



Immunomodulatory and Microbial Effect of *Lactobacillus Casei* Against *Escherichia coli* Infection in Wistar Albino Rats.



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ABSTRACT

Microorganisms such as *Lactobacillus casei* has been extensively studied for its probiotic effects. This bacterium produces lactic acid, antimicrobial peptides, such as bacteriocins, which inhibit the colonization and growth of pathogenic bacteria such as *Escherichia coli*. Twenty (20) Wistar albino rats which were used and shared into five groups: The Control, infected (*Escherichia coli*-infected), Probiotic (*Escherichia coli*-treated), Prophylactic (exposed to *Lactobacillus casei*, next infected with *Escherichia coli*), Antibiotic (ciprofloxacin-treated). At 0, 7, 14 and 21 days, body temperature, weight and faecal samples were collected. While at day 21 (the end of the experiment), blood samples and organs (liver and colon) were collected for hematological analysis and microbial analysis of organ samples respectively. All the groups had temperature range from 36 - 36.3 °C at day 0. At day 14 and 21, all the groups had an increase in temperature, though, there was no statistical difference among the groups with respect to temperature ($p > 0.05$). At day zero (0), the body weight of the experimental animals was within the ranged 150.7 - 191.4 g. The experimental animals in group B had a decline in body weight from 191.4 g at day 0 to 170 g at day 21. While group C wistar rats had an average body weight of 150.7 g at day 0, 169 g at day 7, 156 g at day 14 and a sharp body weight increase of 198 g at day 21 (the end of the experiment). The White blood cells (WBC) range from 8.8 ± 0.7 - 14.7 ± 4.6 $10^3/\mu\text{L}$ and there was statistical difference in WBC counts within the groups ($p = 0.02$). The red blood cells (RBC) ranged from 5.2 ± 2.4 $10^6/\mu\text{L}$ for group D to 7.0 ± 0.3 $10^6/\mu\text{L}$ for group A. For hemoglobin (HGB), group C and D had the lowest of 14.0 ± 1.6 g/dL and group A recorded the highest of 15.8 ± 0.7 g/dL ($p = 0.08$). This results highlighted that probiotic (*Lactobacillus casei*) did not only exhibited antagonistic activities against *E. coli* but also positively have effect on hematological indices which possibly reflect and also enhanced immune response.

Keywords:

Lactobacillus casei,
Escherichia coli,
Probiotics,
Hematological indices,
Ciprofloxacin.

INTRODUCTION

The human gut houses a dense and diverse microbial community that plays an essential role in overall health, including digestion, nutrient absorption, and immune function (Zhang *et al.*, 2015). *Lactobacillus casei* is among the beneficial microorganisms that has been extensively studied as a probiotic. These bacteria lower the pH of the gut environment by producing lactic acid which makes the gut less hospitable to many pathogenic agents. Also, *Lactobacillus casei* produce bacteriocins (antimicrobial peptides) that is known to inhibit the growth of pathogenic bacteria by destroying their cell walls (Jakubczyk and Górska, 2021). These attributes make *Lactobacillus casei* an important part of the gut's natural defence against pathogens.

Aside maintaining a balanced microbial environment, it is reported that *Lactobacillus casei* can actively influence the host's immune responses. Probiotics are well known to interact with intestinal epithelial cells and immune cells of the gut-associated lymphoid tissue (GALT), which are known to house very significant portion of the immune cells of the body (Bodera, 2008). The interaction of probiotics and the immune cells is believed to stimulate immunoglobulin A (IgA) production, activate natural killer (NK) cells and macrophages and regulate pro-inflammatory and anti-inflammatory cytokine levels (Sanders *et al.*, 2016). These immune-modulatory activities of *Lactobacillus* spp highlight their potential to enhance resistance to infections and harmful organisms.

Escherichia spp are diverse bacterial species including both non-pathogen commensals in the gut and pathogenic strains. *E. coli* is known to cause a variety of infections, including food poisoning, gastroenteritis, sepsis, urinary tract infections, and meningitis (Mueller *et al.*, 2023; Tainter *et al.*, 2023; Peng *et al.*, 2024). In animals, most importantly rodents, *E. coli* infections have been used as models to study the immune response and antimicrobial susceptibility due to the similarity to humans in immune responses. Pathogenic *Escherichia coli* strains can freely evade the immune system, and cause disease due to their production of various virulence factors, such as toxins, invasins, and adhesins that allow them to colonize host tissues, (Sora *et al.*, 2021).

