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Hematological modulatory effects of *Weissella* and *Pediococcus* sp. on formalin-induced inflammation in wistar rats

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Abstract. *Oladejo BO, Oluwasola HM. 2022. Hematological modulatory effects of* Weissella and Pediococcus *sp. on formalin-induced inflammation in wistar rats. Asian J Trop Biotechnol 19: 28-34.* The activity of three LAB strains, *Weissella cibaria* II-1-59, *Weissella confusa* JMC 1093, and *Pediococcus pentosaceus* DSM20336, isolated from a Nigerian locally fermented food condiment; 'iru' on blood parameters of acutely inflamed rats using formalin (1%) was investigated in this study. The rats were distributed into six groups (A-F). Rats in Groups A were neither administered formalin nor treated with LAB, while Group B received formalin injection only. Groups C-E were treated with the strains of LABs (2 × 107 CFU/mL), while Group F received diclofenac sodium treatment (150 mg/kg body weight) following formalin administration. In addition, Erythrocyte sedimentation rate **(**ESR), total and differential white blood cell (WBC) count, and total red blood cell (RBC) count were analyzed using standard methods. LAB Treatments significantly reduced the ESR of the blood of LAB-treated rats (1.00±0.00 mm/hr at 1 hour) at $P<0.05$ and regulated leucocyte infiltration in the blood circulation (*W. cibaria* treated group with a neutrophil count of 13.67±1.20% at 336 hrs) compared to diclofenac sodium. These findings revealed that the LAB were good immune modulators and would be effective agents for the treatment of inflammation-induced anemia.

Keywords: Formalin, hematology, inflammation, *Lactobacillus*, edema

INTRODUCTION

Inflammation is a routine immune response used to subdue infectious agents or infections that get into the body tissues (Paramita et al. 2017; Szalay 2018). This inflammatory reaction usually facilitates healing processes, however, it may become fatal if uncontrolled (Sung-Min et al. 2019). Anemia of inflammation (AI), also referred to as anemia of chronic disease or anemia of chronic disorders, is occasionally characterized by a mild to moderately severe anemia that arises during infection, such as in the case of inflammatory disease and or malignancy (Nemeth and Ganz 2014). Unfortunately, there seem to be less efficient laboratory measures required to assess patients suffering from chronic inflammation. Also, diagnoses are only initiated when the inflammation occurs alongside other medical conditions, as observed in the case of cytokine storms linked with the severity of COVID-19 infection.

The development of AI within the first few hours after the commencement of inflammation is sustained by the activation of immune cells and the release of pro-inflammatory chemical mediators such as interferon interferon-γ, interleukin-1, interleukin 9, and tumor necrosis factor-α (TNF-α) (Nairz et al. 2016). As such, alleviating these pro-inflammatory cytokines is highly important for improving inflammatory disorders (Oladejo and Oluwasola 2021). Also, a mild reduction in erythrocyte survival combined with the weakened production of erythrocytes has been linked with AI disorder. This increased destruction of the erythrocytes is mainly associated with the activation of macrophages by inflammatory cytokines; however, other hemolytic mechanisms may play a significant role in specific inflammatory disease conditions. Direct cytokine reaction on the erythropoietic precursors and iron restriction associated with the suppression of erythrocyte production. This can lead to a reduction in the numbers but usually of normal hemoglobin content and size (normocytic, normochromic anemia) (Nemeth and Ganz 2014).

Anemia of inflammation can, therefore, significantly contribute to the morbidity of arrays of inflammatory disorders or diseases occasionally in patients. Treatment options include blood transfusion (although risk associated) as well as the use of iron supplements and erythropoiesis-stimulating agents (ESAs) via intravenous administration (Steinbicker et al. 2011). Although some acceptable outcomes have been achieved with the use of conventional drugs such as non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and herbal supplements in the treatment of inflammatory disorders, a life-threatening condition of chronic cases which poses a major threat to the health status and longevity of individuals persist (Barcelos et al. 2019). As such, there is the need to explore more naturally available alternative therapy for AI., such as in the case of lactic acid bacteria, which are generally regarded as safe (GRAS).

Fermented locust bean ('Iru') is a popular food condiment consumed in Nigeria and other West African countries. It is a very good source of many beneficial microbial strains, particularly the genera of *Bacillus*, *Lactobacillus*, *Leuconostoc*, and *Pediococcus*. Also, it is

often consumed as a good alternative to meat due to its high protein, fat, tannin, vitamins, and mineral contents. These organisms form part of major probiotics, which are live organisms that confer significant health benefits when administered in sufficient amounts in the host (Afolabi et al. 2016). Lactic acid bacteria (LAB) are also known as probiotics and can serve as immune modulators (Pessione 2012). They have been isolated from various fermented foods and used in the production of various fermented foods and beverages. A previous study by Oladejo and Oluwasola (2021) showed that *Weissella* and *Pediococcus* sp. have anti-inflammatory potentials and suppressed the effect of acute inflammation in rats via cytokine regulations (interleukin-10, C-reactive protein, and transforming growth factor-β). Formalin is a strong chemical that has been predominantly explored in the induction of inflammation. Formalin-induced paw edema in a rat is, therefore, one of the most suitable tests to screen for inflammatory responses. Therefore, this study aims to assess the effect of oral administration of lactic acid bacteria isolated from indigenous fermented locust beans ('Iru') on the hematological parameters of Wistar rats induced with formalin.

