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To Investigate the Effects of *Lactobacillus Casei* in Enhancing Immunity against *Staphylococcus sciuri* Infection in Albino Rats (*Rattus Norvegicus*), through Microbiological, Hematological and Histopathological Screening

Saidu, J.Z.[1*] and Agbonwaneten, O.B.[1]

[1]*Department of Microbiology, Faculty of Life Sciences, University of Benin, PMB 1154 Benin City, Edo State, Nigeria.*
**Corresponding author, Email address: joy.saidu@uniben.edu*

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Abstract: Probiotics plays a pivotal role in fermentation processes and possess significant health-promoting properties in enhancing immunity. It has gained significant attention in biomedical research, particularly as global health challenges demand safer and more sustainable therapeutic interventions. This study evaluates the potential of *Lactobacillus casei* as probiotics to enhance immune responses in Wistar albino rats against *Staphylococcus sciuri* infection. Twenty healthy Wistar rats were divided into five groups: Control (group A), group B (*Staphylococcus sciuri*-infected), group C (*Lactobacillus casei*-treated), group D (pre-treated with *Lactobacillus casei* before infected with *Staphylococcus sciuri*), and group E (infected with *Staphylococcus sciuri* and treated with ciprofloxacin). Temperature, body weight and stool samples were collected at 0, 7, 14 and 21 days. At the end of the experiment (21 days), blood samples and organs (liver and spleen) were examined microbiological and pathological following standard methods. It was observed that there was no change in temperature in group A (36.0 ºC) but there was an increase in temperature in group B (36.8 ºC), group C (37.2 ºC), group D (37.3 ºC) and group E (38 ºC) at day 7. At day 21, there was increase in body temperature in group A, B and D, though there was no significant difference in body temperature among the groups ($p>0.05$). The weight of the experimental animals at day zero ranged from 180 to 200 g. At the end of the experiment (at day 21), there was body weight gain in group A, C and D. The White blood cells (WBC) of group A was 19.2 ± 2.00 10^3/µL, while group C was 14.5 ± 4.70 10^3/µL and group E was 9.5± 2.10 10^3/µL, with no statistical significance among the groups ($p=0.68$). while group A had the highest mean Corpuscular Volume (MCV) of 93.6 ± 3.00 fL, followed by group C (80.7 ± 4.00 fL) and there was significant difference among all groups ($p=0.01$). The mean heterotrophic bacteria count of stool samples at day zero ranged from $2.05 \times 10^6 – 2.34 \times 10^6$ cfm/ml. while by day 21 group A had the least mean bacteria count of 4.20×10^6 cfm/ml and the highest occurrence was in group B (6.30×10^6 cfm/ml). It was observed in this study, the spleen tissue in the control group (Group A) exhibited normal architecture, with distinct white and red pulp, while in Group C, the spleen tissue showed some mild lymphocytic infiltration. The findings indicate that *Lactobacillus casei* not only demonstrates antagonistic properties against pathogenic bacteria but also positively affects hematological parameters that reflect and enhanced immune response. I recommend further research should be conducted to explore the long-term effects of *Lactobacillus casei* supplementation on immune system.

Abbreviation

CT: Cell Type
DW: Differential White Blood Cell count
EDTA : ethylenediamine tetraacetic acid
GRAN: Granulocyte percentage
Hb: hemoglobin concentration
HCT: hematocrit
HGB: Hemoglobin
LA: Lactic acid, LAB: Lactic acid bacteria
LCR: Lymphocyte-to-Cell Ratio
LP: Large Unstained Cell count
LYM: Lymphocyte percentage
MRS: Man, Rogosa, and Sharpe
MCHC: mean corpuscular hemoglobin concentration
MCV: mean Corpuscular Volume
MID: Monocyte percentage
PLT platelets
PV: Platelet count
RBC: red blood cells
RDW-CV: Red Cell Distribution Width-Coefficient of Variation
RDW-SD: Red Cell Distribution Width-Standard Deviation
WBC: White blood cells

1. Introduction

Lactic acid bacteria (LAB) encompass a diverse group of Gram-positive, non-spore-forming bacteria that play a pivotal role in food fermentation processes and possess significant health-promoting properties (Anumudu *et al.*, 2024; Hamdaoui *et al.*, 2024). Commonly found in fermented dairy products, vegetables, and other fermented foods, lactic acid bacteria have gained attention for their probiotic potential, contributing to gut health and overall well-being (Fooks and Gibson, 2002; Sanders, 2003; Hamdaoui *et al.*, 2023). In recent years, the application of lactic acid bacteria has expanded into therapeutic domains, particularly in enhancing immune responses and preventing infections (Perdigón *et al.*, 2001; Choukri *et al.*, 2023). Lactic acid bacteria (LAB) include genera such as *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Leuconostoc*, and *Pediococcus*, known for their ability to ferment carbohydrates into lactic acid (Bintsis, 2018). Consequently, the use of LAB as probiotics has been widely investigated for their role in preventing and treating various gastrointestinal disorders, including inflammatory bowel disease, diarrhoea, and other infections (Sazawal *et al.*, 2006; Coeuret *et al.*, 2004; Yassine *et al.*, 2025).

The importance of LA and LAB is reflected in the number of publications on Scopus, which reaches more than 227,000 and 52,000 articles, respectively. Then, a bibliometric study is interesting to visualize the best researchers and countries, as well as their collaborations (Mindeli *et al.*, 2015; Chakir *et al.*, 2023; N'diyae *et al.*, 2022; Salim *et al.*, 2022). In 1908, only 02 articles were indexed, 181 in 1990, 547 in 2000 and to reach 4375 articles on LAB in 2024, we can see the increase interest of countries and researchers as published. **Figure 1** indicated the high increase of publication on LAB from 1990 to present. China is the most country contributing on LAB exceeding 8400 articles, the US in second position with more than 5100 articles… (**Figure 2**). This may be explained by the multiple potential health or nutritional benefits possible from some species of lactic acid bacteria. Among these are: improved nutritional value of food, control of intestinal infections, improved digestion of lactose, control of some types of cancer, and control of serum cholesterol levels (Gilliland, 1990; Anumudu *et al.*, 2024).