In infants *Escherichia coli* typically colonises the gastrointestinal tract within hours of birth (Adlerberth *et al.*, 2003). *E. coli* coexists with its host especially humans in a mutually beneficial association that can go on for decades. These beneficial strains of *E. coli* rarely cause illness except the host's immune system is compromised or there is disruption of the normal gastrointestinal barriers as seen in cases of peritonitis (Kaper *et al.*, 2004). It is believed that the mucous layer of the mammalian colon is the natural habitat of commensal *E. coli*, where it thrives as densely populated intestinal microflora as a dominant facultative anaerobe (Moblely *et al.*, 2004). One intriguing hypothesis proposes that *E. coli* might efficiently be utilizing gluconate, allowing it to occupy a specific metabolic niche in the colon which outcompete other microbial species (Nataro *et al.*, 2004).

Escherichia coli is more than just a commensal intestinal habitant, which have transformed into specialized *Escherichia coli* clones and have developed specific virulence traits that enhance their ability to adapt to new environments and cause a wide range of diseases (Kaper *et al.*, 2004; Mobley *et al.*, 2004). These virulence factors are often carried on genetic materials that can be transfer between strains, leading to newer of virulence traits recombination. In some cases, these genetic elements, often mobile, have evolved to be integrated permanently into the genome. Only the most effective combinations of virulence factors have endured, giving rise to distinct *E. coli* "pathotypes" which have acquired ability to cause disease even in healthy individuals. (James *et al.*, 2004; Harry *et al.*, 2004).

Antibiotics usage have long been the primary treatment for *Escherichia coli* infections; however, the rise of antibiotic-resistant of *E. coli* strains has since posed a significant challenge to public health. The misuse and overuse of antibiotics in humans, agriculture, and animal husbandry have increasingly accelerated the development of resistance, making a good number of *E. coli* infections difficult to treat (Fischbach and Walsh, 2009). This situation has prompted the exploration of alternative therapies, including the use of probiotics and others, which offer a promising non-antibiotic approach to

infection control. The growing interest in natural products and methods of enhancing immunity highlights probiotics as promising candidates for infection prevention. Probiotics like *Lactobacillus* spp. offer a dual function: they support the health of gut flora and enhance immune responses to infections, which is beneficial in preventing infections from *E. coli* and other pathogenic organisms (Markowiak *et al.*, 2017).

Lactobacillus casei offers a multifaceted approach to preventing and mitigating infections through gut health promotion, competitive exclusion of pathogens, and immune modulation (Da Qin *et al.*, 2022). This study aims to explore the microbial and immunomodulatory effect of *Lactobacillus casei* against *E. coli* infection on Wistar albino rats as experimental animals.

MATERIALS AND METHODS

Study Area

The laboratory analysis was carried out in Microbiology Laboratory and Laboratory and animal house of the Faculty of Pharmacy, University of Benin, Benin City, Nigeria. Ethical approval with reference no; CMS/REC/2024/226 was obtained for the use of laboratory and animals. These facilities provided the enabling environments and needed equipment's needed for immunological and microbial investigations of *Lactobacillus casei* against *E. coli* infection on Wistar albino rats as experimental animals.

Experimental Design and Sample Preparation

The species of *Lactobacillus casei* as well as *Escherichia coli* were acquired from Medical and Molecular Laboratory Bayelsa State and transported to Microbiology Laboratory University of Benin under aseptic conditions. The *Lactobacillus casei* was cultured on de Man, Rogosa, and Sharpe (MRS) Agar, while *Escherichia coli* was cultured on Eosin methylene blue Agar (EMB) for confirmation. The isolates were sub-cultured and also maintained as slant for further experimental purpose.

The experimental design was structured to investigate the immunomodulatory and preventive effects of *Lactobacillus casei* as a probiotic on *Escherichia coli* infections induced in animals (Wistar albino rats). A total of twenty (20) Wistar albino rats, of eight (8) weeks old and weigh within the range of 150 to 200 g, were used. The animals were kept/housed in polypropylene cages/house with sterilized bedding to reduce contamination and disease risk, they were supply with 12 hrs light/dark cycle. Standard and commercially purchased rodent chow diet prepared to meet their nutritional needs, which was used to feed the animals, also filtered water was provided. All animals were kept

for 7 days to acclimatized to laboratory conditions before starting the experiments so as to mitigate and reduced stress-induced variability as described by Kuo *et al.* (2013).

