BACTERIAL EMPIRE

2022, VOL. 5, NO. 1, e327

# EFFECT OF *LACTOBACILLUS FERMENTUM* FROM INDIGENOUS DAIRY PRODUCT ON INFLAMMATORY-ASSOCIATED ANEMIA AND LEUCOCYTE HOMEOSTASIS IN CARRAGEENAN-INDUCED INFLAMMATION IN WISTAR RATS

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https://doi.org/10.36547/be.327

**ABSTRACT**

**Introduction**: *Lactobacillus* belongs to a broad classification of Lactic acid bacteria (LAB), which are majorly non-pathogenic living microorganisms often consumed with food. This group of bacteria can confer several health benefits when administrated in adequate amounts to the host.
**Objective**: Activity of two *Lactobacillus fermentum* strains isolated from a Nigerian locally fermented dairy drink products "Nunu" on inflammation-induced anemia condition and leucocyte influx in Wistar rats was studied using paw oedema acute inflammatory model induced by carrageenan.
**Methods**: Oedema was induced with 1% iota-carrageenan in all experimental groups. Apparently healthy rats were distributed into seven groups (A-E). Rats in Groups A were neither administered carrageenan nor treated with LAB, while Group B received carrageenan injection only. Rats in Groups C and D were treated with the strains of *L. fermentum* while Group E received diclofenac sodium treatment following administration of carrageenan. The dose of LAB used for the oral treatment is $5 \times 10^7$ CFU/mL for all the groups while the dose of diclofenac sodium used was 150mg/kg body weight of the rats. Paw thickness (mm) was checked at t = 0, 1, 4, 8, 24, 72, 168 and 336h. ESR, total RBC and WBC was performed on blood samples.
**Results**: This inflammatory model established that carrageenan induced a very strong inflammatory response in the first hour of the experiment due to changes in the paw thickness and ESR measurements. All the *Lactobacillus* treated groups showed a statistically significant decrease in paw thickness at $P<0.05$ and a better ability to minimize effect of inflammation on erythropenia than diclofenac sodium which are generally known to cause anemia, while also regulating leukocyte infiltration in the blood circulation of acutely inflamed rats.
**Conclusion**: This study reveals that *L. fermentum* possesses the ability to play significant role in regulating inflammation-associated anemia and restoring balance to the circulating white blood cells of acutely inflamed rats.

**Keywords:** Inflammation, anemia, *L. fermentum*, leucocytes, carrageenan

## INTRODUCTION

Inflammation is a normal and protective response of the body's immune system to deal with foreign bodies; including pathogens, damaged cells, stress, some pharmaceuticals or vaccines and toxic compounds (**Caceres, 2016; Chen *et al*., 2018**). However, several devastating health conditions like diabetes, cardiovascular diseases, and rheumatoid arthritis e.t.c are also associated with unresolved inflammation, anemia and total leucocytes count (**Farhangi *et al*., 2013; Ma *et al*., 2017; Adeboboye *et al*., 2020**).
Anemia is a condition of lack of adequate healthy red blood cells to carry oxygen to the body's tissues. It is a commonly encountered problem in critically ill patient. In fact, almost 95% of patients admitted to the intensive care unit (ICU) have a hemoglobin level below normal after 3 days of ICU admission (**Straat *et al*., 2012**). Although anemia can result from a number of factors, however inflammatory-associated anemia which is generally known as anemia of inflammation constitutes the second major cause of anemia worldwide (**Weiss *et al*., 2019**).
Circulating white blood cell counts are an important rheostat of altered homeostasis, disease states, and behavioral activity of an organism. For instance, a high white blood cell counts are often an important indicator of inflammation or an active disease state (**Scheiermann *et al*., 2015**). One critical parameter for the measurement of blood cell homeostasis in the circulation is the regulation of cellular numbers. However, since it has been scientifically observed that leucocytes do not undergo apoptosis in blood itself, the key mechanisms responsible for their numbers in blood counts are mobilization and recruitment (**Scheiermann *et al*., 2015**).
*Lactobacillus* belongs to a broad classification of bacteria called Lactic acid bacteria (LAB). LAB are majorly non-pathogenic living microorganisms which are generally regarded as safe (GRAS) and have been consumed with food overtime. They can confer several health benefits when administrated in adequate amounts to the host and are often referred to as probiotics (**Abdulkalam, 2018**). Some of the species of *Lactobacillus*, such as *L. casei, L. fermentum, L. delbrueckii, L. acidophilus, L. plantarum, and L. reuteri*, have been reported to possess potential therapeutic properties since they are capable of preventing the development of some diseases as shown mostly using animal models (**de Le Blanc *et al*., 2011**).
Carrageenan induced inflammatory paw oedema represents a proven classical experimental model for testing and evaluating the anti-inflammatory properties of various substances (**Amdekar *et al*., 2012**). Carrageenan is a sulphated galactan compound that has been described to be capable of inducing inflammatory response, reducing the circulation of red blood cells and causing massive leucocyte infiltrations.
This study therefore was designed to evaluate the *L. fermentum* isolated from a locally fermented dairy product in Nigeria for anti-inflammatory associated effects on blood anemia and leucocyte regulation using carrageenan induced acute inflammatory model in Wistar rats.