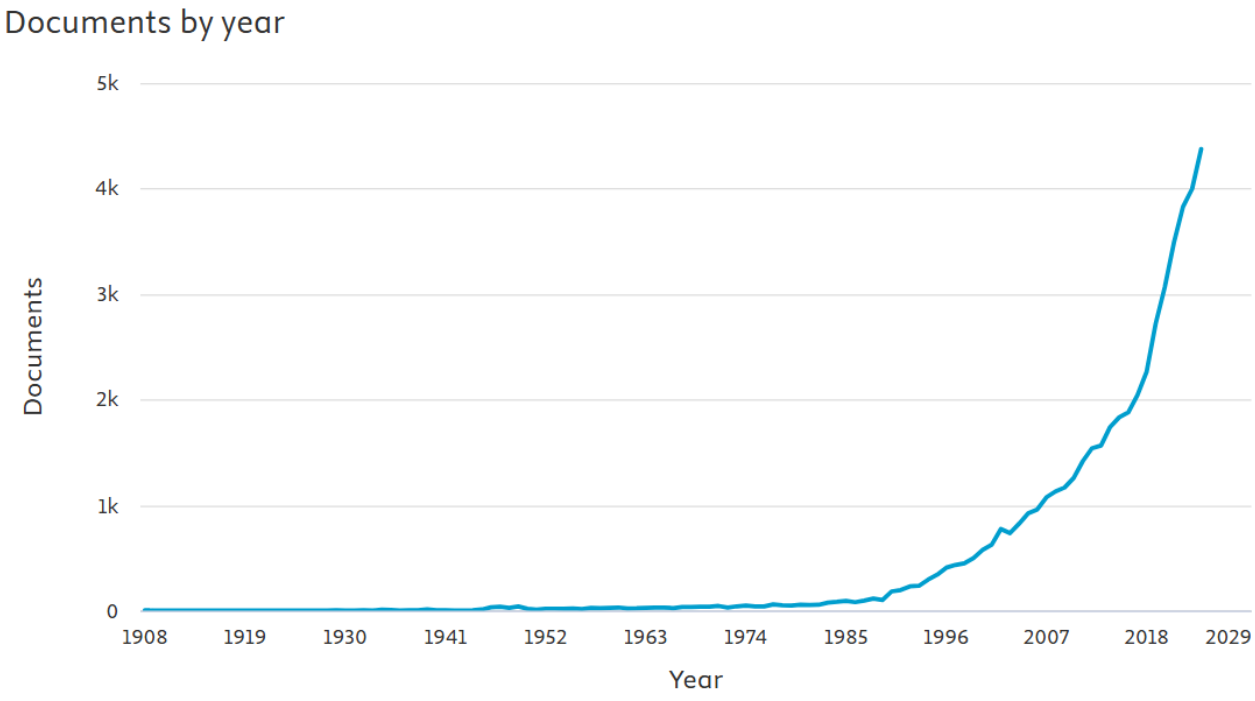


Figure 1: The evolution of publications from 1908 to 2024

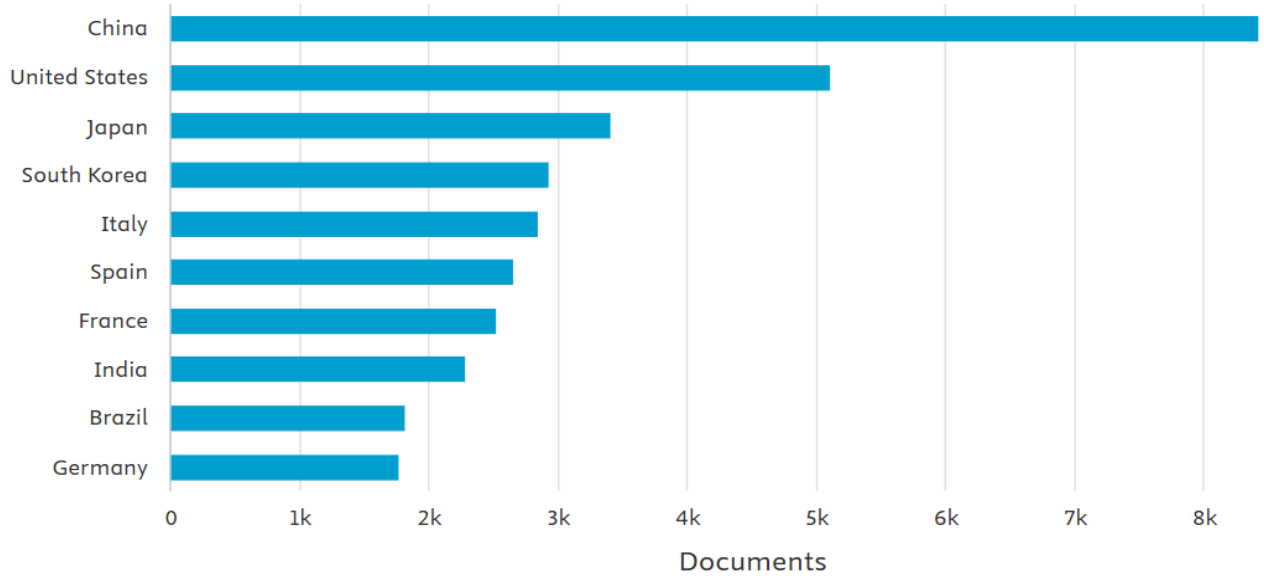


Figure 2: The most Countries contributing on LAB

The ten Scopus authors with the highest contribution (>100 articles) are presented in **Figure 3**. Among the data analyzed, Gobbetti (Free University of Bozen-Bolzano, Italy) published around 250 articles on LAB among his 446 papers with a H-index of 104 and 34,400 citations. The second place is occupied by the Belgian De Vuyst, Luc Vrije Universiteit Brussels, reaching 201 articles (total 392 articles, H=96 and more than 36,600 citations by 17987 documents).

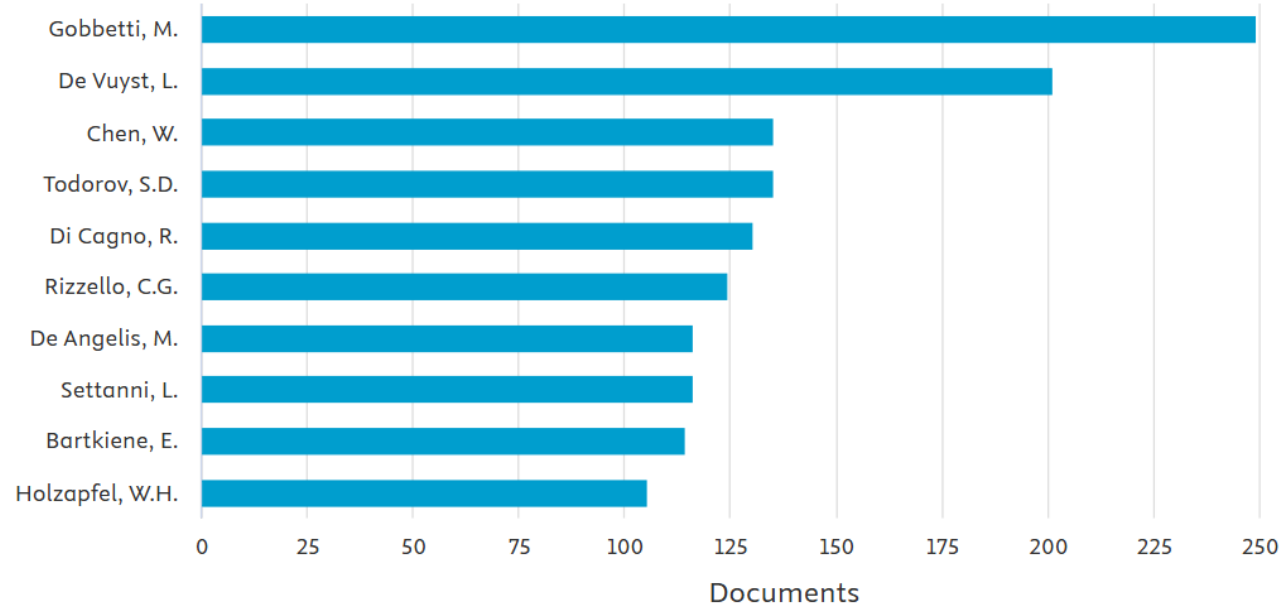


Figure 3: The best researchers on Scopus

This bibliometric analysis can be consolidated by VOS viewer software using data processing and visualization, including co-occurrence analysis and visualization clustering. This analysis identified solo authors, author dyads, author triads, and clusters of these, countries producing collaboratively (Waltman *et al.*, 2010; Perianes Rodriguez *et al.*, 2016;). As VOS viewer limited to less than 20,000 documents, we restricted our analysis toward the period (2020-2024) leading to 18,748 articles.

