Assessment of Lactic Acid Bacteria Treatments on some Biochemical Indices Associated with Ulcerative Colitis Induced in Wistar Rats

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Abstract: Ulcerative colitis (UC) is an idiopathic inflammatory bowel disease that affects the colonic mucosa and it's clinically portrayed by diarrhea, abdominal pain and so on. Lactic acid bacteria (LAB) are one of the most significant groups of probiotic organisms, commonly used in fermented dairy products. These group of organisms enhance lactose digestion, stimulate the immune system, prevent and treat diarrhea. In the present study, the therapeutic effects of *Lactiplantibacillus plantarum* PQ104969 and *Lactiplantibacillus plantarum* PP893151 on acetic acid-induced ulcerative colitis was evaluated in Wistar albino rats. Acetic acid-induced ulcerative colitis was achieved by intrarectal administration of 5% acetic acid after acclimatization. Wistar rats were then treated orally with either 1 ml of normal saline, *L. plantarum* PQ104969 (5×10^7 Cfu/ml), *L. plantarum* PP893151 (5×10^7 Cfu/ml) or prednisolone (2 mg/kg) once a day for 7 days. Disease activity index (DAI) was recorded daily after colitis induction by assessing the symptoms. The rats were sacrificed on day 3 and 7 by cervical dislocation, and colon tissues were isolated for the biochemical analysis of oxidative stress parameters. Depletion of total glutathione (GSH) levels in the colitis group was significantly restored in the *L. plantarum* PP893151 treated groups, while *L. plantarum* PQ104969 regulated the expression of proteins, thus alleviated inflammatory response. Both lactic acid bacteria inhibited neutrophil infiltration to suppress myeloperoxidase activity in order to mitigate inflammatory reaction and oxidative stress development in acetic acid induced ulcerative colitis. Hence, *Lactiplantibacillus plantarum* associated with indigenous fermented foods could be used as an alternative treatment of Ulcerative Colitis.

Key word: Acetic acid, inflammation, ulcerative colitis, *Lactiplantibacillus plantarum*, oxidative stress

INTRODUCTION

Inflammatory bowel diseases (IBD) are chronic inflammatory disorders of the digestive tract, with ulcerative colitis and Crohn's disease being major forms (Rajendiran *et al*., 2018). These diseases can negatively impact tissues and organs. Some research suggests that chronic inflammation could also play a role in a range of conditions, from cancer to asthma (Oladejo *et al*., 2024). Ulcerative colitis (UC) is an idiopathic inflammatory bowel disease that affects the colonic mucosa and is clinically portrayed by diarrhea, abdominal pain and hematochezia. The predominance of IBD including ulcerative colitis, is generally higher with an estimation of 250 cases per 100,000 individuals in western countries however, it is becoming common in other parts of the world due to the adoption of western lifestyle (Baumgart and Sandborn, 2007).

Lactic acid bacteria (LAB) are non-sporing, non-respiring Gram-positive cocci or rods that produce lactic acid as the primary end product during carbohydrate fermentation. Lactic acid bacteria and their probio-active substances have numerous beneficial effects on the gastrointestinal tract, preventing the adherence, establishment, and replication of various enteric mucosal pathogens through various antimicrobial mechanisms (Naidu *et al*., 2010). Lactic acid bacteria are a well-known type of probiotic bacteria, including the genera such as *Lactobacillus*, *Lacticaseibacillus*, *Lactiplantibacillus* and so on. Lactic acid bacteria can produce high levels of lactic acid and other metabolites, which possess anti-inflammatory properties (Hao *et al*., 2023).

Steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) are used to manage inflammatory conditions, but their use is limited due to high costs and adverse effects (Jargalsaikhan *et al*., 2019). The NSAIDs include naproxen, ibuprofen, and aspirin and so on. Corticosteroids, a type of steroid hormone, decrease inflammation and suppress the immune system. But, long-term use of corticosteroids can cause vision problems, high blood pressure, and osteoporosis (Santos-Longhurst, 2018).