## MATERIALS AND METHODS

### Collection of samples

A Nigerian indigenous dairy product 'Nunu' was purchased from a local farm, Akure, South-West, Nigeria. The samples were collected in sterile containers and transported immediately to the Department of Microbiology laboratory, Federal University of Technology, Akure for isolation.

### Isolation and identification of Bacteria

*L. fermentum* CIP 102980 and *L. fermentum* NBRC 15885 were isolated from the purchased 'Nunu' using Mann Rogosa Sharpe Agar (MRS) at 37◦C in anaerobic condition and identified using standard microbiological methods as described by (**Bin Masalam *et al*., 2018**). Colony morphology of isolates on MRS agar was determined visually. Gram staining was performed to determine the cell morphology, catalase test was also performed. As for molecular identification, cell pellets were harvested from 2 mL of overnight cultures (up to $2 \times 10^9$ bacterial cells) of LAB grown in MRS broth. DNA extraction was done using a Jena bioscience DNA Purification Kit following the manufacturer's instructions. PCR was carried out to amplify nearly the entire region of the 16S rDNA gene. The 16SrRNA gene of the bacteria was amplified using the primer pair 27F-5′-AGAGTTTGATCCTGGCTCAG-3', and 1492R 5'GGTTACCTTGTTACGACTT-3'. The amplification product was separated on a 1.5% agarose gel and electrophoresis was carried out at 80V for 1 hour 30 minutes. After electrophoresis, DNA bands were visualized by ethidium bromide staining. 100bp DNA ladder (Solis Biodyne) was used as DNA molecular weight marker. They were BLAST-searched to detect similar sequences in the NCBI database (https://www.ncbi.nlm.nih.gov). The evolutionary history was inferred

using the Neighbor-Joining method (**Saitou and Nei, 1987)**. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (**Felsenstein, 1985**). The evolutionary distances were computed using the Jukes-Cantor method (**Jukes and Cantor, 1969**) and are in the units of the number of base substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). All ambiguous positions were removed for each sequence pair (pairwise deletion option). Evolutionary analyses were conducted in MEGA X v 10.1.2 (**Kumar *et al*., 2018**).

**Drugs and Chemicals Used**

Iota-carrageenan (CAS 9064-57-7) was purchased from Tokyo Chemical Industry (TCI), diclofenac sodium drug (manufactured by Impulse Pharma PVT. Ltd, Boisar, India. Expiry date; March, 2023) was purchased from pharmaceutical a government approved shop, Akure, Nigeria.