MATERIALS AND METHODS

Collection of fermented locust bean ('Iru')

Fermented locust bean ('Iru') was purchased from Oba market in Akure, Ondo State (7°10'N 5°05'E), Nigeria. They were put in a sterile polythene bag and transported immediately to the Department of Microbiology Laboratory, Federal University of Technology, Akure, Nigeria, for bacteriological analysis.

Isolation and identification of lactic acid bacteria (LAB)

Lactic acid bacteria from the fermented locust bean ('Iru'), including *Weissella cibaria* II-1-59, *Weissella confusa* JMC 1093, and *Pediococcus pentosaceus* DSM20336 were isolated and identified as described in the previous work of Oladejo and Oluwasola (2021). Briefly, molecular identification of the isolated LAB was carried out by harvesting cell pellets from 2 mL of overnight cultures (up to 2×10^9 cells) of LAB grown in MRS broth and DNA extraction was done using a JenaBioscience DNA Purification Kit following the manufacturer's instructions. Polymerase chain reaction (PCR) was carried out to amplify the 16SrRNA gene of the bacteria using the primer pair 27F-5′-AGAGTTTGATCCTGGCTCAG-3', and 1492R 5'GGTTACCTTGTTACGACTT-3'. The amplified product was separated on a 1.5% agarose gel and electrophoresis was carried out at 80 V for 1 hour 30 minutes. Following electrophoresis, DNA bands were visualized by ethidium bromide staining with 100 bp DNA ladder (Solis Biodyne) used as DNA molecular weight marker (Oladejo and Oluwasola 2021). They were BLAST-searched to detect similar sequences in the NCBI database (https://www.ncbi.nlm.nih.gov).

Inflammatory drugs and chemicals

Formalin (Pascal Biosciences) and diclofenac sodium drug (Impulse Pharma Pvt. Ltd, Boisar, India. Expiry date; March, 2023) were purchased in Akure, Nigeria.

Animal and experimental design

Eighty-four (84) male Wistar rats weighing 120 -150 kg used in this research were obtained from the Department of Microbiology, Federal University of Technology, Akure (FUTA), Nigeria. They were housed in cages with wire screen tops and maintained under adequate ventilation and environmental temperature. The animals were maintained on a commercial rat chow with tap water, food (finisher) provided and acclimatized for one week before the experimental session. All the experimental procedures were carried out following the guidelines of the Institutional Animals Ethics Committee of the Federal University of Technology Akure, Nigeria. Ethical permission was sought from FUTA Research Ethical Committee (FUTA/ETH/21/06).

Evaluation of anti-inflammatory activity

Inflammation was induced by sub-plantar injection of 0.1 mL of 1% freshly prepared formalin into the right hind paws of all the rat groups except the Group A rats (formalin control). After inflammation had been induced, the rats were divided into 5 groups (Groups B-F) of 14 rats each. Rats in Group B were not treated following inflammation (negative control); rats in Group C, D, and E were treated orally with 2×10^7 CFU/mL of *W. cibaria* II-1-59, *W. confusa* JMC 1093, and *P. pentosaceus* DSM20336 respectively while rats in Group F (positive control) were treated with diclofenac sodium (150 mg/Kg body weight) after the development of paw edema in all the rats. Changes in rat paw thickness were measured at 20 min before and after injection at different time intervals (0, 1, 4, 8, 24, 72, 168 and 336 h) using a digital vernier caliper and were measured in millimeters (mm) (Amdekar et al. 2012).

Blood sample collection

At earlier intervals, the rats were sacrificed through cardiovascular bleeding according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA). Then, blood samples were collected into an EDTA bottle for hematological assays. The hematological parameters such as erythrocyte sedimentation rate **(**ESR), total and differential white blood cell count, and total red blood cell count were analyzed using the method of Valentini et al. (2015) as stated below.

Determination of erythrocyte sedimentation rate (ESR)

Erythrocyte Sedimentation Rate (ESR) was estimated by adding dilution of the blood sample with sodium citrate anticoagulant into a Westergren tube until the blood level reached 100 millimeters (mm). The tubes were stored vertically and allowed to sit at room temperature for an hour. The distance between the top of the blood mixture and the top of the sedimentation of RBCs was measured. The ESR (mm) was calculated using the formula: ESR (mm) = Final reading – initial reading.