Regarding country/region distribution, **Figure 4** illustrated the dominance of China with the highest number of publications and citations, as shown with large pink node. The orange node for the United

States contributed significantly to this field. South Korea, India and Italy indicated by red, blue and brown nodes, respectively. The lines suggested the strengthen collaboration and communication among different countries and regions (Jiang *et al.*, 2023; Oyewola & Dada, 2022).

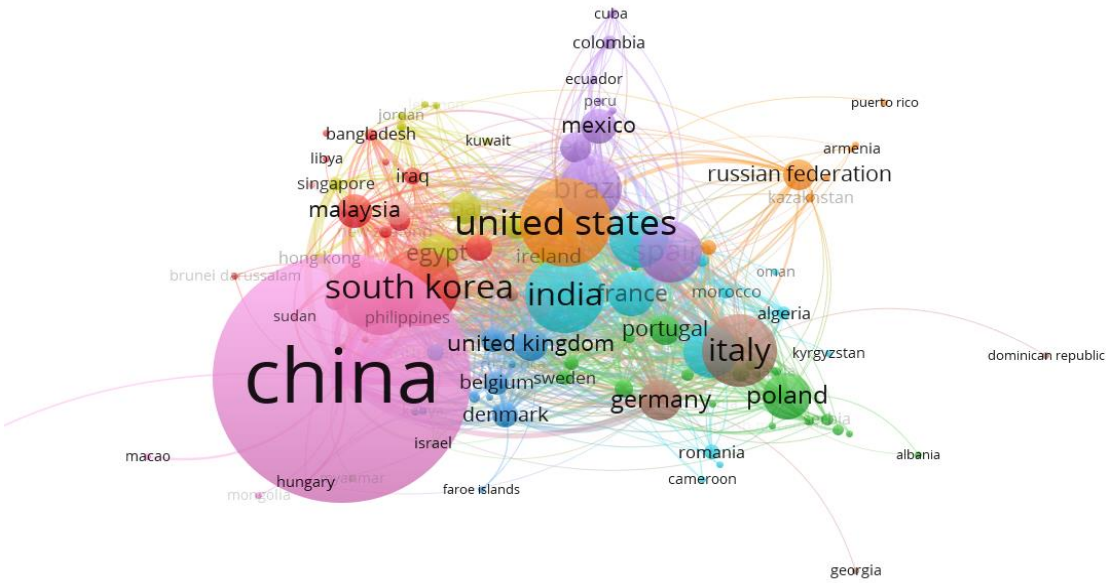


Figure 4: The best researchers on LAB and interconnecting Clusters on VOS viewer (2020-2024)

Figure 5 shows the mapping of authors and their clusters by colored nodes where the size indicating the number of articles. In this period, the list of the authors are: Bartkiene E. (67 articles), Pan (56 articles), Chan (55 articles), Rocha (53 articles), Gobbetti (52 articles) …

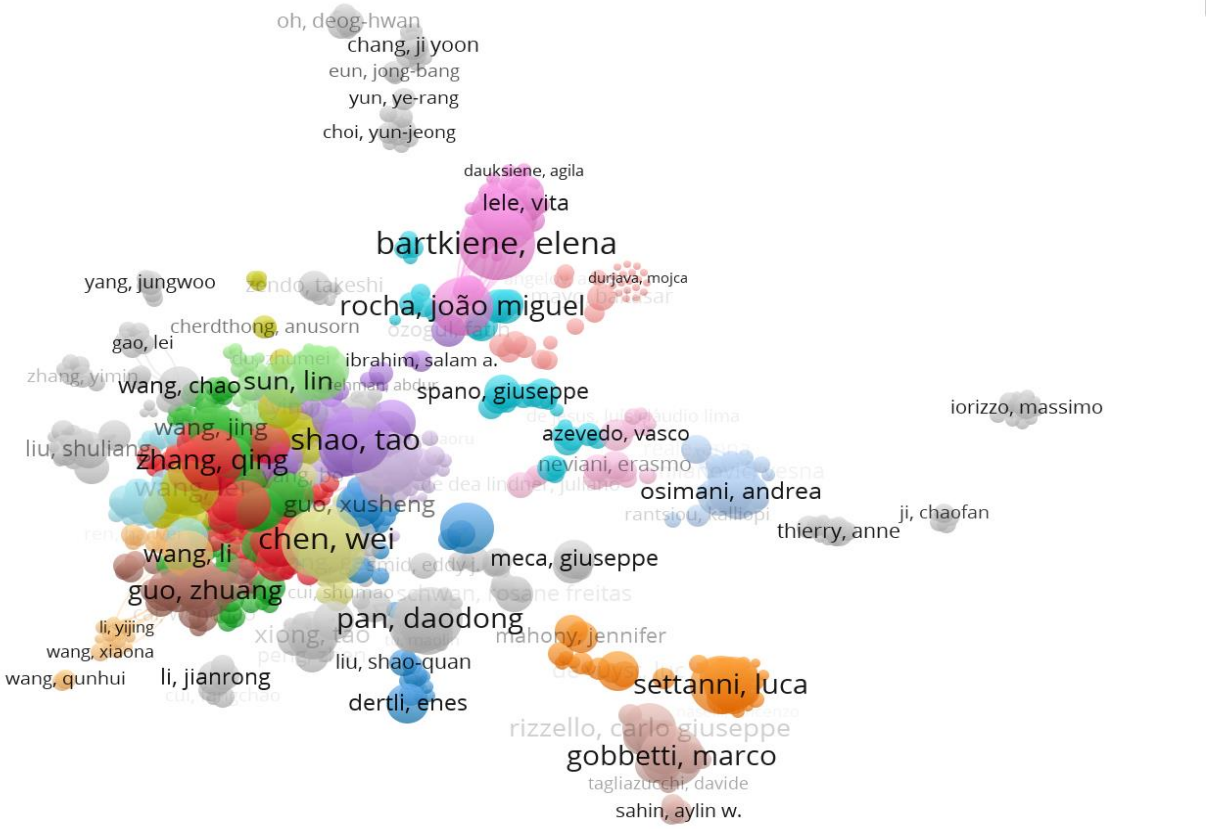
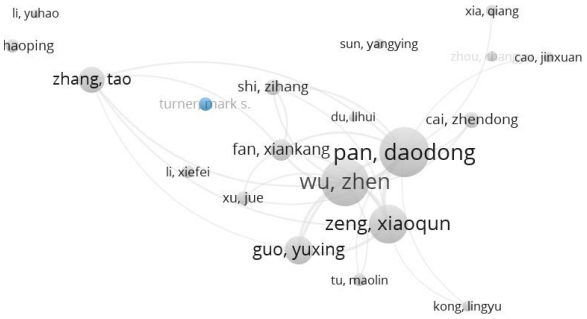
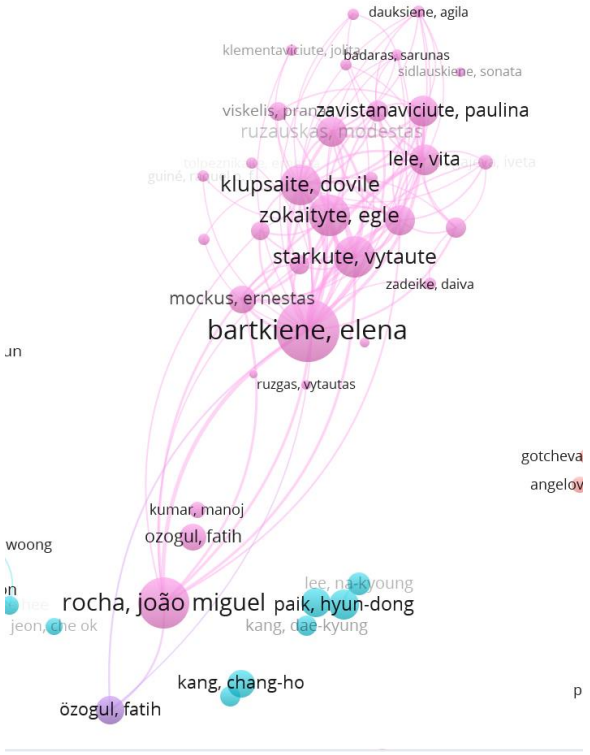