Thus, there is a need to come up with other substances or drugs that can treat or reduce inflammatory reactions (Mohd *et al*., 2021). The discovery of anti-inflammatory drugs obtained from natural sources is a rational and effective approach to the treatment of inflammatory conditions because they are safe, effective, biocompatible, and cost-efficient treatment options for inflammatory diseases (Oladejo *et al*., 2024). The purpose of this study was to assess the potential of *Lactiplantibacillus plantarum* strains in mitigating oxidative stress associated with acetic acid-induced ulcerative colitis in Wistar rats.

MATERIALS AND METHODS

Preparation of LAB species isolated from "Pap" (maize gruel): Strains of *Lactiplantibacillus plantarum* were isolated from "Pap" (Maize gruel; prepared from fermented maize-Yellow or white and are popularly consumed in Tropical Africa) samples in this study. The organisms were identified using morphological characterization and biochemical tests. The strains were confirmed by molecular characterization as *Lactiplantibacillus plantarum* PQ104969 and *Lactiplantibacillus plantarum* PP893151 (Bin Maslam *et al*., 2018).

Animals, chemicals, and treatments: The study involved 50 Wistar albino male rats, each weighing 160g. They were kept in cages for 2 weeks to acclimate before the experiments. The rats were fed with standard rat food and water, following the ethics committee's requirements of the Federal University of Technology Akure, Nigeria. Ethical permission was sought from FUTA Research Ethical Committee (FUTA/ ETH/23/101). The study involved rats in different groups, including Group A, which was neither induced nor treated, Group B, which was colitis-only, Group C and D, which were colitis-induced and treated with 5×10^7 Cfu/ml of *L. plantarum* PQ104969 and *L. plantarum* PP893151 respectively. Group E, which was colitis-induced and treated with prednisolone (2 mg/kg). Colitis was induced in rats by using 5% acetic acid through rectal administration. This was achieved through the use of a flexible plastic catheter (outer diameter of 2 mm) which was inserted rectally into the colon 8 cm proximal to the anus of each fasted rats. The rats were then sacrificed on days 3 and day 7 and their blood and colon were analyzed for oxidative stress (Omayone *et al*., 2018)

Biochemical assays of the colon: The colon was removed and homogenized in a Tris-KCl buffer solution of pH 7.00. The homogenate was then centrifuged at 5000 g for a duration of 10 minutes. The resulting supernatants were collected for the assessment of protein levels and various enzymatic activities (Omayone and Olaleye 2022).

Determination of protein concentration in the colon: The Biuret method, as described by Gornal *et al.* (1949), was used to determine protein concentrations in various samples, with a slight modification involving potassium iodide being added to prevent precipitation of Cu^{2+} ions as cuprous oxide as reported by Omayone and Olaleye 2022. The sample homogenates were diluted 10 times with distilled water, 1 ml was then added to 3 ml of Biuret reagent in triplicate. The mixture was incubated at room temperature for 30 minutes. Absorbance was read at 540 nm from spectrophotometer using distilled water as blank.

Determination of myeloperoxidase (mpo) activity in the colon: Myeloperoxidase activity was determined according to the method of Xia and Zweier (1997). Two thousand (2000) µl of O-dianisidine and H_2O_2 mixture was pipetted in the cuvette and 70 µl of sample (serum) was subsequently added to it. The reaction mixture was read at 0 second, 30 seconds and 60 seconds respectively at 460 nm wavelengths. One unit of MPO activity can be defined as the quantity of enzyme able to convert/degrade 1 µmol of hydrogen peroxide to water in one minute at room temperature.

Determination of glutathione (gsh) level in the colon: The method of Beutler *et al*.

(1963) was followed in estimating the level of reduced glutathione (GSH). Sample of 0.2 ml was added to 1.8 ml of distilled water and 3 ml of the precipitating agent was mixed with the sample. This was centrifuged at 3,000 g for 4 minutes. Thereafter, 0.5 ml of the supernatant was added to 4.5 ml of Ellman reagent. A blank was prepared with 0.5 ml of the diluted precipitating agent and 4 ml of phosphate buffer and 0.5 ml of Ellman's reagent. The absorbance of the reaction mixture was read from spectrophotometer within 30 minutes of colour development at 412 nm against a reagent blank.