Determination of white blood cell count (WBC)

The blood was first diluted in ratio 1: 20 and 0.02 mL of the blood was pipetted into 0.38 mL of diluting fluid, Turk's reagent (3 % acetic acid with crystal violet dye). Then, a small portion was charged into the counting chamber and observed using x40 objective to count the cell in cells/cubic mm. Finally, the total number of WBCs counted was calculated using the formula:

WBCs = number of cells counted x depth factor (10) x dilution factor x area factor (0.25).

Determination of red blood cell count (RBC)

The blood sample was diluted 1: 200 and mixed properly. About 0.02 mL of the blood was pipetted into 4 mL of diluting fluid in a bijou bottle and was mixed thoroughly by alternately drawing up expelling fluid. A Pasteur pipette was used to fill the counting chamber and red blood cells were counted under x40 objective. The total number of RBCs counted was calculated using the formula:

RBCs = number of cells counted x dilution factor (200) x 1/5 x area factor (0.1 mm^3).

Determination of leucocyte differential count

A drop of blood was thinly spread over a glass slide and air dried. The smear was covered with Leishman stain and allowed to stand for 2 minutes after which it was buffered with distilled water and allowed to stand for 10 minutes. The slide was then rinsed thoroughly with distilled water, air dried and viewed under a microscope. Different WBC (neutrophil, eosinophil, lymphocyte and monocyte) were counted and numbers recorded.

Statistical analysis

Data were expressed as the mean ± standard error mean (SEM) calculated over an independent time frame of experiments performed in duplicate. One-way analysis of variance (ANOVA) was applied, followed by a post hoc test; Bonferroni's Multiple Comparison Test for difference between treatments groups compared with control (Group B) using GraphPad Prism version 5.0.

RESULTS AND DISCUSSION

Effects of oral treatment of LAB on erythrocyte sedimentation rate (ESR)

The sub-plantar injection of formalin into the right hind paw of the rats caused an increase in the erythrocyte sedimentation rate (ESR) of the injected groups reaching the maximum rate at 1 hour and then reducing continuously till the end of the experiment, as shown in Figure 1. In Group A rats (formalin control), the ESR was 0.30±0.00 mm/hr at 1 hour, which remained the same throughout the period of the experiment. The highest erythrocyte sedimentation rate (4.00±0.00 mm/hr) was observed in group B and C rats 1 hour after the injection, which was significant at $p<0.05$. Group E and F rats showed the lowest ESR of 1.00±0.00 mm/hr at 1 hour. A significant decrease was immediately observed in every group except Group A after 1 hour and continued till the end of 336 hours.

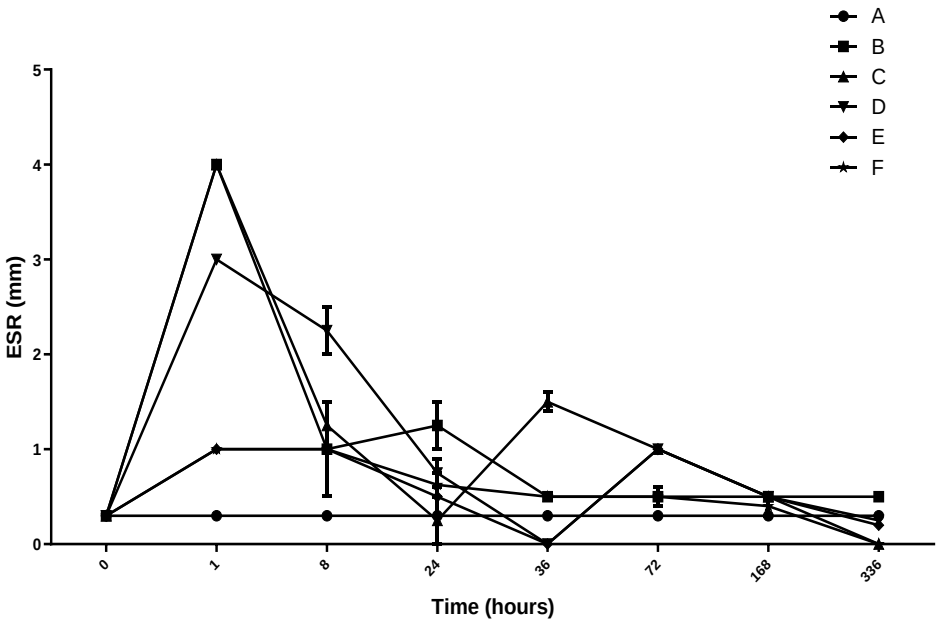

Figure 1. Effect of oral treatment of LAB on erythrocyte sedimentation rate [ESR] (mm). Note: n =2 (significant at P<0.05). Data are expressed as mean ± standard error of two rats per group. Group A: formalin control; Group B: formalin only (negative control); Group C: treated with *W. cibaria* II-1-59; Group D: treated with *W. confusa* JMC 1093; Group E: treated with *P. pentosaceus* DSM20336; Group F: treatment with diclofenac sodium (positive control)