Figure 5: The best researchers on LAB and interconnecting Clusters on VOS viewer (2020-2024)

Bartkiene from Lietuvos Sveikatos Mokslų Universitetas, Kaunas, Lithuania is shown by light pink node with several co-authors as Rocha J.M. (Universidade do Porto, Porto, Portugal) and others indicating the European collaboration.

The second author Pan, D., Ningbo University, China is a young researcher from China (56 articles) reached a totally of 541 articles, an H-index of 54 and more than 11,000 citations by 8573 documents.





The role of probiotics in enhancing immunity has gained significant attention in biomedical research, particularly as global health challenges demand safer and more sustainable therapeutic interventions. Among the numerous probiotics studied, *Lactobacillus casei* has been highlighted for its remarkable ability to regulate host immune responses and provide protection against pathogenic bacteria (La Fata *et al*., 2018; Sellam *et al*., 2024). *Lactobacillus casei*, a lactic acid bacterium commonly found in fermented dairy products, exhibits robust survival in the gastrointestinal tract due to its acid and bile tolerance (Leite *et al*., 2015). This resilience enables it to exert immunomodulatory effects by enhancing phagocytic activity, modulating cytokine production, and reinforcing the intestinal epithelial barrier (Sánchez *et al*., 2017). In addition to its gastrointestinal benefits, *Lactobacillus casei* has been shown to influence systemic immunity, potentially reducing the severity of infections caused by various pathogens.

Conversely, *Staphylococcus sciuri* is emerging as an opportunistic pathogen of significant concern. Once regarded as a commensal species found in the skin and mucosal surfaces of animals, it is now increasingly associated with zoonotic infections in humans. Its pathogenicity is heightened by its capacity to acquire and disseminate antibiotic resistance genes, posing a serious threat to both veterinary and public health (Foster, 2019). *S. sciuri* has been implicated in cases of endocarditis, wound infections, and septicemia, with evidence suggesting its potential to evade host immune defenses, establish persistent infections, and compromise therapeutic outcomes (Becker *et al*., 2020; Abebe & Birhanu, 2023).

The interplay between beneficial microbes like *Lactobacillus casei* and pathogens such as *Staphylococcus sciuri* offers a promising area of study for developing alternative therapeutic strategies. As an alternative, probiotics offer a promising solution due to their ability to restore microbial balance, enhance immune function, and directly antagonize pathogenic bacteria through competitive exclusion and production of antimicrobial compounds (Ouwehand *et al*., 2002). Despite the growing interest in probiotic therapy, there is limited research on the specific effects of *Lactobacillus casei* in counteracting

infections caused by *Staphylococcus sciuri*. The hematological and immunological responses elicited by this probiotic in the context of such infections remain underexplored. Understanding these interactions is critical for developing safe, natural, and cost-effective interventions that address the dual challenges of infection control and antibiotic resistance. The aim of this study is to investigate the effects of *Lactobacillus casei* on enhancing immunity against *Staphylococcus scuri* infection in Albino rats (*Rattus norvegicus*), through hematological, microbiological, and histopathological screening.

2.Methodology

2.1 Study Area

The laboratory analysis for this study was conducted at the Microbiology Laboratory, University of Benin, and the Animal House and Laboratory of the Faculty of Pharmacy, University of Benin, Edo State, Nigeria. Ethical approval was obtained for the use of laboratory and animals. These facilities provided the controlled environments and equipment's necessary for microbial, pathological, and immunological investigations.

2.2 Sample Preparation and Experimental Design

Lactobacillus casei and *Staphylococcus sciuri* were obtained from Medical and Molecular Laboratory at Bayelsa State and were transported to the laboratory under aseptic conditions and cultured on de Man, Rogosa, and Sharpe (MRS) Agar and Manitol Salt Agar for confirmation of the isolates. The isolate was sub-cultured and maintained for subsequent experimental used.

The experimental design was structured to evaluate the immunomodulatory and therapeutic effects of *Lactobacillus casei* in mitigating *Staphylococcus sciuri* infections in Wistar albino rats. Twenty healthy Wistar albino rats, of eight weeks old and weighing between 180 to 200 grams, were selected for uniformity in physiological parameters. Animals were housed in polypropylene cages with sterilized bedding to reduce contamination risk and with 12hours light/dark cycle. Rats were fed with a standard rodent chow diet formulated to meet their nutritional needs and were provided with filtered water. All animals were acclimatized to laboratory conditions for 7 days before starting the experiments to mitigate stress-induced variability as described by Kuo *et al.* 2013.

The study involved five groups, each comprising four rats each. Serial dilution was carried out for *Lactobacillus casei* and *Staphylococcus sciuri*, the 1 x 10^8 CFU/ml suspension was used to challenge the animals orally by the used of gavage.

Group A (Control Group): Received no treatment or infection, serving as the baseline.

Group B (Infected Group): Exposed to only *Staphylococcus sciuri* infection at day 7 and without any treatment.

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

Group D (Prophylactic Group): Pre-challenged with *Lactobacillus casei* at day 7 before being challenged with *Staphylococcus sciuri*at day 14 to assess preventive effects.

Group E (Antibiotic Group): Challenged with *Staphylococcus sciuri* at day 7and treated with a standard antibiotic (ciprofloxacin) at day 14.

2.3 Blood Collection and Hematological Analysis

Rats were sacrificed and blood samples were collected at the end of the experiment (at day 21). Blood was taken through a cardiac puncture procedure into ethylenediamine tetraacetic acid (EDTA) tubes for hematological analysis. White blood cells (WBC), Lymphocyte percentage (LYM), Monocyte percentage (MID), Granulocyte percentage (GRAN), red blood cells (RBC), Hemoglobin (HGB), platelets (PLT), hemoglobin concentration (Hb), hematocrit (HCT), mean corpuscular volume (MCV),

mean corpuscular hemoglobin concentration (MCHC), Red Cell Distribution Width-Standard Deviation (RDW-SD), Red Cell Distribution Width-Coefficient of Variation (RDW-CV), Large Unstained Cell count (LP), Platelet count (PV), Differential White Blood Cell count (DW), Cell Type (CT), Lymphocyte-to-Cell Ratio (LCR) where analyzed as described by Galdeano and Perdigón.(2006).