Ethical approval: Ethical approval was obtained from the Centre for Research and Development (CERAD) of The Federal University of Technology, Akure; FUTA/ETH/23/101.

Statistical analysis of the results: All data were expressed as mean ± standard error mean (SEM). Statistical comparison was performed across the groups using Graph Pad Prism 9 and Microsoft Excel 2019. The differences across the group were accessed by means of analysis of variance (ANOVA). For all tests, the significant differences were taken as $P<0.05$.

RESULTS

Effect of Lactobacilli administration on body weights of rats

Weights of rat's post-colitis induction showed no significant changes across all groups as shown in Figure 1.

Effect of Lactobacilli administration on myeloperoxidase (mpo) activity in rat's colon

The study revealed on day 3, group A (negative control) maintained a low MPO activity, while group B (colitis only) maintained a very high level. Group D (plantarum B2 treated group) had a relatively lower MPO activity as compared to group C (plantarum B3 treated group), while the standard drug (group E) had the highest MPO activity among the treatment groups. Following 7 days treatment, there was a tremendous decrease of MPO activity in group D and group E than that of group C as shown in Figure 2.

Protein concentration in rat's colon

The concentration of protein in the colon of Group A (negative control) remained constant throughout the period of the experiment, with an observation of a highly similar effect on the protein concentration in the Group C (plantarum B3 treated group) and Group E (Prednisolone treated group). Group D (plantarum B2 treated group) and Group B (colitis only group) showed increase protein concentration. Groups of rats that were sacrificed on day 7, showed no significant difference from the control group. This is shown in Figure 3.

Effect of Lactobacilli administration on glutathione (gsh) level in rat's colons

The concentration of glutathione antioxidant molecule in Group A rats' colon remained constant, with a slight decrease in the colitis group on day 3. The *L. plantarum* PQ104969 treatment exhibited similar effects to colitis only, while oral *L. plantarum* PP893151 administration significantly increased GSH levels, with slight increases observed at day 7. This is shown in Figure 4.