**Evaluation of anti-inflammatory activity**

Apparently healthy Wistar rats with an average weight of 150g were used for this study. The rats were housed in stainless steel cages. They were fed with standard rat chow and water and allowed to acclimatize 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. For the anti-inflammatory assay, inflammation was first induced using iota-carrageenan in the rat's paw tissues. This was done by sub-plantar injection of 1 ml of 1 % Iota-carrageenan dissolved in sterile saline into the right hind paws of all the rat groups except for the general control group (Group A). After the induction of the inflammation, rats were divided into 4 groups (Groups B-E) of 3 rats each. Rats in Group B were administered carrageenan only without any treatment (negative control); rats in Groups C and D were administered carrageenan and were treated orally with $5 \times 10^7$ CFU/mL of the isolated *L. fermentum* CIP 102980 and *L. fermentum* NBRC 15885 respectively while rats in Group E (positive control) were administered carrageenan and treated with diclofenac sodium (150 mg/Kg body weight) after the development of paw oedema in the rats.
The rat's paw thickness was measured at 20 min before carrageenan injection and after at different time intervals (0, 1, 4, 8, 24, 72, 168 and 336 h) with an electronic digital vernier caliper (Daytona DVC150 150mm (6") Sydney, Australia) and was measured in millimeters (mm) according to the method of (**Amdekar *et al.*, 2012**).

**Blood sample collection**

Blood was collected for the hematology after each time interval at 1, 8, 24, 36, 72 hrs, and 1 and 2 weeks, animals were sacrificed with cardiovascular bleeding according to the guidelines of the Institutional Animals Ethics Committee of the Federal University of Technology Akure, Nigeria, Centre for research and development (CERAD) (**Amdekar *et al*., 2012**).

**Hematological Parameters**

Total red blood cell (RBC), white blood cell (WBC) counts and erythrocyte sedimentation rate (ESR) were estimated to check for presence of inflammation.
ESR was estimated by adding blood samples into a Westergren ESR pipette (Citotest Scientific 5830-0002, Jiangsu, China) until the blood level reaches 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 as described by (**Valentini *et al*., 2015**). For the Total Red Blood Cell count, the collected blood was drawn to the 0.5 mark of the standard pipette and mixed with Hayem's solution to fill the pipette tube to the 101 mark. The mixture was transferred into a counting chamber (Hemacytometer Neubauer improved, China), and the total number of RBC was counted under a microscope (x40). 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 (0.1mm3). On the other hand, the total circulating WBC count was carried out by mixing 0.02 ml of the collected blood with 0.38ml of Turk's reagent (3% acetic acid with crystal violet dye) in a tube. The mixture was transferred into a counting chamber, and the total number of WBC counted under a microscope (x40). The total number of WBCs counted was calculated, using the formula: WBCs = number of cells counted x depth factor (10) x dilution factor (20) x area factor (0.25).

**Statistical Analysis**

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

**RESULTS**

**Isolation and identification of *Lactobacillus***

Two (2) different strains of *Lactobacillus fermentum* strains were isolated from 'Nunu' and identified. They are *L. fermentum* CIP 102980 and *L. fermentum* NBRC 15885. As shown by phylogenetic tree analysis, *L. fermentum* NBRC 15885 were clustered together as one clade and shared common ancestor with *L. gorilla* KZO1, while *L. fermentum* CIP 102980 were clustered together as one clade shared common ancestor with *L. fermentum* NCDO 1750 as shown below.

**Table 1** Molecular Identities of *Lactobacillus fermentum* strains isolated from 'Nunu'

| Isolate code | Biochemical identity | Molecular identity | % similarity | Strain no. | Accession no. |
|---|---|---|---|---|---|
| COVNU2 | Lactobacillus sp. | L. fermentum | 98.52 % | CIP 102980 | NR-104927.1 |
| COVNU1 | Lactobacillus sp. | L. fermentum | 82.68 % | NBRC 15885 | NR-11335.1 |

