conducted on control groups. Bacterial strains were confirmed through fecal isolation. Data analysis was performed using one-way ANOVA (Tukey's test), with a p-value of less than 0.05 considered statistically significant. Histopathological examinations showed no damage to the liver or thymus. Probiotic viability and colonization were confirmed by re-isolating bacteria from fecal samples. This study provides valuable evidence that probiotics can enhance intestinal health and immune function, particularly when consumed together. This supports the use of probiotics as safe and effective supplements in managing host health.



This study aimed to evaluate the histological impact of probiotics on intestinal morphology in Wistar rats, highlighting their potential to improve gut health and mucosal immunity. This research evaluated the isolated versus combined probiotic strains influence on structural changes in the gut. To investigate this, histopathological analysis was performed on the intestines of probiotic-treated and control rats. Parameters such as villus height and width, crypt height and width, and the thickness of the mucosa and submucosa in the jejunum were measured. In the medial colon, the mucosa tunic, muscle tunic, total wall thickness, and crypt depth were also examined. The results showed that these structural features were significantly improved in probiotic-treated groups compared to both 0-day and negative control groups. Additionally, fecal analysis confirmed the presence of the administered *Lactobacillus* strains, indicating successful colonization. These findings suggest that probiotics not only enhance intestinal morphology but also contribute to better mucosal immune function. Importantly, the combined use of multiple probiotic strains produced more pronounced benefits than individual strains, emphasizing the synergistic potential of multi-strain probiotic formulations in promoting gut health. This research provides valuable evidence for the role of probiotics in structural and functional enhancement of the gastrointestinal system.