2.4 Histopathology

Rats were dissected, and organs (spleen and liver) were harvested and immediately kept in plastic tubes containing 10% formalin. The organs were dehydrated by repeatedly immersing in 70% ethanol. Xylene was used to aid tissues penetration and also to make the tissues transparent. After that the xylene-infused tissues were infiltrated with molten embedding paraffin wax and kept at 60 ^{0}C in the oven for the solvent to evaporate and to solidify. Next, a section of the tissues was suspended on water and placed on glass slides and stained with hematoxylin and eosin stains. The plates were examined under a microscope at X400 magnification as described by Kuo *et al*. (2013) and Esposito *et al*. (2009).

2.5 Microbial Analysis of Stool Samples

Fecal samples were collected on 0, 7, 14 and 21 days. Approximately 1 g of stool was homogenized in 9 mL of sterile Phosphate buffer saline (PBS) and a tenfold serial dilution was performed creating dilutions from 10^{-1} to 10^{-6}. A 0.1 mL of 10^{-6} dilution was plated onto Nutrient agar plates in triplicates and incubated at 37°C for 24hrs. Following incubation, distinct colonies were counted, and the bacterial load was calculated. Results were expressed as colony-forming units (CFU) per gram using the formula below (NRC, 2011):

$$\frac{cfu}{ml} = \frac{numberofcoloniesxdilutionfold/series}{volumeofinoculum}$$

2.6 Microbial Analysis of Organ Samples

Also, tissue samples (spleen and liver) were aseptically collected from euthanized rats and weighed. Each tissue sample (1 g) was homogenized in9 mL of sterile PBS and a tenfold serial dilution was performed creating dilutions from 10^{-1} to 10^{-6}. A 0.1 mL of 10^{-6} dilution was plated onto Nutrient agar plates in triplicates and incubated at 37°C for 24hrs. Following incubation, distinct colonies were counted, and the bacterial load was calculated. Results were expressed as colony-forming units (CFU) per gram using the formula below (NRC, 2011):

$$\frac{cfu}{g} = \frac{numberofcoloniesxdilutionfold/series}{volumeofinoculum}$$

2.7 Statistical analysis

Analysis of variance (Mean ± S.D) was used to analyze the data. The Statistical Pack initial Age for Social Sciences (SPSS) program version 20.0 was utilized. P value < 0.05 was used as the significance level, and was analyzed by Turkey's test.

3.Results and Discussion

3.1 Body weight (g) and temperature (^{0}C) of wistar albino rats challenged with *Lactobacillus casei* and *Staphylococcus sciuri.*

The body temperature (^{o}C) of different groups of wistar albino rats challenged with *Lactobacillus casei* and *Staphylococcus sciuri* at different days (**Figure 6**). The temperature at day 0 was observed to be 36 ^{0}C in group A, B and E. At day 7, it was observed that there was no change intemperature in group A

(36.0 ^{o}C) but there was an increase in temperature in group B (36.8 ^{o}C), group C (37.2 ^{o}C), group D (37.3 ^{o}C) and group E (38 ^{o}C). At day 21, there was increase in body temperature in group A, B and D. Similarly, rats in group C maintained a stable body temperature of 37.6 °C, while the infected group showed an elevated temperature of 38.6°C, indicating a febrile response to infection, though there was no significant difference in temperature among the groups ($p>0.05$). Elevated body temperature in the infected group indicates an inflammatory response to the *Staphylococcus sciuri* infection. The maintenance of body temperature in the probiotic-administered and prophylactic groups suggests a potential protective effect of *Lactobacillus casei* against infection-induced fever. Probiotics have been reported to modulate immune responses, potentially influencing body temperature regulation (Raheem *et al.*, 2021). These findings align with previous research demonstrating that *L. casei* can enhance host resistance against infections.

Figure 6 showed weight of wistar rats at day 0, 7, 14 and 21 days challenged with *Lactobacillus casei* and *Staphylococcus sciuri.* The weight of the experimental animals at day zero ranged from 180 to 200 g. At day seven, It was observed that there was body weight gain of 220 g and 209.5 g in group A and C (control and probiotic groups) respectively. There was decrease in the body weight of the Wistar albino rats in group B (173 g), group D (173.3 g) and in group E (159 g) at day 7. Also, at day seven there was reduction in body weight (159 g) of group E, but after treatment at day 14, there was weight gain of 179.2 g at the end of 21 days.

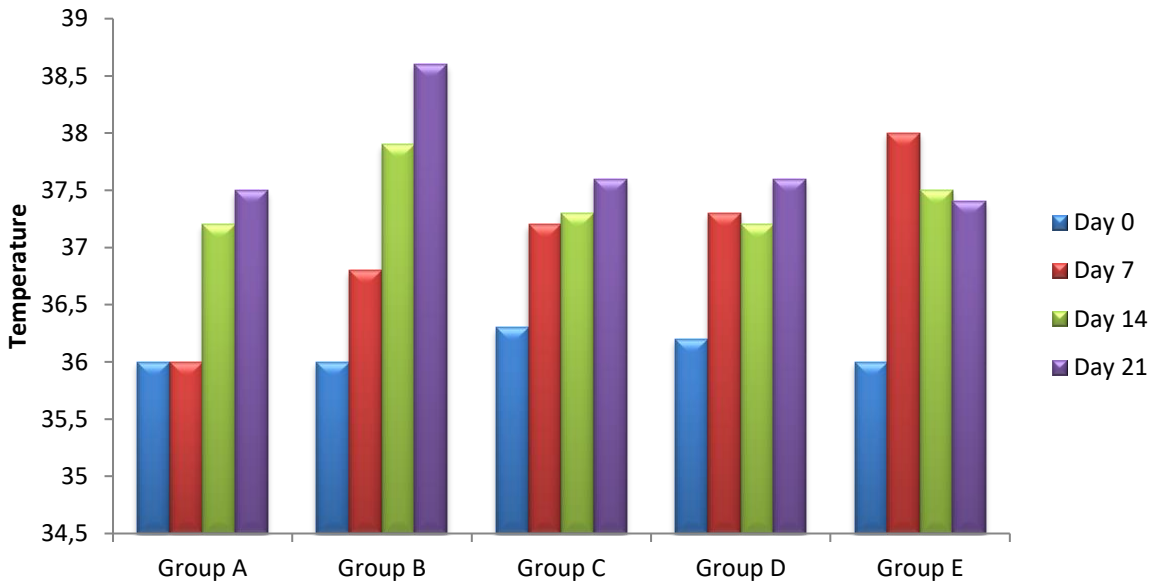


Figure 5: The body temperature (^{o}C) of different groups of wistar albino rats challenged with *Lactobacillus casei* and *Staphylococcus sciuri* at different days ($p>0.05$)
Key: A = Control group, B= Infected group (with only *Staphylococcus sciuri*), C= Probiotic group (Challenged with only *Lactobacillus casei*), D= Prophylactic group (Firstly, challenged with *Lactobacillus casei* and later with *Staphylococcus sciuri*) and E= Antibiotic group (Firstly, challenged with *Staphylococcus sciuri* and later treated with antibiotic).