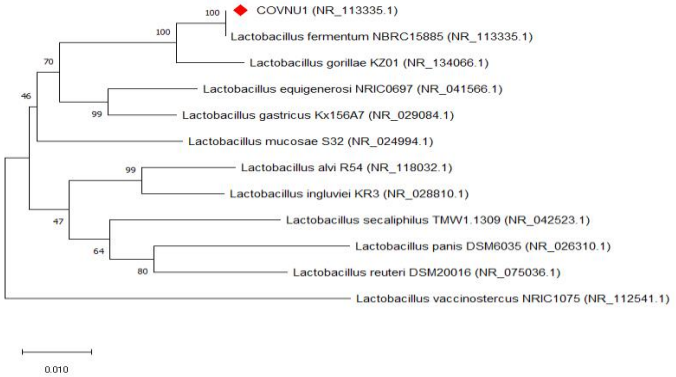


**Figure 1** Phylogenetic analysis of 16S rRNA gene of isolate COVNU1 (red) and other related *Lactobacillus spp.* By Neighbor-joining method. Numbers at the nodes indicate the bootstrap support (%) based on 1000 replicates. The scale bar indicates 0.010 nucleotide substitutions per nucleotide position. Genebank accession numbers are given in parenthesis. In the phylogenetic tree, COVNU1 (red) and *L. fermentum* were clustered together as one clade and shared common ancestor with *L gorillae.*

**Effect of lactobacilli administration on Carrageenan induced inflammation in rat paw tissues**

The administration of lactobacilli caused a reduction in the paw oedema size of rats treated with it in a fashion very similar to that of diclofenac (Fig. 3). The paw size started to reduce by 1h. On the other hand, Group B rats which only receive carrageenan with no treatment had the highest paw size throughout the duration of the experiment. Although like the other treatment groups, the paw size also started to reduce by 1h but it was slow and gradual till the end of the duration of the experiment unlike the paw oedema of LAB treated rats in Groups C and D and diclofenac treated rats which resolves better. Out of the three treated groups however, the treatment mediated with *L. fermentum* NBRC 15885 in Group D rats is highly comparable with the treatment mediated with diclofenac in Group E rats. There was a rapid and significant decrease ($p < 0.05$) in paw size by 72h of oral

treatment which was very similar to that mediated with Diclofenac as observed in group E rats (Figure 3).

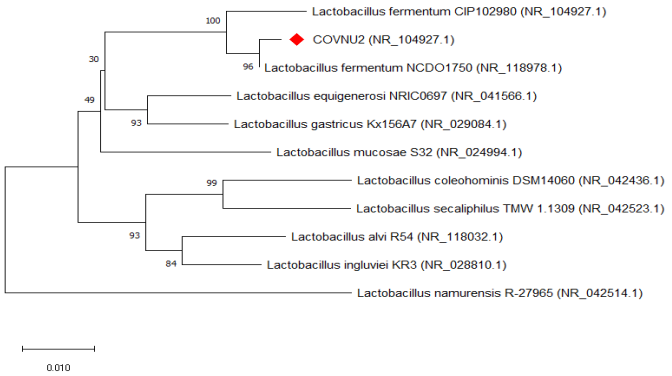


**Figure 2** Phylogenetic analysis of 16S rRNA gene of isolate COVNU2 (red) and other related *Lactobacillus spp.* By Neighbor-joining method. Numbers at the nodes indicate the bootstrap support (%) based on 1000 replicates. The scale bar indicates 0.010 nucleotide substitutions per nucleotide position. Genebank accession numbers are given in parenthesis. In the phylogenetic tree, COVNU1 (red) and *L. fermentum* NCDO1750 were clustered together as one clade. The tree indicates that COVNU1 (red), *L. fermentum* NCDO 1750, and *L. fermentum* CIP 102980 share common ancestor.