Effects of oral treatment of LAB on the total white blood cell count of rats

In Group A (formalin control group), total white blood cell count at 1 hour was $4.68\pm0.00 \times 10^3$ μL^{-1} which remained the same throughout the period of the experiment. The WBC count of Group B rats at 1 hour was $3.40\pm0.38 \times 10^3$ μL^{-1} which increased gradually till the end of the experiment. LAB treated Groups C, D and E rats showed WBC counts of $2.85\pm0.00 \times 10^3$ μL^{-1}, $5.55\pm0.00 \times 10^3$ μL^{-1} and $3.95\pm1.60 \times 10^3$ μL^{-1}, respectively, at 1 hour which increased up to the end of 36 hrs, followed by a decrease at 72 hours till 336 hours. Group F rats (Diclofenac treated group) showed the most decrease in the WBC count at 1 hour to be $2.43\pm0.75 \times 10^3$ μL^{-1} as shown in Table 1.

Effects of oral treatment of LAB on the total red blood cell count of rats

Total red blood cell count of Group A rats (formalin control) at 1 hour was $8.65\pm0.41 \times 10^6$ μL^{-1} which remained constant throughout the period of the experiment. In untreated Group B rats, a continuous low RBC count was observed until after 168 hrs. LAB treated Groups C, D and E rats showed RBC counts of $6.00\pm0.03 \times 10^6$ μL^{-1}, $4.83\pm0.12 \times 10^6$ μL^{-1} and $4.33\pm0.06 \times 10^6$ μL^{-1}, respectively, within the first hour of the experiment. An increase in RBC count was observed at 8 hours and then continued till the end of the experiment. Relatively low RBC count was observed in Group F (Diclofenac treated group) until the end of 72 hours compared with other treatment groups, as shown in Table 2.

Effects of LAB treatment on the percentage of circulating lymphocytes in rats

The percentage circulating lymphocyte count of 80.50±2.02% was observed in Group A rats (formalin control) at 1 hour, which remained constant throughout the period of the experiment. After 1 hour, a significant reduction in the population of circulating lymphocytes was observed in all other groups. In untreated Group B rats, there was a continuous reduction in the percentage of circulating lymphocyte count till the end of 72 hours. But in Group C, D, E, and F, a significant increase was observed after 24 hours and continued till the end of the experiment. The percentage of circulating lymphocyte count observed in Group F (Diclofenac treated group) at 1 hour was lower compared with other treatment groups, as shown in Table 3.

Table 1. Effects of LAB treatment on the total white blood cell count (WBC) x 10^3 μL^{-1}

Groups	Time (hrs)						
	1	8	24	36	72	168	336
A	4.68±0.00**	4.68±0.00**	4.68±0.00**	4.68±0.00**	4.68±0.00**	4.68±0.00**	4.68±0.00**
B	3.40±0.38	3.55±2.08	4.00±0.18	5.05±0.25	5.25±0.38	5.40±0.15	5.50±0.05
C	2.85±0.00**	5.50±0.00**	9.05±0.03	11.40±0.00**	9.10±0.00**	8.55±0.00**	4.85±1.83**
D	5.55±0.00	5.40±0.00**	10.07±0.60**	11.95±0.05**	9.45±0.00**	4.75±0.00**	4.15±1.73**
E	3.95±1.60	6.45±0.00	8.20±1.68	8.65±0.67	8.55±0.00	7.60±0.00	5.15±0.00
F	2.43±0.75**	3.85±0.00	6.20±0.90	9.55±0.00	3.52±1.17	2.50±0.00	5.00±0.00

Note: Data are expressed as mean ± standard error of two rats per group. Group A: formalin control; Group B: formalin only (negative control); Group C: treated with *W. cibaria* II-1-59; Group D: treated with *W. confusa* JMC 1093; Group E: treated with P. pentosaceus DSM20336; Group F: treatment with diclofenac sodium (positive control). **Difference between comparison treatment group was considered statistically significant at $P < 0.05$ when compared with control group (B)

Table 2. Effects of LAB treatment on the total red blood cell count (RBC) x 10^6 μL^{-1}

Groups	Time (hrs.)						
	1	8	24	36	72	168	336
A	8.65±0.41**	8.65±0.41**	8.65±0.41**	8.65±0.41**	8.65±0.41**	8.65±0.41**	8.65±0.41**
B	4.15±0.43	4.48±0.50	4.30±0.65	5.23±0.50	5.69±0.42	5.50±0.75	8.05±0.54
C	6.00±0.32**	6.10±0.03**	6.82±0.08**	7.45±0.31**	6.09±0.11	7.40±0.20**	8.24±1.10
D	4.83±0.12	6.05±0.34**	6.15±0.33**	7.25±0.28**	7.55±0.06	7.73±0.36**	7.91±0.28
E	4.33±0.06	6.52±0.66**	7.08±0.90**	7.21±0.15**	7.86±1.33**	7.48±0.26**	8.25±0.27
F	3.81±0.75	4.71±0.13	5.65±0.18	5.74±0.52	6.64±0.18	9.72±1.15	9.11±1.08