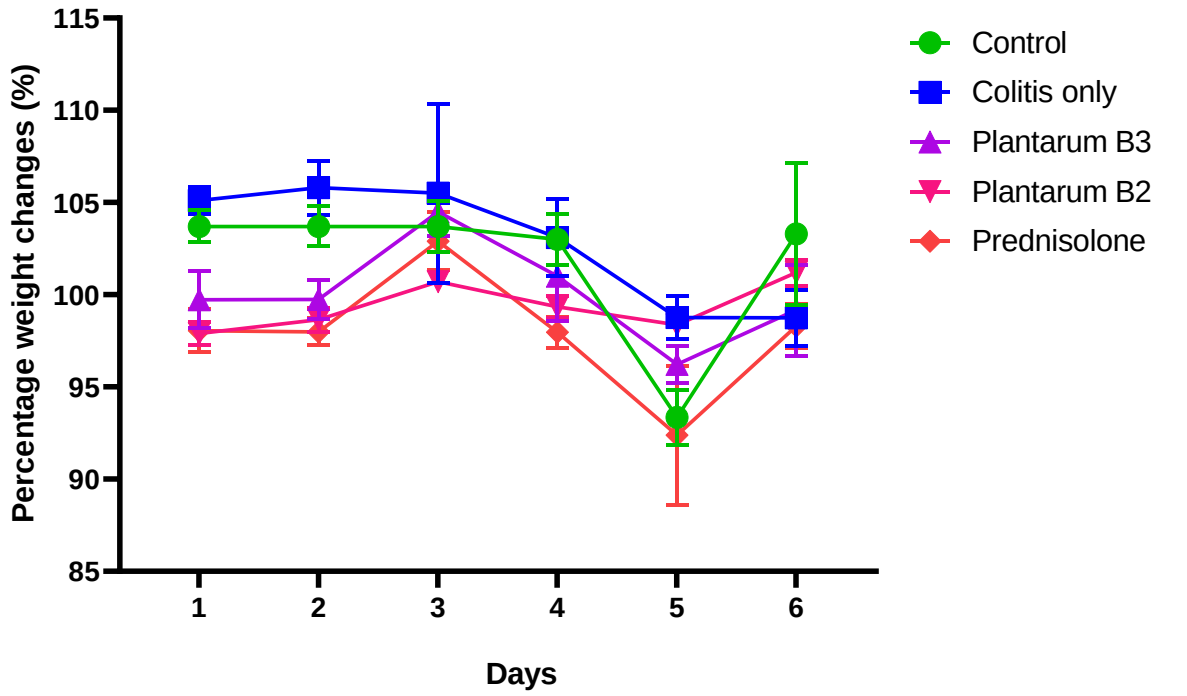
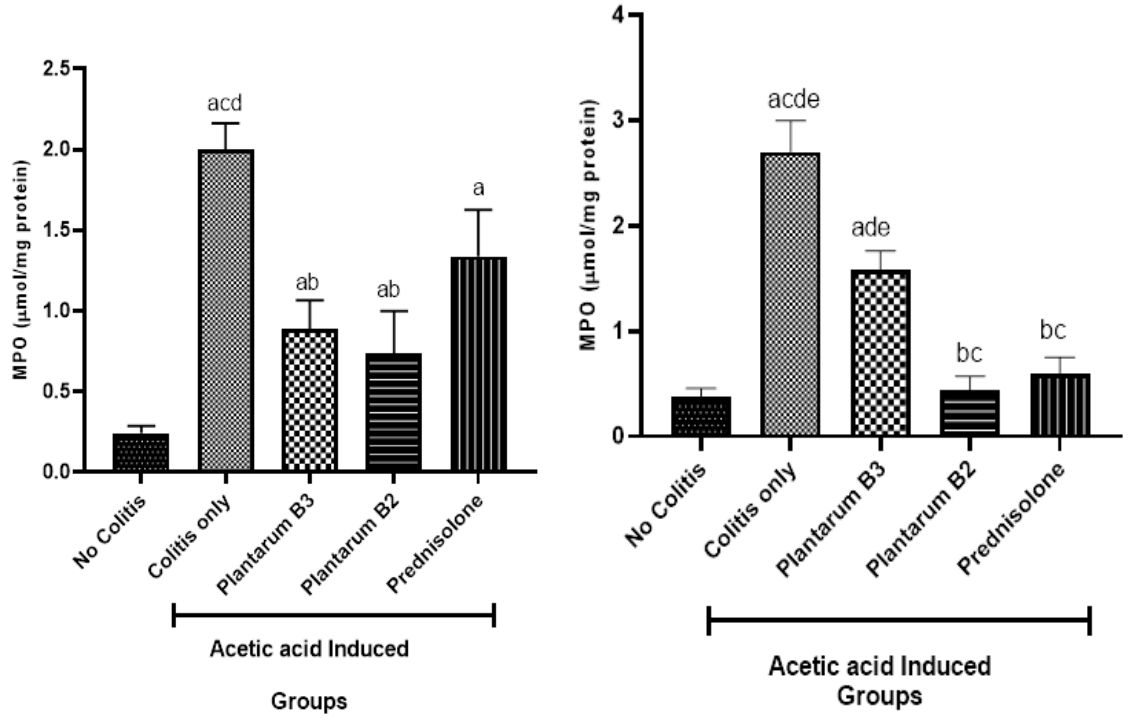