The experimental design involved five (5) groups, each group comprising four (4) rats each. Serial dilution of suspension 1×10^8 cfu/ml were administered to the animals orally with the aid of gavage.

Control Group (Group A): No exposure to *Lactobacillus casei* nor *E. coli*.

Infected Group (Group B): Exposed to only *Escherichia coli* at day 7 and with no treatment.

Probiotic Group (Group C): Received *Lactobacillus casei* only at day 7

Prophylactic Group (Group D): Pre-challenged with *Lactobacillus casei* at day 7 and challenged with *Escherichia coli* at day 14 to ascertain preventive majors.

Antibiotic Group (Group E): exposed to *Escherichia coli* at day 7 and later administered antibiotic (ciprofloxacin) at day 14.

Hematological Analysis

At the end of day 21(end of the experiment), the wistar albino rats in each of the groups were sacrificed and blood samples were collected. The blood samples taken for blood cell counts through a cardiac puncture process into ethylenediamine tetraacetic acid (EDTA) tubes for analysis. White blood cells (WBC), red blood cells (RBC), Lymphocyte percentage (LYM), Platelet count (PV), Monocyte percentage (MID), mean corpuscular volume (MCV), Granulocyte percentage (GRAN), Hemoglobin (HGB), hematocrit (HCT), platelets (PLT), hemoglobin concentration (Hb), mean corpuscular hemoglobin concentration (MCHC), Red Cell Distribution Width-Standard Deviation (RDW-SD), Lymphocyte-to-Cell Ratio (LCR), Red Cell Distribution Width-Coefficient of Variation (RDW-CV), Cell Type (CT), Differential White Blood Cell count (DW), Large Unstained Cell count (LP), where analysed as described by Galdeano and Perdigon.(2006).

Microbiological Analysis of Stool Samples

At 0, 7, 14 and 21 days of the experiment, faecal samples were collected from the rats. Approximately 1 g of stool each from three (3) rats were homogenized, each in 9 mL of sterile Phosphate buffer saline (PBS) and a tenfold serial dilution was performed. A 0.1 mL of 10^{-6} dilution was plated onto Nutrient agar plates in triplicates under standard microbiological method and incubated at 37°C for 24hrs. After 24hrs, distinct colonies were observed, counted and the bacterial load were calculated using the formula below. Results were expressed as colony-forming units (CFU) per gram (NRC, 2011).

$$\frac{\text{cfu}}{\text{ml}} = \frac{\text{number of colonies} \times \text{dilution fold/ series}}{\text{volume of inoculum}}$$

Microbiological Analysis of Organ Samples

Also, tissue samples (liver and colon) were aseptically collected from euthanized rats and weighed. Each tissue sample (1 g) was homogenized in 9 mL of sterile PBS and a tenfold serial dilution was performed. A 0.1 mL of 10^{-6} dilution was plated onto Nutrient agar plates in triplicates under standard microbiological method and incubated at 37°C for 24hrs. After 24hrs, distinct colonies were observed, counted and the bacterial load were calculated using the formula below. Results were expressed as colony-forming units (CFU) per gram (NRC, 2011).

$$\frac{\text{cfu}}{\text{g}} = \frac{\text{number of colonies} \times \text{dilution fold/ series}}{\text{volume of inoculum}}$$

Statistical analysis

Analysis of variance (Mean \pm S.D) was used to analyse the data. The Statistical Pack Initial Age for Social Sciences (SPSS) software version 20.0 was utilized. P value less than 0.05 was used as the statistical significance level, and was analysed by Turkey's test. Figures were plotted using Originpro 2024.

RESULTS AND DISCUSSION

Temperature ($^{\circ}\text{C}$) and weight (g) of albino rats challenged with *Lactobacillus casei* and *E. coli*

The effect of temperature ($^{\circ}\text{C}$) on the different groups of wistar albino rats exposed to *Lactobacillus casei* and *E. coli* is presented on Figure 1. For all the groups (A, B, C, D and E), temperature range was $36 - 36.3^{\circ}\text{C}$ at day 0. There was an increase in temperature for only group B, C, D and E at day 7. All the groups had an increase in temperature at day 14 and 21, although the highest temperature recorded was 38.6°C at day 21 by wistar albino rats in group B. There was no statistical difference in temperature change among the groups ($p > 0.05$). The weight of the animals at day zero ranged from 150.7 to 191.4 g. The control group (group A) recorded a steady body weight increase from day 0 (172.7 g), day 7 (176.2 g), day 14 (180.1 g), and day 21 (185.3 g). The rats in group B had a steady decrease in body weight from 191.4 g at day 0 to 170 g at day 21. The weight of Wistar rats challenged with *Lactobacillus casei* and *E. coli* at different days is presented in Figure 2. Group C wistar rats had an average body weight of 150.7 g at day 0, 169 g at day 7, 156 g at day 14 and a sharp body weight increase of 198 g at the end of 21 days (end of the experiment).