Note: Data are expressed as mean ± standard error of two rats per group. Group A: formalin control; Group B: formalin only (negative control); Group C: treated with *W. cibaria* II-1-59; Group D: treated with *W. confusa* JMC 1093; Group E: treated with *P. pentosaceus* DSM20336; Group F: treatment with diclofenac sodium (positive control). **Difference between comparison treatment group was considered statistically significant at $P < 0.05$ when compared with control group (B)

Table 3. Effects of LAB treatment on total percentage circulating lymphocyte count (%)

Groups	Time (hrs.)						
	1	8	24	36	72	168	336
A	80.50±2.02	80.50±2.02**	80.50±2.02**	80.50±2.02**	80.50±2.02	80.50±2.02	80.50±2.02
B	76.00±2.08	61.33±0.88	61.67±2.73	64.67±1.20	73.33±1.45	75.33±2.40	81.67±0.88
C	71.00±1.00	68.00±1.15	58.67±2.91	75.67±1.20**	78.67±1.20	80.00±1.15	83.33±1.45
D	72.00±1.73	70.67±2.33**	64.00±1.73	76.66±1.33**	75.33±2.40	78.00±1.53	79.67±3.28
E	69.34±1.33	67.00±1.53	63.00±1.15**	74.67±0.33**	78.33±1.20	82.00±1.73	85.33±1.33
F	62.00±1.53	60.00±0.58	69.00±1.15	68.00±1.53**	71.00±0.57	80.34±1.45	81.00±0.58

Note: Data are expressed as mean ± standard error of two rats per group. Group A: formalin control; Group B: formalin only (negative control); Group C: treated with *W. cibaria* II-1-59; Group D: treated with *W. confusa* JMC 1093; Group E: treated with *P. pentosaceus* DSM20336; Group F: treatment with diclofenac sodium (positive control). **Difference between comparison treatment group was considered statistically significant at P <0.05 when compared with control group (B)

Effects of LAB treatment on the percentage of circulating neutrophils in rats

Sub-plantar injection of formalin into the rat paw caused infiltration of neutrophils in blood circulation, which led to an increase in the percentage circulating neutrophil count in all the injected Groups within 8 hours of the experiment. In Group A rats (formalin control), the total percentage neutrophil count was 17.50±1.44% at 1 hour, which remained the same throughout the period of the experiment. The highest percentage circulating neutrophil count (38.33±4.33%) was observed in Group B rats at 8 hours, while the lowest was observed in Group C rats (13.67±1.20%) at 336 hours. In LAB-treated Groups C, D, and E rats, a significant decrease in percentage circulating neutrophil count was observed after 24 hours and then continued till the end of the experiment. The percentage neutrophil count observed in Group F (Diclofenac treated group) was higher when compared with other treatment groups, as shown in Table 4.

Effects of LAB treatment on the percentage of circulating monocyte in rats

In Group A rats (formalin control), the percentage circulating monocyte count was 1.67±0.33% at 1 hour, which remained the same throughout the period of the experiment. A significant increase in the population of circulating monocyte was observed in all other groups after 1 hour. The highest percentage of circulating monocyte was observed in Group B rats (3.82±0.00%) at 8 hours, while the lowest was observed in Groups C and E rats (0.67±0.33%) at 336 hours. In LAB treated groups, a significant reduction in the population of circulating monocyte was observed after 8 hours which continued till the end of 336 hours, as shown in Table 5.

Table 4. Effects of LAB treatment on total percentage circulating neutrophil count (%)

Groups	Time (hrs)						
	1	8	24	36	72	168	336
A	17.50±1.44**	17.50±1.44**	17.50±1.44**	17.50±1.44**	17.50±1.44**	17.50±1.44**	17.50±1.44
B	24.33±2.33	38.33±4.33	35.00±2.89	32.33±1.20	22.67±1.20	24.67±0.88	16.00±1.00
C	25.67±0.33	31.66±1.20	26.67±1.76**	22.00±1.15**	19.00±0.58	16.67±0.88**	13.67±1.20
D	27.00±1.15	32.33±2.67**	29.67±3.84**	19.00±1.15**	22.00±3.51	17.67±0.88	15.67±2.85
E	27.33±1.20	32.33±2.02	24.00±1.16**	23.33±1.45**	18.67±1.76	15.33±1.76**	14.67±1.45
F	36.33±1.67**	33.00±0.58	36.00±1.16	27.00±2.08**	23.00±1.73	18.67±1.45	18.00±0.58

Note: Data are expressed as mean ± standard error of two rats per group. Group A: formalin control; Group B: formalin only (negative control); Group C: treated with *W. cibaria* II-1-59; Group D: treated with *W. confusa* JMC 1093; Group E: treated with *P. pentosaceus* DSM20336; Group F: treatment with diclofenac sodium (positive control). **Difference between comparison treatment group was considered statistically significant at P <0.05 when compared with control group (B)