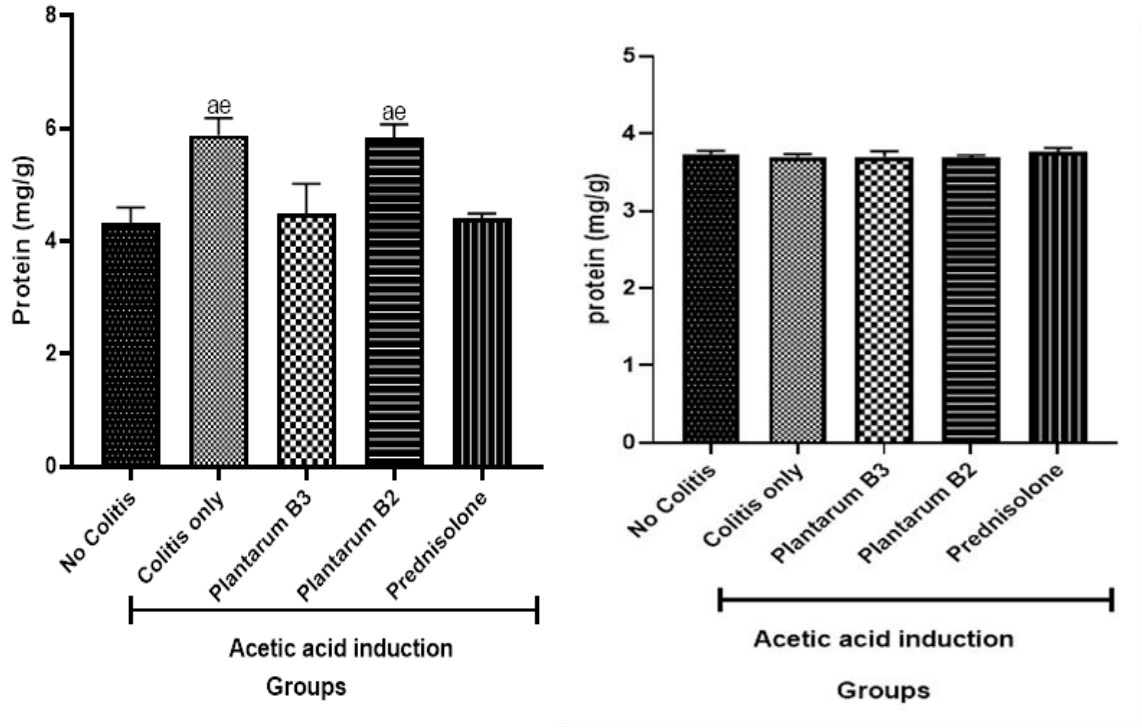