The weight measurements, did not present any significant variations between the different treatment groups. The probiotic-administered group exhibited a significant weight increase, reaching 224.3 g by Day 21 and also the prophylactic group had increase in body weight (209 g) at day 21. This suggests that *L. casei* supplementation may support weight gain during infection. For instance, de Waard *et al.* (2002) reported that *L. casei* supplementation significantly reduced *Listeria monocytogenes* counts in various organs of infected rats, suggesting improved host defense mechanisms This result suggests that *Lactobacillus casei* may enhance weight gain by improving gastrointestinal health, nutrient absorption, and overall immune function, as reported by Nazir *et al.* (2018).

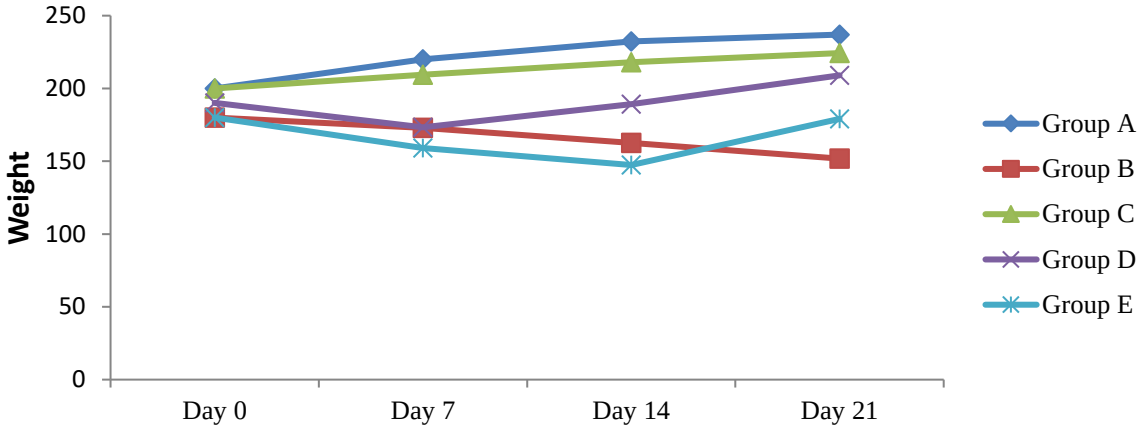


Figure 6: Body Weight of Wistar albino rats at day 0, 7, 14 and 21 days challenged with *Lactobacillus casei* and *Staphylococcus sciuri* (p>0.05).
Key: A = Control group, B= Infected group (with only *Staphylococcus sciuri*), C= Probiotic group (Challenged with only *Lactobacillus casei*), D= Prophylactic group (Firstly, challenged with *Lactobacillus casei* and later with *Staphylococcus sciuri*) and E= Antibiotic group (Firstly, challenged with *Staphylococcus sciuri* and later treated with antibiotic).

Conversely, the lowest weight was observed in the infected group (150 g), which can be attributed to the debilitating effects of the infection. This outcome aligns with studies by Kinsman and Arbuthnott (1980) who reported that staphylococcal infections in newborn mice inhibited normal weight gain, serving as an index of virulence. This is due to the pathogen's adverse impact on metabolism, appetite, and overall health. These findings emphasize the detrimental effects of *S. scuri* infection on the body's ability to sustain weight gain.

3.2 Hematological parameters of blood samples of the experimental animals

The Hematological parameters from blood samples of experimental animals at the end of the experiment is presented on Table 1. The White blood cells (WBC) of group A was 19.2 ± 2.00 10^3/µL, while group C was 14.5± 4.70 10^3/µL and group E was 9.5± 2.10 10^3/µL, with no statistical significance among the groups (p=0.68). The Lymphocyte percentage (LYM) was 84.3± 3.00 % for the control group and 73.4± 8.70 % for group E (p=0.19). The red blood cells (RBC) ranged from 9.87± 4.00 10^6/µL for group A to 5.7± 0.70 10^6/µL for group E. For hemoglobulin (HGB), control had the highest of 18.4± 1.00 g/dL followed by group C 15.9± 2.00 g/dL and group D had the least HGB (13.77± 1.35 g/dL), though not statistically significant (p=0.26). For Mean Corpuscular Volume (MCV), group A had the highest MCV of 93.6 ± 3.00 fL, followed by group C (probiotic group) with 80.7 ± 4.00 fL and there was significant difference among all groups (p=0.01).

The haematological analysis revealed significant variations in immune-related parameters among the different treatment groups. The probiotic-treated group (group C) exhibited a notable increase in total white blood cell (WBC) count (14.5± 4.70 103/µL) compared to the infected group (group B) (9.2 ± 2.00 103/µL), indicating an enhanced immune response. This finding corroborates earlier reports that probiotics stimulate leukocyte proliferation and enhance innate immunity (Fijan, 2023).

Lymphocyte counts were highest in the group A and C (84.3± 3.00% and 70.5± 5.00%, respectively), supporting previous findings that probiotics enhance adaptive immunity by promoting T-lymphocyte activity (Galdeano and Perdigón, 2006). Granulocyte counts were lower in the group C compared to group B, suggesting reduced inflammation and effective pathogen clearance (Yan and Polk, 2011). Red blood cell (RBC) counts, hemoglobin (HGB), and hematocrit (HCT) values were significantly lower in group B compared to group A and C, indicating the impact of bacterial infections on erythropoiesis. This aligns with previous reports that infections can cause hemolysis or anemia

through inflammatory cytokines (Weiss and Goodnough, 2005). The improved RBC and HGB levels in group C suggest that *Lactobacillus casei* supplementation aids in hematopoiesis and reduces infection-induced anemia (Asemi *et al*., 2011).

Table 1. Hematological parameters of experimental animals

Parameters	Group A	Group B	Group C	Group D	Group E	p value
WBC (10^3/µL)	19.2± 2.00	11.1 ± 4.10	14.5± 4.70	9.20 ± 2.00	9.50 ± 2.10	0.68
LYM (%)	84.3± 3.00	68.05 ± 7.40	70.5± 5.00	68.8 ± 6.80	73.4 ± 8.70	0.19
MID (%)	25.5± 5.00	17.55 ± 2.01	19.7± 1.00	19.00 ± 2.66	14.0 ± 3.12	0.07
GRAN (%)	20.2± 2.00	14.40 ± 6.00	14.6± 2.00	12.10 ± 5.10	12.5 ± 6.00	0.24
RBC (10^6/µL)	9.87± 4.00	6.63 ± 0.50	7.30± 1.00	5.89 ± 1.00	5.70 ± 0.70	0.09
HGB (g/dL)	18.4± 1.00	14.45 ± 1.37	15.9± 2.00	13.77 ± 1.35	14.2 ± 1.40	0.26
HCT (%)	53.6± 3.00	43.10 ± 2.80	48.1± 3.00	42.60 ± 3.33	44.6 ± 4.60	0.86
MCV (fL)	93.6± 3.00	65.10 ± 2.40	80.7± 4.00	73.6 ± 9.20	78.5 ± 6.50	0.01
MCH (pg)	32.4± 2.00	21.7 ± 0.74	20.1± 4.00	23.65 ± 2.37	24.9 ± 2.00	0.04
MCHC (g/dL)	39.3± 3.00	33.45 ± 1.00	39.4± 5.00	32.25 ± 1.30	31.8 ± 0.70	0.01
RDW-SD (fL)	38.5± 2.00	39.0 ± 2.00	43.7± 3.00	47.0 ± 6.30	48.1 ± 3.73	0.07
RDW-CV (%)	27.2± 1.00	17.1 ± 0.60	29.5± 3.00	18.72 ± 0.60	18.2 ± 0.64	0.03
PLT (10^3/µL)	712± 12.00	798.2 ± 397	562±18.00	821 ± 109	1143±876	0.31
MPV (fL)	8.20 ± 2.00	8.4 ± 0.50	11.8± 2.00	8.7 ± 0.47	10.0 ± 1.00	0.11
PDW (%)	9.70 ± 3.00	11.4 ± 1.60	15.2± 2.00	11.6 ± 0.87	15.5 ± 2.65	0.01
PCT (%)	0.58± 0.00	0.68 ± 0.37	0.52± 0.00	0.71 ± 0.10	1.20 ± 1.00	0.03
P-LCR (%)	7.0± 1.00	15.5 ± 1.00	21.2± 3.00	12.77 ± 2.91	21.6 ± 4.10	0.01