Table 5. Effects of LAB treatment on total percentage circulating monocyte count (%)

Groups	Time (hrs)						
	1	8	24	36	72	168	336
A	1.67±0.33	1.67±0.33	1.67±0.33	1.67±0.33	1.67±0.33	1.67±0.33	1.67±0.33
B	1.00±0.33	3.82±0.00	3.00±0.33	2.00±0.58	2.33±0.66	1.67±0.33	1.33±0.33
C	1.67±0.33	3.33±0.33**	2.66±0.33	2.33±058	2.00±0.33	1.33±0.58	0.67±0.00**
D	2.33±0.33	3.67±0.33**	3.34±0.33**	2.67±0.33**	2.33±0.33	1.67±0.33	1.70±0.00
E	2.34±0.33**	2.67±0.33	2.33±0.33	2.00±0.58	1.33±0.33	1.00±0.33	0.67±0.00**
F	1.67±0.33	2.00±0.58	3.00±0.58	2.33±0.33	0.33±0.00**	1.33±0.00	1.00±0.33

Note: Data are expressed as mean ± standard error of two rats per group. Group A: formalin control; Group B: formalin only (negative control); Group C: treated with *W. cibaria* II-1-59; Group D: treated with *W. confusa* JMC 1093; Group E: treated with *P. pentosaceus* DSM20336; Group F: treatment with diclofenac sodium (positive control). **Difference between comparison treatment group was considered statistically significant at P <0.05 when compared with control group (B)

Discussion

Lactic acid bacteria isolated from African fermented locust beans (Iru) in this study appear similar to other studies that have successfully isolated and identified different types of LAB from fermented food products, including African locust beans (Uaboi-Egbenni et al. 2009; Ouoba et al. 2010; Ajayi 2014; Afolabi et al. 2016; Oyetola et al. 2017). Therefore, they are regarded as a major group of probiotic bacteria with a sufficient number of studies revealing their health-promoting ability in man and animals.

Blood cells play a major significant role in the onset of inflammation. The aggregation of white blood cells (Leucocytes) at the site of inflammation is a key elemental event in the inflammatory process, where adhesion and cell mobility processes could lead to cell migration (Umapathy et al. 2010). Treatment with *W. cibaria* II-1-59; *W. confusa* JMC 1093, and *P. pentosaceus* DSM20336 significantly decrease the WBC count, which suggests that these LAB strains can regulate the migration of WBCs to the site of inflammation which might have led to their significant decrease in the paw thickness of LAB treated Groups C, D and E rats. However, it was observed that *W. cibaria* II-1-59 and *W. confusa* JMC 1093 had a better ability to regulate this leucocyte infiltration in the inflamed rats' blood circulation than *P. pentosaceus* DSM20336. Moreover, studies by Szabo et al. (2011) and Tsai et al. (2014) further supported that treatment with LAB strains had a statistical significance on the hematological parameters in rats.

Red blood cells (Erythrocytes) and hemoglobin are also important for oxygen transportation. It is well known that inflammation can cause a significant decrease in the RBC, leading to erythropenia (Straat et al. 2012). LAB strains in this study showed a better ability to regulate the decrease in the RBC of the inflamed rats. An increase in RBC count was observed in LAB-treated rats compared to diclofenac sodium. This result was in line with the study of Bikheet et al. (2021), which showed that oral administration of LAB (*P. acidilactaci*) caused a significant increase in the RBC count of treated rats. A high level of RBC shows that the rats were not anemic, while a lower level indicates a sign of anemia (Cheesborough 1991). LABs may have used mechanisms to disrupt RBC-endothelial interactions, thereby modulating the rats' excessive development of anemic conditions as described by several researchers (Adeboboye et al. 2022). They might have also been able to avoid prolonged erythropenia by controlling the release of acute phase proteins like fibrinogen, as shown in the ESR results of this study.

Erythrocyte sedimentation rate (ESR) measures the rate at which RBCs settle in anticoagulated blood, which is a process influenced by the presence of fibrinogen, an acute phase protein associated with inflammatory response (Bray et al. 2016). An elevated ESR due to an increase in the level of fibrinogen serving as a significant mediator of RBC aggregation, therefore, indicates the existence of inflammation. The sub-plantar injection of formalin into the right hind paws of the rats in this study induced an inflammatory response within the first hour based on the swelling of the rat paw and an increase in the ESR. However, a significant ESR decrease ($P<0.05$) in the blood of LAB-treated rats indicated that *W. cibaria* II-1-59; *W. confusa* JMC 1093, and *P. pentosaceus* DSM20336 were able to control the release of fibrinogen in the blood thereby alleviating inflammatory response.