Figure 1: Change in body weights of experimental rats (g) for a period of time (Days). Mean values ± standard error for five rats per group (n = 5) are shown for each group. Group A: control; Group B: Colitis only; Group C-D: fed with *L. plantarum* PQ104969 and *L. plantarum* PP893151 respectively; Group E: treatment with prednisolone (positive control) (significant at P<0.05).



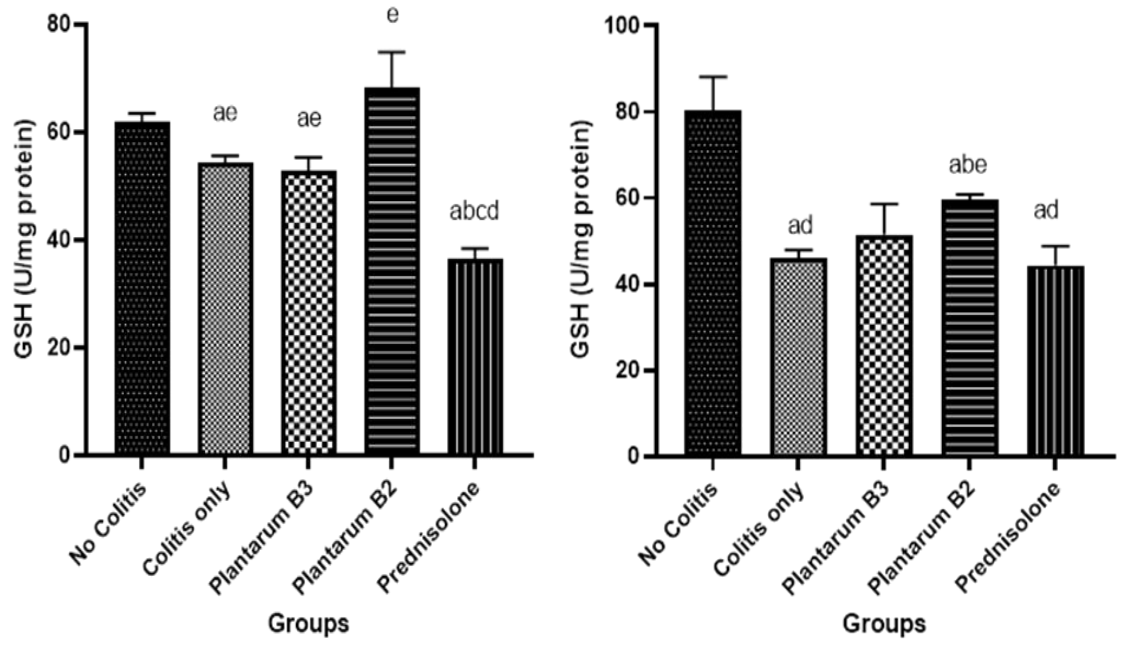
A **B**

Figure 2: Effect of *Lactobacilli* administration on myeloperoxidase (MPO) activity in the colons of rats sacrificed on day three (A) and seven (B). Mean values ± SEM of MPO values in acetic acid-induced colitis ($p < 0.05$; $n = 5$). a, b and c indicate significant difference when compared to control, colitis only, plantarum B3, plantarum B2, prednisolone respectively from One-way ANOVA and Tukey's post-tests.



A **B**

Figure 3: Effect of *Lactobacilli* administration on protein concentration in the colon of rats sacrificed rats sacrificed on day three (A) and seven (B). Mean values ± SEM of protein concentration values in acetic acid-induced colitis ($p < 0.05$; $n = 5$). a, b and c indicate significant difference when compared to control, colitis only, plantarum B3, plantarum B2, prednisolone respectively from One-way ANOVA and Tukey's post-tests



A **B**

Figure 4: Effect of lactobacilli administration on glutathione (GSH) concentration in the colon of rats sacrificed on day three (A) and seven (B). Mean values ± SEM of GSH values in acetic acid-induced ulcerative colitis ($p < 0.05$; n = 5). a, b, c, d and e indicate significant difference when compared to No colitis, colitis only, plantarum B3, plantarum B2, Prednisolone respectively from One-way ANOVA and Tukey's post-tests.

DISCUSSION

Acetic acid-induced ulcerative colitis is a reproducible model for inducing colitis in rats, similar to human ulcerative colitis, potentially useful for studying its pathophysiology and treatment (Renata *et al*., 1992). *Lactiplantibacillus plantarum* has been reported for its use in the treatment of various inflammatory disorders (Hao *et al*., 2023). The study examines the therapeutic effect of *Lactiplantibacillus plantarum* on acetic acid-induced ulcerative colitis in Wistar albino rats.

In this study, the body weights of the rats in the treatment groups showed no significant variation from those of the control group. An increased level of MPO in the blood have been linked to inflammation and oxidative damage (Davies and Hawkins, 2020). Inflammatory reactions result in raising myeloperoxidase levels, through the significant influx of neutrophils into the tissue (Oladejo *et al*., 2024). Lactic acid bacteria treatment reduced MPO levels in rats treated with LAB compared to colitis-only groups, suggesting inhibiting neutrophil activation and infiltration.

Increase in protein concentration indicates potential malabsorption or malnutrition, linked to chronic gastro-intestinal diseases like Crohn's and ulcerative colitis. This offers valuable information regarding the severity of the inflammation (Oladejo *et al*., 2024). The study found that *L. plantarum* PQ104969 treatment reduced protein concentration, suggesting it relieves inflammation by inhibiting protein increase.

The Glutathione (GSH), a non-enzymatic antioxidant, maintains cellular redox homeostasis by scavenging reactive oxygen species (ROS) (Oladejo *et al*., 2024). It supports immunological functions and eliminates electrophilic compounds and peroxidases through catalytic activities. In this study, *Lactiplantibacillus plantarum* PP893151 enhances GSH antioxidant activity, protecting the colon from lipid peroxidation caused by oxidative stress.

CONCLUSION

The study found that lactic acid bacteria treatment can mitigate inflammatory reactions and oxidative stress in acetic acid-induced ulcerative colitis. The *L. plantarum* PQ104969 regulates protein expression, while *L. plantarum* PP893151 increases GSH concentration, scavenging reactive oxygen species, suggesting it could be a potential therapeutic agent.

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