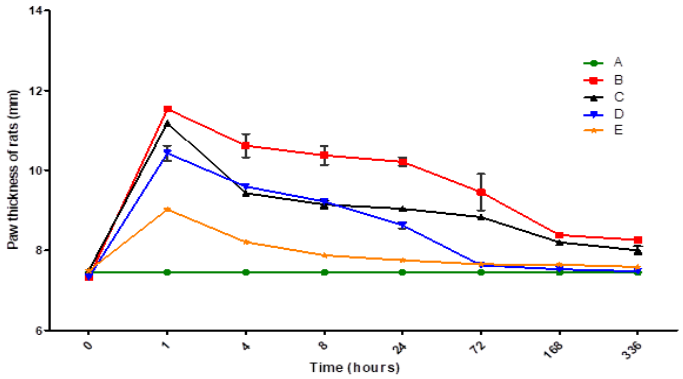


**Figure 3** Change in paw thickness (mm) at t = 0, 1, 4, 8, 24, 72, 168, and 336 hours. n =3 (significant at P<0.05). Edema was induced by injecting 0.1mL of 1% solution of iota-carrageenan into the sub-plantar surface of right-hind paw. Data are expressed as mean ± standard error of two rats per group. Group A: Carrageenan control; Group B: Carrageenan only (negative control); Group C: fed with *L. fermentum* CIP 102980; Group D: fed with *L. fermentum* NBRC 15885; Group E: treatment with diclofenac sodium (positive control).

**Effects of administration of Lactobacilli on Erythrocyte sedimentation rate of rat's blood**

The injection of carrageenan into the right hind paw induced massive increase in the erythrocyte sedimentation rate of the various rat groups within the first hour of the experiment as compared to the control rats (group A), which had a constant ESR value of 0.30±0.00 mm/h through the experiment. The value which was highest in the untreated Group B rats, 3.88±0.13 mm/hr at the first hour started to decrease after 4h. Although, the same trend was observed in the *Lactobacillus* treated Groups C and D rats and diclofenac treated Group E rats, the ESR value return to normal values observed in Group A rats by the 4th hour of treatment with *L. fermentum* NBRC 15885 having the best effect (Figure 4).

**Effects of administration of Lactobacilli on Red blood cell count (RBC)**

Group A rats (carrageenan control) showed a total red blood cell count of 8.65±0.41× 106µL-1at t= 1hr which remained the same all through the time course of the experiment. After, the injection of carrageenan into the right hind paw of other groups, a state of erythropenia (low RBC count) was immediately observed in all the injected groups after the first hour, with the total RBC count falling below the normal rat standard. Diclofenac treated Group E rats showed the greatest decrease at 3.81±0.75 × 106µL-1. *Lactobacillus* treated Groups C (6.56±0.00 ×106µL-1) and D (6.39±0.01×106µL-1) rats began to show improved RBC counts after 36hours and 8hours respectively while untreated group B rats maintained a continuous low RBC count until after 168h (Table 2).

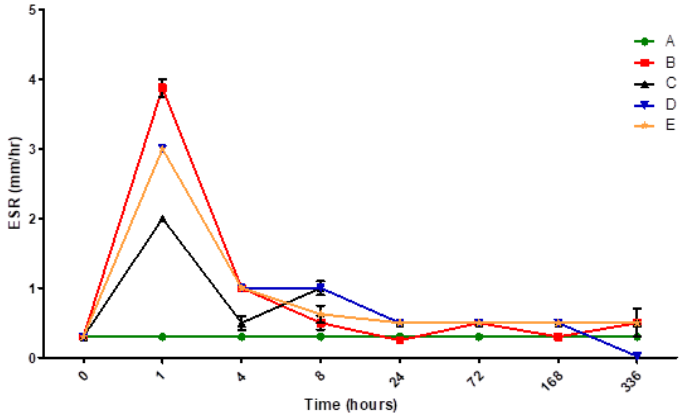


**Figure 4** Change in Erythrocyte sedimentation rate test (mm/hr) at t = 0, 1, 4, 8, 24, 72, 168, and 336 hours. n =3 (significant at P<0.05). Edema was induced by injecting 0.1mL of 1% solution of iota-carrageenan into the sub-plantar surface of right-hind paw. Data are expressed as mean ± standard error of two rats per group. Group A: Carrageenan control; Group B: Carrageenan only (negative control); Group C: fed with *L. fermentum* CIP 102980; Group D: fed with *L fermentum* NBRC 15885; Group E: treatment with diclofenac sodium (positive control).