Mean \pm SD Weight of Harvested Organs of Experimental Rats

Figure 3 shows the Mean \pm SD Weight of Harvested Organs (liver and colon) of the Experimental Rats. At the end of 21 days (end of the experiment) the organs were

weighed and group D recorded the highest weight of 6.83 ± 0.21 g followed by group B with 6.46 ± 0.24 g and the least liver weight was observed in group E (5.47 ± 0.09 g). The highest Mean \pm SD Weight of colon was seen in group C (2.6 ± 0.11 g), while it was observed that group A (0.89 ± 0.05 g) had the least weight of colon. although there was no difference statistically among the groups.

Heterotrophic Bacterial Count ($\times 10^6$ CFU/ml) from the Stool Samples of Wistar albino rats.

The Heterotrophic Bacterial Count ($\times 10^6$ CFU/ml) from the Stool Samples of the rats shows their mean and error bar as presented in Figure 4. At day 0, group C recorded the least bacterial load from stool samples ($4.5 \pm 0.5 \times 10^6$ CFU/ml), while group E had the highest of bacterial load $8.4 \pm 0.5 \times 10^6$ CFU/ml. The highest bacterial load was observed in group B ($9 \pm 0.25 \times 10^6$ CFU/ml) at day 7. At day 14 the highest bacterial load was observed among group D ($5.6 \pm 0.4 \times 10^6$ CFU/ml). at the end of 21 days (end of the experiment), group E had the highest bacterial load in stool samples ($11.5 \pm 0.3 \times 10^6$ CFU/ml), followed by group B ($11 \pm 0.4 \times 10^6$ CFU/ml), while group C recorded the least bacterial load of $3.5 \pm 0.2 \times 10^6$ CFU/ml.

Hematological assessment of blood samples from the experimental animals

Table 1 represent the Hematological parameters from blood samples of experimental animals. The White blood cells (WBC) range from 8.8 ± 0.7 - $14.7 \pm 4.6 \times 10^3/\mu\text{L}$, where group A had the lowest WBC of $8.8 \pm 0.7 \times 10^3/\mu\text{L}$ and group E had the highest of $14.7 \pm 4.6 \times 10^3/\mu\text{L}$. There was statistical difference in WBC counts within the groups ($p = 0.02$). The Lymphocyte percentage (LYM) for group A was $60.5 \pm 11.6\%$, group B was $66.6 \pm 9.7\%$, group C was $73.8 \pm 3.9\%$, group D was $72.5 \pm 7.5\%$, and group E was $71.0 \pm 5.1\%$ ($p = 0.05$). The red blood cells (RBC) was observed to be from $5.2 \pm 2.410^6/\mu\text{L}$ for group D to $7.0 \pm 0.3 \times 10^6/\mu\text{L}$ for group A, with no statistical significant difference among groups. For hemoglobin (HGB), group C and D had the lowest of 14.0 ± 1.6 g/dL and group A recorded the highest of 15.8 ± 0.7 g/dL ($p = 0.08$). For hematocrit (HCT) percentage, group A recorded the highest ($44.9 \pm 1.7\%$), while group D and E had $43.3 \pm 4.8\%$ and $43.1 \pm 2.4\%$ respectively. There was no statistical difference in HCT within the groups. The Mean Corpuscular Volume (MCV) among the groups showed that group D had the highest MCV of 72.9 ± 5.0 fL and group A recorded the least value of 64.6 ± 1.1 fL and a significant difference was observed ($p = 0.03$). It was observed that Mean Corpuscular Hemoglobin, Large Unstained Cell count, Width-Standard Deviation, Red Cell Distribution, Red Cell Distribution Width-Coefficient of Variation, Platelet count and Cell Type recorded no significant difference within the groups ($p > 0.05$). While Differential White Blood Cell count, Mean

Corpuscular Hemoglobin Concentration and Lymphocyte-to-Cell Ratio recorded statistical difference within the groups ($p < 0.05$).