The reduction in the population of neutrophils and monocytes after the treatment with these LAB strains showed their involvement in suppressing the inflammatory response. The *W. cibaria* II-1-59 had the best ability to control the influx of neutrophils, which is essential for sustaining inflammatory response and the release of pro-inflammatory cytokines. The control of leucocyte infiltration could result from the production of specific cytokines responsible for regulating the responses of arrays of immune cells (Scheiermann et al. 2015). Also, the lymphocyte counts of LAB-treated rates were higher compared to diclofenac-treated rats (positive control group). The primary role of lymphocytes is associated with antibody formation (humoral) and cellular immunity. Therefore, a marked increase in the lymphocyte count observed in LAB-treated rats showed signs of an immunostimulatory effect. In a previous study, Aboderin and Oyetayo (2006) reported that the lactic acid bacterium (*Lactobacillus plantarum*) isolated from fermenting corn slurry has immunostimulatory properties due to raised lymphocyte count in rats. Also, Aattouri et al. (2002) had also earlier reported that oral ingestion of lactic acid bacteria increases lymphocyte proliferation and interferon-γ production.

These immune cell responses can be due to findings of our previous study, Oladejo and Oluwasola (2021), which reported that the oral administration of *Weissella* and *Pediococcus* sp. significantly decreased inflammatory responses in rats. This showed that treatment with these LAB had a very consistent anti-inflammatory effect and thus compared favorably with diclofenac sodium (standard NSAID drug) through the ability to modulate inflammatory responses via control of the balance between the pro- and anti-inflammatory cytokines (Groeger et al. 2013), as our treatments with *Weissella* and *Pediococcus* sp. significantly decreased the secretion of pro-inflammatory biomarker (CRP) and increased the serum IL-10 and TGF-β concentration of LAB treated rats (Oladejo and Oluwasola 2021).

In conclusion, the results of oral administration of W. cibaria II-1-59; *W. confusa* JMC 1093, and *P. pentosaceus* DSM20336 on the hematological parameters in the rats showed that these LAB strains could ameliorate inflammatory-induced anemia. Therefore, treating anemia of inflammation with lactic acid bacteria would be a great breakthrough, considering the risks associated with the treatment, as mentioned earlier.

REFERENCES

Aattouri N, Bouras M, Tome D, Marcos A, Lemonnier D. 2002. Oral ingestion of lactic acid bacteria by rats increases lymphocyte proliferation and interferon-γ production. Br J Nutr 87 (4): 367-373. DOI: 10.1079/bjnbjn2001527.

Aboderin FI, Oyetayo VO. 2006. Hematological studies of rats fed different doses of probiotic, *Lactobacillus plantarum*, isolated from fermenting corn slurry. Pak J Nutr 5 (2): 102-105. DOI: 10.3923/pjn.2006.102.105.

Adeboboye CF, Oladejo BO, Adebolu TT. 2022. Effects of *Lactobacillus fermentum* on inflammatory-associated anaemia and leucocyte homeostasis in carrageenan-induced paw edema in wistar rats. Bact Emp 5 (1): e327. DOI: 10.36547/be.327.

Afolabi FT, Abdulkadir M, Onilude A. 2016. Isolation and screening of microorganisms associated with locust bean (Iru) for the ability to ferment soya bean to produce soy Iru. Br Microbiol Res J 13 (6): 1-10. DOI: 10.9734/BMRJ/2016/24593.

Ajayi AO. 2014. Bacteriology and Qualitative study of African locust bean (*Parkia biglobosa*). Open J Soc Sci 2: 73-78. DOI: 10.4236/jss.2014.211010.

Amdekar S, Roy P, Singh V, Kumar A, Singh R, Sharma P. 2012. Anti-inflammatory activity of *lactobacillus* on carrageenan-induced paw edema in male wistar rats. Intl J Inflam 75: 1-6. DOI: 10.1155/2012/752015.

Barcelos IP Troxel RM, Graves JS. 2019. Mitochondrial dysfunction and multiple sclerosis. Biology 8 (2): 37. DOI: 10.3390/biology8020037.

Bikheet MM, Mahmoud ME, Hassan HM, Yassien EE. 2021. The effect of two strains of LAB (*P. acidilactaci* and *L. lactis*) in laboratory rats. SJAS 3 (1): 137-143. DOI: 10.21608/sjas.2021.73876.1093.

Bray C, Bell LN, Liang H, Haykal R, Kaiksow F, Mazza JJ, Yale SH. 2016. Erythrocyte sedimentation rate and c-reactive protein measurements and their relevance in clinical medicine. WMJ 115 (6): 317-321.

Cheesborough M. 1991. Medical Laboratory Manual for Tropical Countries. 2nd Edition. Tropical Health Technology and Butterworth Scientific Limited, London.

Groeger DO, Mahony L, Murphy EF, Bourke JF, Dinan TG, Kiely B, Shanahan F, Quigley EM. 2013. *Bifidobacterium infantis* 35624 modulates inflammatory processes beyond the gut. Gut Microbes 4 (4): 325-339. DOI: 10.4161/gmic.25487.