Key: Values are presented as mean ± SD, **WBC** (White Blood Cellcount), **LYM** (Lymphocyte percentage), **MID** (Monocyte percentage), **GRAN** (Granulocyte percentage), **RBC** (Red Blood Cellcount), **HGB** (Hemoglobin), **HCT** (Hematocrit), **MCV** (Mean Corpuscular Volume), **MCH** (Mean Corpuscular Hemoglobin), **MCHC** (Mean Corpuscular Hemoglobin Concentration), **RDW-SD** (Red Cell Distribution Width-Standard Deviation), **RDW-CV** (RedCell Distribution Width-Coefficient of Variation), **LP** (Large Unstained Cellcount), **PV** (Platelet count), **DW** (Differential White Blood Cellcount), **CT** (Cell Type), **LCR** (Lymphocyte-to-Cell Ratio),A = Control group, B= Infected group (with only *Staphylococcus sciuri*), C= Probiotic group (Challenged with only *Lactobacillus casei*), D= Prophylactic group (Firstly, challenged with *Lactobacillus casei* and later with *Staphylococcus sciuri*) and E= Antibiotic group (Firstly, challenged with *Staphylococ cussciuri* and later treated with antibiotic).

3.3 The Mean heterotrophic bacteria count of stool Samples and organs of Wistar albino rats.

The Mean heterotrophic bacteria count of stool Samples of Wistar albino rats is presented on Table 2. The mean heterotrophic bacteria count at day zero ranged from $2.05 \times 10^6 – 2.34 \times 10^6$ cfm/ml. while the mean heterotrophic bacteria count of Wistar albino rat at day 21 showed that group A had the least mean bacteria count of 4.20×10^6 cfm/ml and the highest occurrence was in group B (6.30×10^6 cfm/ml). The probiotic group had intermediate counts, indicating a potential role in controlling bacterial proliferation. This aligns with findings by de Waard *et al*. (2002), who reported that *L. casei* supplementation reduced *L. monocytogenes* counts in the gastrointestinal tract and systemic organs, suggesting enhanced antimicrobial defenses. This outcome supports the hypothesis that probiotics can exert antimicrobial effects by competing with pathogens for resources, producing antimicrobial peptides, or modulating the host immune system (Parvez *et al*., 2006; Nair *et al*., 2017; Raheem *et al*., 2021).

Table 3; The examination of organ weights reveals a marked difference within the groups. The weights of liver was highest in group A (6.42 g) and lowest was in group E (2.53 g). The probiotic-administered group (group C) had a liver weight of 5.93 g, indicating that *L. casei* may help preserve liver mass during infection. Spleen weights was highest in group E (1.12 g) and lowest in group A (0.86 g). The probiotic group had a spleen weight of 0.98 g, suggesting a potential role in maintaining spleen health. This finding is consistent with reports of Kuo *et al*. (2013) and Esposito *et al*. (2009), which noted that probiotics can affect liver weight as part of their broader physiological influence.

The Mean heterotrophic bacteria count from organs (liver and spleen) of Wistar albino rats represented on Table 4. Group B had the highest bacteria count of 25.40 x 10^3 CFU/ml for liver and group D had the

highest count for spleen. Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits to the host. They play a pivotal role in maintaining the balance of the gut microbiota, thereby supporting digestive health and modulating the immune system (Sánchez *et al.*, 2017). Infections caused by pathogenic bacteria such as staphylococcal infections pose significant health challenges worldwide. *Staphylococcus spp.* are capable of causing a range of infections, from mild skin conditions to severe systemic diseases. The increasing prevalence of antibiotic-resistant strains has further complicated the treatment and management of these infections (Choudhur *et al.*, 2012). Consequently, there is a growing interest in alternative therapeutic strategies, including the use of probiotics, to enhance the body's natural defense mechanisms against such pathogens.

Table 2. Mean heterotrophic bacteria count (x 10^6 cfu/ml) of stool Samples of Wistar albino rats

	Days			
Groups	**0**	**7**	**14**	**21**
Group A	2.05± 0.85	2.17± 0.41	4.30± 0.30	4.20± 0.00
Group B	3.35± 0.45	3.50± 0.30	4.00± 1.41	6.30± 0.50
Group C	2.33± 0.42	2.35± 0.45	4.50± 0.50	5.21± 0.10
Group D	3.03± 0.35	3.49± 0.35	5.60± 0.60	5.20± 0.00
Group E	2.34± 1.03	4.00± 0.50	5.42± 0.28	4.30± 0.43

Key: Values are presented as mean ± SD,A = Control group, B= Infected group (with only *Staphylococcus sciuri*), C= Probiotic group (Challenged with only *Lactobacillus casei*), D= Prophylactic group (Firstly, challenged with *Lactobacillus casei* and later with *Staphylococcus sciuri*) and E= Antibiotic group (Firstly, challenged with *Staphylococcus sciuri* and later treated with antibiotic).

Table 3. Mean Weight of Harvested Organs of Experimental Rats

Groups	Liver Weight (g)	Spleen Weight (g))
A	6.42	0.86
B	5.93	0.98
C	5.62	1.105
D	5.33	0.91
E	2.53	1.12

Key: Values are presented as mean = A = Control group, B= Infected group (with only *Staphylococcus sciuri*), C= Probiotic group (Challenged with only *Lactobacillus casei*), D= Prophylactic group (Firstly, challenged with *Lactobacillus casei* and later with *Staphylococcus sciuri*) and E= Antibiotic group (Firstly, challenged with *Staphylococcus sciuri* and later treated with antibiotic).

Table 4. Mean heterotrophic bacteria count (× 10^3cfu/g) from organs of Wistar albino rats

Groups	Liver	Spleen
A	5.70± 0.00	4.90± 0.00
B	25.40± 0.00	6.15± 0.30
C	17.60± 0.50	6.60± 0.30
D	9.30± 0.00	16.15± 0.30
E	4.20± 0.00	2.45± 0.30

Key: Values are presented as mean ± SD, A = Control group, B= Infected group (with only *Staphylococcus sciuri*), C= Probiotic group (Challenged with only *Lactobacillus casei*), D= Prophylactic group (Firstly, challenged with *Lactobacillus casei* and later with *Staphylococcus sciuri*) and E= Antibiotic group (Firstly, challenged with *Staphylococcus sciuri* and later treated with antibiotic).