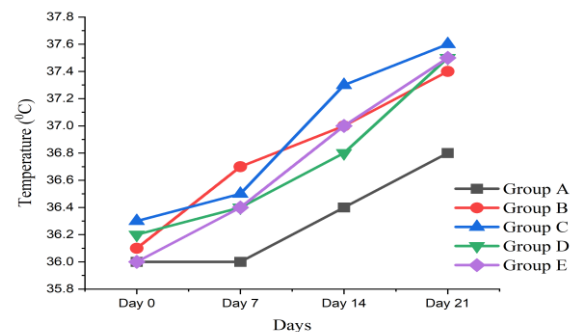


Figure 1: Effect of temperature ($^{\circ}\text{C}$) on different groups of albino rats challenged with *Lactobacillus casei* and *E. coli*

Key: A- Control group, B- Infected group (challenged with only *E. coli*), C- Probiotic group (exposed to only *Lactobacillus casei*), D- Prophylactic group (exposed *Lactobacillus casei* and later with *E. coli*) and E- Antibiotic group (exposed to *E. coli* and later treated with Ciprofloxacin antibiotic).

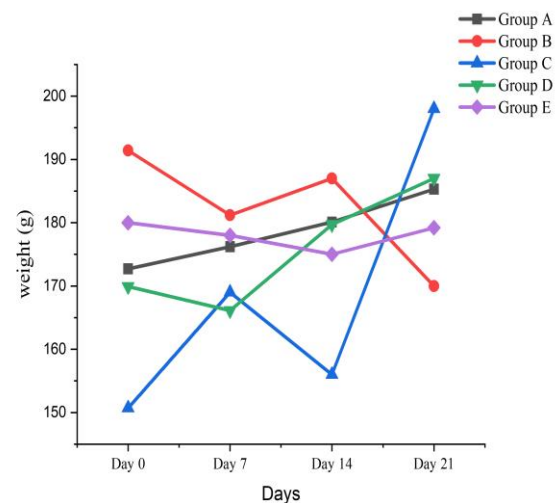


Figure 2: Weight of Wistar rats exposed to *Lactobacillus casei* and *E. coli* at different days interval.

Key: A- Control group, B- Infected group (challenged with only *E. coli*), C- Probiotic group (exposed to only *Lactobacillus casei*), D- Prophylactic group (exposed *Lactobacillus casei* and later with *E. coli*) and E- Antibiotic group (exposed to *E. coli* and later treated with Ciprofloxacin antibiotic).

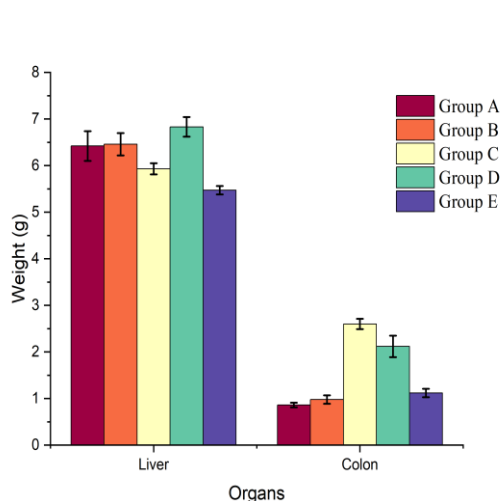


Figure 3: Mean ± SD Weight of Harvested Organs (liver and colon) of Experimental Rats

Key: A- Control group, B- Infected group (challenged with only *E. coli*), C- Probiotic group (exposed to only *Lactobacillus casei*), D- Prophylactic group (exposed *Lactobacillus casei* and later with *E. coli*) and E- Antibiotic group (exposed to *E. coli* and later treated with Ciprofloxacin antibiotic).

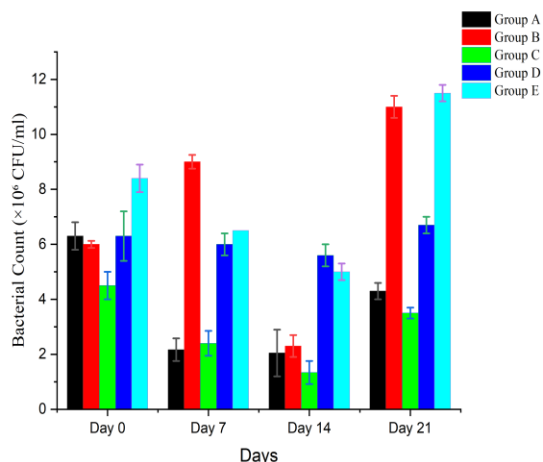


Figure 4: Heterotrophic Bacterial Count (x10⁶ cfu/ml) from the Stool Samples of Wistar albino rats.