Nairz M, Theurl I, Wolf D, Weiss G. 2016. Iron deficiency or anemia of inflammation? Differential diagnosis and mechanisms of anemia of inflammation. Wien Med Wochenschr 166: 411-423. DOI: 10.1007/s10354-016-0505-7.

Nemeth E, Ganz T. 2014. Anemia of inflammation. Hematol Oncol Clin North Am 28 (4): 671-681. DOI: 10.1016/j.hoc.2014.04.005.

Oladejo BO, Oluwasola HM. 2021. Oral administration of *Weissella* and *Pediococcus* sp. alleviates formalin induced inflammation in rats by cytokine modulation. Ife J Sci 23 (2): 1-10. DOI: 10.4314/ijs.v23i2.10.

Ouoba LI, Nyanga-Koumou CA, Parkouda C, Sawadogo H, Kobawila SC, Keleke S, Diawara B, Louembe D, Sutherland JP. 2010. Genotypic diversity of lactic acid bacteria isolated from African traditional alkaline-fermented foods. J Appl Microbiol 108: 2019-2029. DOI: 10.1111/j.1365-2672.2009.04603.x.

Oyetola MT, Agarry O, Oyetola S. 2017. Screening of indigenous bacterial isolates from dawadawa (African locust beans) in North Central Nigeria. Biochem Mol Biol 2(6): 67-72. DOI: 10.11648/j.bmb.20170206.11.

Paramita S, Kosala K, Dzulkifli D, Saputri DI, Wijayanti E. 2017. Anti-inflammatory activities of ethnomedicinal plants from Dayak Abai in North Kalimantan, Indonesia. Biodiversitas 18: 1556-1561. DOI: 10.13057/biodiv/d180434.

Pessione E. 2012. Lactic acid bacteria contribution to gut microbiota complexity: Lights and shadows. Front Cell Infect Microbiol 2 (86): 1-15. DOI: 10.3389/fcimb.2012.00086.

Scheiermann P, Frenette PS, Hidalgo A. 2015. Regulation of leucocyte homeostasis in the circulation. Cardiovasc Res 107 (3): 340-351. DOI: 10.1093/cvr/cvv099.

Steinbicker AU, Sachidanandan C, Vonner AJ, Yusuf RZ, Deng DY, Lai CS, Rauwerdink KM, Winn JC, Saez B, Cook CM, Szekely BA, Roy CN, Seehra JS, Cuny GD, Scadden DT, Peterson RT, Bloch KD, Yu PB. 2011. Inhibition of bone morphogenetic protein signaling attenuates anemia associated with inflammation. Blood 117 (18): 4915-4923. DOI: 10.1182/blood-2010-10-313064.

Straat M, van Bruggen R, de Korte D, Juffermans NP. 2012. Red blood cell clearance in inflammation. Transfus Med Hemother 39: 353-360. DOI: 10.1159/000342229.

Sung-Min H, Gehoon C, Yong HK, Chul-Kyu P. 2019. The role of maresins in inflammatory pain: Function of macrophages in wound regeneration. Intl J Mol Sci 20 (5849): 1-16. DOI: 10.3390/ijms20235849.

Szabo NJ, Laurie C, Dolana GA, Burdocka TS, Satob HS, Tohru U, Satoko Y, Masamichi T, Hirofumi U. 2011. Safety evaluation of *Lactobacillus pentosus* strain b240. Food Chem Toxicol 49 (1): 251-258. DOI: 10.1016/j.fct.2010.10.027.

Szalay J. 2018. Inflammation: Causes, Symptoms and Anti-inflammatory Diet. www.livescience.com Accessed 07/04/2020.

Tsai YT, Cheng PC, Pan TM. 2014. Anti-obesity effects of gut microbiota are associated with lactic acid bacteria. Appl Microbiol Biotechnol 98: 1-10. DOI: 10.1007/s00253-013-5346-3.

Uaboi-Egbenni PO, Okolie PN, Sobande AO, Alao O, Teniola O, Bessong PO. 2009. Identification of subdominant lactic acid bacteria in dawadawa (a soup condiment) and their evolution during laboratory-scale fermentation of *Parkia biglobosa* (African locust beans). Afr J Biotechnol 8 (25): 7241-7248. DOI: 10.5897/AJB2009.000-9558.

Umapathy E, Ndebia EJ, Meeme A, Adam B, Menziwa P, Nkeh-Chungag BN, Iputo JE. 2010. An experimental evaluation of *Albuca setosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. J Med Plant Res 4 (9): 789-795. DOI: 10.5897/JMPR10.056.

Valentini L, Pinto A, Bourdel-Marchasson I, Ostan R, Brigidi P, Turroni S. 2015. Impact of personalized diet and probiotic supplementation on inflammation, nutritional parameters and intestinal microbiota - The "RISTOMED project": Randomized controlled trial in healthy older people. J Clin Nutr 34: 593-602. DOI: 10.1016/j.clnu.2014.09.023.