**Table 2** Effect of oral treatment of LAB on the total red blood cell count (RBC) × $10^6$µL$^{-1}$ of blood

| | Time (h) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Groups** | **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 | 4.04±0.26 | 5.68±1.66 | 5.28±0.22 | 5.38±0.17 | 5.89±0.10 | 6.83±0.09 | 7.82±0.12 |
| C | 4.03±0.03 | 4.22±0.07 | 4.87±0.05 | 6.56±0.00 | 7.29±0.06* | 7.86±0.02 | 9.12±0.24 |
| D | 4.83±0.12 | 6.39±0.01 | 6.48±0.02* | 7.88±0.05* | 7.25±0.06* | 8.07±0.10* | 8.24±0.06 |
| E | 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 |

Data are expressed as mean ± SEM of three rats per group. Group A: Carrageenan control; Group B: Carrageenan only (negative control); Group C: fed with *L. fermentum* CIP 102980; Group D: fed with *L. fermentum* NBRC 15885; Group E: 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 administration of Lactobacilli on Total white blood cell count (WBC)**

Out of all the treatments groups, administration of *L. fermentum* CIP 102980 to carrageenan treated rats in Group C caused the WBC value which had started to increase significantly by 24h as a result of the inflammation caused by carrageenan to reduce. However, the untreated Group B had a progressively massive influx of leucocytes (leucocytosis) starting from 24h to 36h at 8.63±2.08 and 18.25±0.25×$10^3$µL-1 respectively before the value started to reduce after 72h. Rats in Groups D and E however had similar WBC values as that of the general control, Group A rats by 338h of treatment. This is shown in Table 3.

**Table 3** Effect of oral treatment of LAB on the total white blood cell count (WBC) × $10^3\mu L^{-1}$ of blood

| | Time (h) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Groups** | **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 | 3.78±0.38 | 4.18±0.18 | 8.63±2.08 | 18.25±0.25 | 12.38±0.38 | 7.25±0.15 | 8.05±0.05 |
| C | 2.45±0.00 | 2.00±0.00* | 4.78±0.03 | 5.95±0.00 | 8.33±1.83 | 5.40±0.00* | 3.75±0.00* |
| D | 3.55±0.00 | 3.05±0.00* | 16.30±0.60* | 16.55±0.05* | 7.53±1.73 | 3.4±0.00* | 4.75±0.00* |
| E | 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* |

Data are expressed as mean ± SEM of three rats per group. Group A: Carrageenan control; Group B: Carrageenan only (negative control); Group C: fed with *L. fermentum* CIP 102980; Group D: fed with *L. fermentum* NBRC 15885; Group E: 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

The presence of *L. fermentum* in fermented dairy drink product such as "Nunu" have been previously reported as the most abundant among a total of 373 LAB isolated and identified from spontaneously fermented "Nunu" in Ghana (**Akanbanda *et al*., 2013**). *L. fermentum* was also described to possess the ability to produce yoghurt with desirable consumer sensory characteristics, which illustrate its importance in the production of "Nunu" (**Akanbanda *et al*., 2014**).

Carrageenan induced acute inflammation occurs in a biphasic step. Injection of a compound like carrageenan causes the release of inflammatory mediators such as histamine, serotonin, kinins e.t.c which starts the first phase and leads to the accumulation of fluids in the endothelial tissues. Within 2-3 hours, the second phase kicks off with further release of prostaglandins and related substances. This is also characterized by leukocyte infiltration at the site of infection or damage, following the activation of PRRs like Toll like receptors in a series of inflammatory cascade (**Amdekar *et al*., 2012; Silva *et al*., 2010**).