Key: A- Control group, B- Infected group (challenged with only *E. coli*), C- Probiotic group (exposed to only *Lactobacillus casei*), D- Prophylactic group (exposed *Lactobacillus casei* and later with *E. coli*) and E- Antibiotic group (exposed to *E. coli* and later treated with Ciprofloxacin antibiotic).

Table 1: Hematological parameters from blood samples of experimental animals

Parameters	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E	P value
WBC (10 ³ /μL)	8.8±0.7	12.1±2.0	11.4±1.8	10.8±1.2	14.7±4.6	0.02
LYM (%)	60.5±11.6	66.6±9.7	73.8±3.9	72.5±7.5	71.0±5.1	0.05
MID (%)	18.5±2.4	19.1±3.8	15.0±2.9	16.4±4.7	20.8±5.4	0.78
GRAN (%)	21.0±9.8	14.6±6.7	11.3±3.0	11.1±3.2	10.8±2.0	0.02
RBC (10 ⁶ /μL)	7.0±0.3	6.5±0.5	6.4±0.5	5.2±2.4	6.3±0.3	0.18
HGB (g/dL)	15.8±0.7	14.6±1.6	14.0±1.6	14.0±1.8	14.1±2.4	0.08
HCT (%)	44.9±1.7	44.3±3.3	43.9±3.9	43.3±4.8	43.1±2.4	0.37
MCV (fL)	64.6±1.1	67.8±0.5	69.2±2.2	72.9±5.0	68.3±2.8	0.03
MCH (pg)	22.7±0.2	22.3±0.7	21.9±1.0	23.4±1.0	22.3±0.9	0.84
MCHC (g/dL)	35.2±0.4	32.9±1.3	31.7±1.5	32.2±0.8	32.7±1.5	0.01
RDW-SD (fL)	38.0±1.1	42.8±3.0	42.8±4.6	46.0±5.4	39.6±1.2	0.35
RDW-CV (%)	16.8±0.5	18.3±1.4	18.0±1.9	18.5±1.2	16.8±0.2	0.94
LC (10 ³ /μL)	653.3±104.7	645.8±67.6	822.0±114.0	735.8±190.8	701.5±280.2	0.49
PV (fL)	8.2±0.5	8.5±0.3	8.8±0.4	8.7±0.4	8.7±0.8	0.17
DW (%)	9.6±0.7	11.1±1.3	12.5±1.0	11.1±1.5	14.6±3.7	0.01
CT (%)	0.5±0.1	0.8±0.3	0.9±0.4	0.6±0.2	1.3±1.0	0.13
LCR (%)	8.0±0.7	12.3±2.7	15.3±1.2	14.2±4.2	21.5±4.7	0.00

Key: Values are presented as mean ± SD, WBC (White Blood Cell count), RBC (Red Blood Cell count), LYM (Lymphocyte percentage), GRAN (Granulocyte percentage), MID (Monocyte percentage), HGB

(Hemoglobin), MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Hemoglobin), HCT (Hematocrit), MCHC (Mean Corpuscular Hemoglobin Concentration), RDW-CV (Red Cell Distribution Width-Coefficient of

Variation), DW (Differential White Blood Cell count), RDW-SD (Red Cell Distribution Width-Standard Deviation), PV (Platelet count), CT (Cell Type), LP (Large Unstained Cell count), LCR (Lymphocyte-to-Cell Ratio). A- Control group, B- Infected group (challenged with only *E. coli*), C- Probiotic group (exposed to only *Lactobacillus casei*), D- Prophylactic group (exposed *Lactobacillus casei* and later with *E. coli*) and E- Antibiotic group (exposed to *E. coli* and later treated with Ciprofloxacin antibiotic).

Temperature variations also reflected immune response differences among the groups. The *E. coli*-infected group (group B) had the highest temperature increase, indicative of an infection-induced fever response (Katharina *et al.*, 2003). The *Lactobacillus casei* group showed a slight increase, suggesting a mild immune activation. The control group maintained a stable temperature, indicating no infection. The fever response in *E. coli*-infected rats aligns with immune activation against bacterial invasion. The probiotic-treated group displayed mild temperature variations, suggesting that *Lactobacillus casei* provided protection without inducing excessive inflammation. This supports the immunomodulatory role of probiotics in balancing pro- and anti-inflammatory responses Zhang *et al.* (2021). The temperature fluctuations observed in this study are consistent with the findings of Karami *et al.* (2020), who examined the immune response in rats exposed to various pathogens. Their research indicated that infections frequently cause a rise in body temperature, primarily due to cytokine release as part of the body's systemic inflammatory reaction (Karami *et al.*, 2020).