3.4 Histopathology of the organs of Wistar albino rats

The pathological screening of spleen sections is presented in **Figure 7**. The Control group showed normal white pulp with lymphocytes, and red pulp with red blood cells, indicating healthy splenic tissue, whilethe probiotic group which shows dense lymphocyte population, minimal damage, and no significant inflammatory cell infiltration. Histopathological findings offer valuable insights into the structural integrity of tissues in response to infection and treatment. It was observed in this study, the spleen tissue in the control group (Group A) exhibited normal architecture, with distinct white and red pulp, consistent with healthy immune tissue. In the probiotic-treated group (Group C), the spleen tissue showed some mild lymphocytic infiltration, suggesting that *Lactobacillus casei* induced a mild immune response, likely enhancing immune surveillance without causing excessive inflammation. This is consistent with findings by Sherwood and Toliver-Kinsky, (2004), who noted that bacterial infections often causes tissue damage due to excessive immune activation and the release of inflammatory mediators. **Figure 8** shows pathological screening of liver sections. Group A showed normal hepatocytes with eosinophilic cytoplasm, while group Bshowed mild inflammatory infiltration.

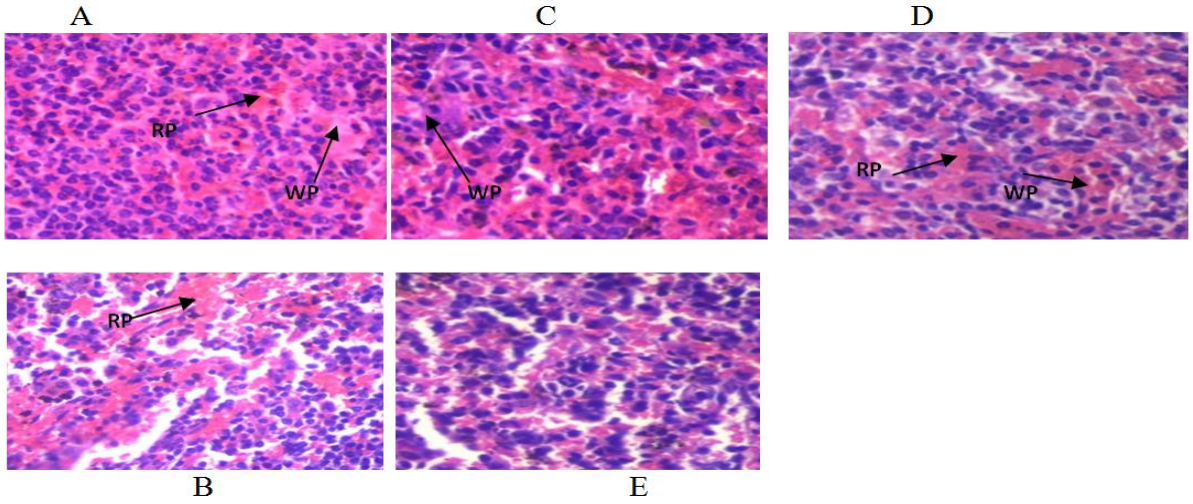


Figure 7: Micrograph of Wistar albino rats spleen at X400with arrows pointing at histological structures of spleen white pulp (WP), red pulp (RP).

Key:A = Control group, B= Infected group (with only *Staphylococcus sciuri*), C= Probiotic group (Challenged with only *Lactobacillus casei*), D= Prophylactic group (Firstly, challenged with *Lactobacillus casei* and later with *Staphylococcus sciuri*) and E= Antibiotic group (Firstly, challenged with *Staphylococcus sciuri* and later treated with antibiotic)

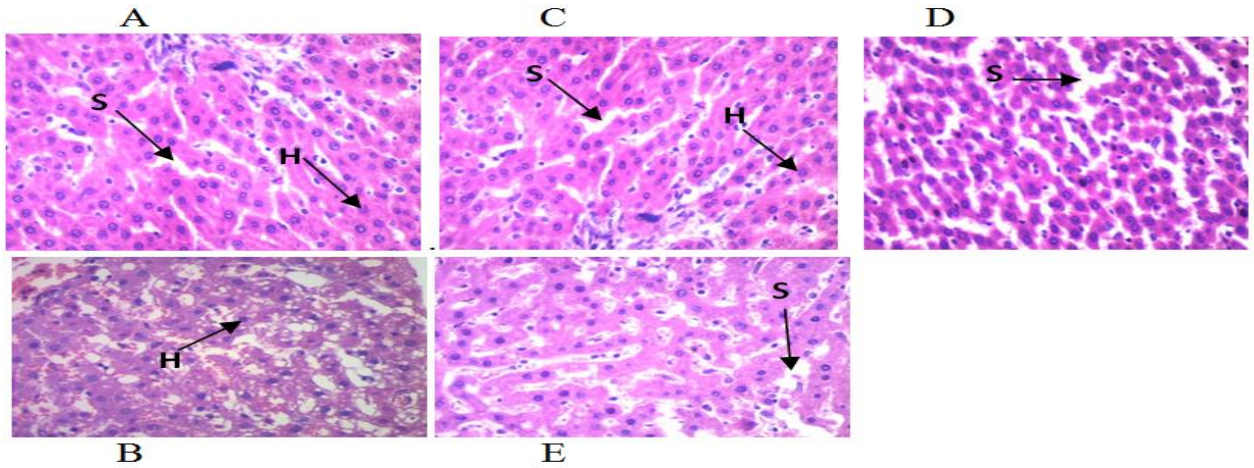


Figure 8: Micrograph of Wistar albino rats liver at X400with arrows pointing at histological structures of Liver Hepatocytes (H), Sinusoids (S)

Key:A = Control group, B= Infected group (with only *Staphylococcus sciuri*), C= Probiotic group (Challenged with only *Lactobacillus casei*), D= Prophylactic group (Firstly, challenged with *Lactobacillus casei* and later with *Staphylococcus sciuri*) and E= Antibiotic group (Firstly, challenged with *Staphylococcus sciuri* and later treated with antibiotic).

The probiotic group (group C) is showing normal hepatocytes with eosinophilic cytoplasm with no tissue damage. *Lactobacillus casei* is known to provide protective effects against the pathogenic infection. In the current study, the milder tissue damage in the probiotic-treated group (Group B) suggest that *Lactobacillus casei* not only enhanced the immune response but also contributed to the resolution of the infection by inhibiting the growth of *S. scuri*. The histopathological analysis further supports the notion that *Lactobacillus casei* can reduce immune-mediated tissue damage. The mild inflammatory changes observed in the probiotic-treated group (Group C) suggest that *Lactobacillus casei* modulates the immune system to prevent excessive inflammation, a common problem in bacterial infections (Qin *et al*., 2022). This is consistent with findings from Lukic *et al*. (2017) and Yousefi *et al*. (2019), who reported that probiotics help in the regulation of immune responses, thereby reducing inflammation and promoting tissue repair.

Conclusion

The findings indicate that *Lactobacillus casei* not only demonstrates antagonistic properties against pathogenic bacteria but also significantly affects hematological parameters that reflect an enhanced immune response. This highlights the probiotic's promise as a valuable adjunct in the prevention and management of bacterial infections. This study recommends that further clinical and preclinical research to assess the effectiveness of probiotic treatment

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