Erythrocyte sedimentation rate (ESR) estimates the suspension stability of RBC in plasma, it measures the release of acute phase protein (fibrinogen, α and β gobulins) in the blood which is short lived. It is a crucial inflammatory biomarker and also confirms an ongoing disease state within an animal. (**Bray *et al*., 2016; Shobana *et al*., 2017**). Elevated ESR levels shows presence of inflammation in the blood due to the presence of fibrinogen which was described as an important mediator of RBC aggregation. Our carrageenan injection induced a massive elevation in the erythrocyte sedimentation rate of the various groups within the first hour of the experiment. However, this was immediately resolved in all groups, with *L. fermentum* CIP 102980 showing a slightly better regulation of RBC clearance from circulation in the first hour. They could have prevented the prolonged erythropenia by modulating the release of acute phase proteins.

Acute inflammations have been reported overtime to be associated with anemia and increased WBC count. A high WBC count is an important marker of inflammatory activity in the body and inflammation is also an important cause of blood vessel damage. The result of this inflammatory model shows that carrageenan induced a very strong inflammatory response in the first hour of the experiment based on the paw thickness and ESR measurements. *L. fermentum* CIP 102980 and *L. fermentum* NBRC 15885 both showed better ability to minimize effect of inflammation on erythropenia by gradually restoring the circulating levels of RBC than observed in diclofenac sodium, which are generally known to cause anemia. *L. fermentum* CIP 102980 showed a better ability to regulate leukocyte infiltration in the blood circulation of acutely inflamed rats. **Ghazanfarpour *et al*. (2019)** also described similar protective effects of *L. fermentum* on heamatological parameters of rats inflamed with Lead toxicant. This may have contributed to the observed significant decrease in the paw thickness of rat Group C and D rats. Several studies have described the anti-inflammatory activities of *lactobacillus* in the gut of different animals (**Amdekar *et al*., 2012; Abdul Kalam, 2018**), although there is still a dearth of information on their effect on inflammatory anemia and regulation of leucocytes, and the mechanisms involved in the process.

Generally, increased hemolysis is thought to only play a minor role in the anemia caused by inflammation, because parameters of hemolysis are usually undisturbed. Therefore, researches propose that decreased RBC population in inflammation is rather due to an altered morphology of the RBCs which results in increased adherence to the endothelium and clearance from the circulation (a process which is aided by the release of inflammatory mediators) (**Straat *et al*., 2012**). *L. fermentum* CIP 102980 and *L. fermentum* NBRC 15885 could have both engaged mechanisms to disrupt RBC-endothelial interactions thereby modulating excessive development of anemic conditions in the rats.

The control of leukocyte infiltration may however be associated with the release of specific cytokines which are responsible for modulating the response of various immune cells (lymphocytes, granulocytes, macrophages, mast cells) (**Abdul Kalam, 2018**). Modulation of inflammatory mediators by *L. fermentum* could be another mechanism for regulating inflammatory based anemic conditions. For instance, stimulation with TNF-α caused increased adherence of RBCs to endothelial monolayers during the incubation of both endothelial cells and RBCs with endotoxin (**Anniss and Sparrow, 2007**).

## CONCLUSION

This present study shows that *L. fermentum* CIP 102980 and *L. fermentum NBRC* 15885 possess protective effect against inflammation-associated anemia, while *L. fermentum* CIP 102980 is most effective in restoring balance to the circulating white blood cells and suppresses the effect of inflammation of acutely inflamed rats.

**Authors' inputs:** BOO designed the study. CFA developed the methodology, acquired the data, analysed and interpreted the data. CFA wrote the manuscript, BOO and TTA corrected the manuscript. TTA reviewed and revised the manuscript, provided administrative support and aptly supervised the study. All authors read and approved the final manuscript.

**Competing interest:** Authors declare no competing interest.

**Acknowledgement:** Authors appreciate the laboratory technologists of the Microbiology department, Federal University of Technology, Akure for providing some necessary paraphernalia useful for the protocols employed in this study.

**Funding:** This study was funded independently by the authors.

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