The effects of *Lactobacillus casei* on body weight and general health is seen to be very important. The control group exhibited stable weight gain of 172.7g at day 0 to 185.3g at day 21. The probiotic group (group C) also showed increase in body weight of 150.7 at day 0 to 214g at day 21. This study aligns with the study by Mender *et al.* (2021) which showed that the administration of *Lactobacillus spp* significantly increases weight gain in rats subjected to enteric infections (Mender *et al.*, 2021). The *E. coli*-infected group showed a decline in body weight, indicating that *E. coli* infection led to metabolic stress, inflammation, and potential nutrient malabsorption. The weight gains in the probiotic group suggest that *Lactobacillus casei* contributed to metabolic balance and gut health maintenance. This is in resonance with the study by Ruan *et al.* (2021) that dietary inclusion of *Lactobacillus casei* improved growth performance in piglets under stressful conditions, ultimately suggesting the potential for probiotics to mitigate the impact of pathogens on growth (Ruan *et al.*, 2021). The prophylactic and antibiotic-treated groups also showed weight gain, indicating some level of protection against *E. coli*-induced weight loss. Weight loss in *E. coli*-infected rats suggests that pathogenic bacteria disrupt

normal gut function, leading to inflammation and nutrient deficiencies (Christiane *et al.*, 2008). The supplementation of *Lactobacillus spp* helped maintain body weight, possibly by improving gut microbiota composition, enhancing nutrient absorption, and reducing infection severity (Paul *et al.*, 2020).

The variations in liver and spleen weights reported in the study are corroborated by findings from Ebrahim *et al.* (2020), who noted that infections can lead to both hepatic and splenic hypertrophy as a physiological response to pathogen load (Ebrahim *et al.*, 2020). The reduced liver weight in the infected group aligns with the literature suggesting that severe infections can compromise liver function (Furtado *et al.*, 2019). Furthermore, studies by Gandía-Herrero *et al.* (2019) highlighted the importance of maintaining organ health through probiotic intervention, where organisms such as *Lactobacillus plantarum* supported liver function and anti-inflammatory responses in model organisms (Gandía-Herrero *et al.*, 2019)

The comparative analysis of heterotrophic bacterial counts highlights the competitive interactions occurring within the gut microbiome. The findings that the bacterial load from stool samples decreased in the probiotic-administered group ($4.5 \pm 0.5 \times 10^6$ to $3.5 \pm 0.2 \times 10^6$ CFU/ml) emphasize the competitive exclusion hypothesis. Ahn *et al.* (2018) demonstrated that *Lactobacillus species* inhibit the growth of pathogenic bacteria through the production of bacteriocins and other metabolites, providing a protective barrier in the gut against infection. This mechanism, coupled with improved gut integrity, supports the concept of using probiotics as a preventive measure against gastrointestinal infections (Ahn *et al.*, 2018).

Animal models, particularly rat models, are frequently used to study hematological changes associated with immune responses, as they offer insights that are often translatable to human physiology. In studies where rats are administered *Lactobacillus casei* and subsequently exposed to *Escherichia coli*, researchers have observed beneficial changes in hematological parameters, suggesting that probiotics may enhance immune resilience (Sanders *et al.*, 2016). The immunostimulative action of probiotics is known to reduce intensity risk of intestinal diseases (Ford *et al.*, 2014). Hematological parameters, such as white blood cell (WBC) count, hemoglobin concentration, and lymphocyte levels, are critical indicators of immune health (Tarvernit and Guglielmetti, 2011). Changes in these parameters can reflect an organism's response to infection and inflammation. For instance, an increase in WBC count and neutrophil activity typically signifies an immune response to bacterial infection. Lymphocytes, on the other hand, are crucial for adaptive immunity, with B cells producing antibodies and T cells helping to clear infections (Schwarz *et al.*, 2013). Probiotics like

Lactobacillus casei can influence these hematological parameters, potentially enhancing the host's immune defences against pathogens.

For White blood cells (WBC), this is the number of WBC presented per blood microliter (μL). The reference limit in adults is within the range $4.5 - 11 \times 10^3$ cells/ μL , while in rats is given as $5.5-16.5 \times 10^3$ cells/ μL . In this study, all the studied groups recorded WBCs within the reference interval stated out by National Research council for care and use of Laboratory animals (NRC, 2011). This work is in corroboration with the work of Herich *et al.* (1999) and Kim *et al.* (2020).

In this study, the LMY were within the range 60.5 ± 11.6 to 72.5 ± 7.5 %. This is within the reference limit of lymphocytes (LYM) in rat mammals (60% to 80%) of the absolute WBC. Lymphocytes are a type of WBC that act as a crucial part of the immune system responsible for fighting infections and diseases, while lymphocytes below the normal range is referred to as Lymphocytopenia, which is a decrease in the lymphocyte count and is often attributed to viral infection, primary immunodeficiency diseases or medications, bacterial infection, myeloproliferative disease, autoimmune disease malignancy, lymphoproliferative disease, (Ruggiero, 2014; Hoffman *et al.*, 2020).

These hematological indices [Hemoglobin, Red blood cells, mean corpuscular volume (MCV), Mean corpuscular hemoglobin concentration (MCHC), Hematocrit, mean corpuscular hemoglobin (MCH)] are often used spuriously as indicative of anemia (Hill *et al.*, 2018; Afzaal *et al.*, 2022). The reference limit is within 4.2 to 5.4 million cells/ μL in adult female and 7.5 to 12.5 million cells/ μL in adult men. Hemoglobin measures the amount of hemoglobin in whole blood and is expressed as g/dL. It was observed that, the Hemoglobin (Hb) ranged from 14.0 ± 1.6 to 15.8 ± 0.7 g/dL and there was no statistically significant difference within the groups. The references limit for hemoglobin levels is within 12 to 18 g/dL in rats as stated by World Health Organization (WHO), Hemoglobin within the acceptable limits indicates that RBC are adequately transporting oxygen throughout the body (WHO, 2011). It is reported that those with hypervolemia may often exhibit a Hb lower than their true level expected, while severely dehydrated people may have a falsely elevated Hb. The cause of anemia is expansive and also attributed to blood cell destruction, blood loss, deficient blood cell production, or defective blood cell production. (Li *et al.*, 2020; Amengialue *et al.*, 2023)

Mean corpuscular volume (MCV) is the average volume of the red blood cells present and the acceptable limit is 50 -70 fL. It was observed that all the groups had MCV within the acceptable limit except group D that recorded an MCV of 72.9 ± 5.0 above the normal limits. A normal Mean corpuscular volume (MCV) is referred to as

normocytic indicating that RBC are of normal size, while an elevated MCV above the normal range is referred as macrocytic, suggesting larger than normal RBC (Martín *et al.*, 2013). The common causes of macrocytic are vitamin B12 or folate deficiency, alcoholism or certain medications (Hoffman *et al.*, 2020). Mean corpuscular hemoglobin (MCH) is given as the amount of hemoglobin per red blood cell and is between range 17 to 24 pg. while MCHC is the mean hemoglobin concentration per unit volume of RBC (30 to 36 g/dL) and it measures the average concentration or density of hemoglobin within a given volume of packed RBC. In this study all the groups have an MCHC within the acceptable limits and there was statistical difference within the group ($p = 0.01$). A low MCHC is known as Hypochromia, which may be due to decreased Hb production, the most common cause is often being iron deficiency (less commonly copper deficiency or lead poisoning or pyridoxine deficiency) (van Niel *et al.*, 2022). While MCHC above the normal range indicates hyperchromia, suggesting RBC are more concentrated with hemoglobin, which occurs in conditions like hereditary spherocytosis (Hoffman *et al.*, 2020).

The study conducted by Vilela *et al.* (2015) reported that *Lactobacillus casei* can enhance immunity against infections in Wistar albino rats by positively by improving various hematological indices, where these cells are essential for the immune response, thereby improving the body's defence mechanism to fight bacterial infections. This effect is attributed to the probiotic's ability to stimulate the immune system by activating immune cells and promoting cytokines production that are vital for immune responses in a host (Ruggiero, 2014).

CONCLUSION

The study of the investigation of the microbial and immunomodulatory effect of *Lactobacillus casei* against *E. coli* infection on Wistar albino rats indicated that probiotics can improve and enhance blood indices in a given host. Also highlight the preventive ability of probiotics to bacterial infections.

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