

Full Length Research Paper

## Safety and protective effect of *Lactobacillus acidophilus* and *Lactobacillus casei* used as probiotic agent *in vivo*

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The protective effect of *Lactobacillus acidophilus* and *Lactobacillus casei*, isolated from fresh cow milk, was studied *in vivo*. Toxicological data of rat serum revealed that the *Lactobacillus* isolates had liver improvement functions. Serum alanine aminotransferase (ALT) activities of the rats dosed with *Lactobacillus* isolates alone were lower (15.50 and 18.27 iu/l) than the control. There was a reduction in the count of enterobacteria in rats dosed with *L. casei* after 3 days of feeding trials. Protection of the gastrointestinal tract (GIT) by these isolates was also observed. Histopathological data confirmed partial protection of the GIT in rats dosed with *Lactobacillus* isolates and simultaneously infected with *Escherichia coli*. *L. casei* was generally observed to have a better effect than *L. acidophilus* in terms of liver function improvement, anticholesterolaemic effect, and reduction of enterobacteria in the GIT.

**Key words:** Probiotic, protection, *Lactobacillus*.

### INTRODUCTION

Gastrointestinal disorders are caused by various factors including antibiotic administration (Van der Waaij et al., 1982), or as a result of infectious agents such as toxigenic *Escherichia coli*, *Salmonella enteritidis*, *Entamoeba histolytica*, and viruses (Silva et al., 1999).

Innovative approaches have been tried as alternative to antibiotics in treating gastrointestinal diseases and these include using live biotherapeutic agents such as yeast (*Saccharomyces* spp.) and bacterial isolates (*Lactobacillus* spp.) or faecal enemas (Fuller, 1992).

*Lactobacilli* are important for the maintenance of the intestinal microbial ecosystem (Sandine, 1979). Colonisation of the gut with *Lactobacilli* starts within the first week of life (Salminen et al., 1995). The presence of

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this group of bacteria in the gut is considered to have several potential benefits such as growth enhancement of farm animals (Baird, 1977), protection from pathogens (Casas and Dobrogosz, 2000), alleviation of lactose intolerance (Jiang et al., 1996), relief of constipation (Walker and Duffy, 1998), anticholesterolaemic effect (Bertazzoni et al., 2001) and immunostimulation (Aattouri et al., 2001). *Lactobacilli* exert their protective or therapeutic effect through production of antimicrobial compounds (Dodd and Gasson, 1994), reduction of gut pH by stimulating the lactic acid producing microflora (Langhendries, 1995), competition for binding of receptor sites that pathogens occupy (Kailasapathy and Chin, 2000), stimulation of immunomodulatory cells (Rolfe, 2000) and competition with pathogens for available nutrients (Rolfe, 2000).

Walker and Duffy (1998) suggested that current perspectives on biotechnological applications of probiotic products require further *in vitro* and *in vivo* investigation to evaluate the safety of using wild type organisms or those obtained by genetic engineering. The present study is therefore aimed at understanding the protective effect of *L. acidophilus* and *L. casei* from fresh cow milk, and their ability to reduce the toxicological and pathological consequences associated with enterotoxigenic *E. coli* used to experimentally infect rats.

## MATERIALS AND METHODS

### *Lactobacillus* culture

*L. acidophilus* and *L. casei* were isolated from fresh cow milk on deMann Rogosa and Sharpe (MRS) agar. The isolates were characterised using colonial, morphological, and biochemical methods. Preliminary studies show that these two isolates are capable of inhibiting food spoilage and pathogenic bacteria. These *Lactobacillus* species were also found to adhere to the ileal epithelial cell (IEC) of albino rat. The isolates were cultured in MRS broth and incubated at 37°C for 2 days to obtain large cell concentration of approximately 10<sup>10</sup> cfu/g. The cells were washed, suspended in rehydrated skim milk (10% w/v), lyophilised, and stored at -20°C until use (Fujiwara et al., 2001). The concentration of the viable cells was determined by serial dilution techniques (Taylor, 1962).

### *In vivo* feeding

Twenty four albino rat (Wistar strain) aged 5 – 6 weeks were obtained from Department of Physiology, University of Ibadan. The rats were fed on basal diet purchased from Bendel feed, Edo State, Nigeria for 1 week *ad libitum* before the treatment. They were randomly assigned to 6 treatment groups designated as C, SA, SC, CA, CC and CT. Each was made up of 4 rats per group. Lyophilised *Lactobacillus* cells were reconstituted by dissolving 1 g in 10 ml of sterile water (approximately 10<sup>10</sup> cfu/ml). Group C was kept on the basal diet alone (control). Groups SA and SC were fed on the basal diet and were also dosed with 0.3 ml of *L. acidophilus* and *L. casei*, respectively. Groups CA and CC were fed the basal diet, dosed with 0.3 ml of *L. acidophilus* and *L. casei*, respectively, and infected

with 0.3 ml of 10<sup>5</sup> cfu/ml enterotoxigenic *E. coli* obtained from culture collection of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. Group CT was fed on basal diet and also infected with 0.3 ml of 10<sup>5</sup> cfu/ml of *E. coli*. The treatment above was repeated the second day. A post ingestion period of 18 days was observed after the administration of the cultures. The rats were killed by cervical dislocation and the blood samples of the rats were collected into EDTA bottles for analyses of serum biomarkers.

### Biochemical assay

Reflotron M06.02<06.00 (Boehringer Mannheim company, Germany) was used for the analyses of some major serum biochemical markers that can reveal the effects of the administered culture on the rat. The biomarkers assayed for were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total cholesterol of the serum. Standardised amount of the sample were automatically pipetted and applied on the test strip. The strip was inserted into the test chamber and result was displayed after some seconds on the computer monitor. The tests were carried out at 25°C.

### Bacterial count in faeces of rats

Freshly voided faecal materials were collected and pooled from each rat (1 g/rat) at days zero and 3. This was done to confirm if the *Lactobacillus* species were able to survive the stress within the gastrointestinal tract (GIT). The faeces were homogenised in normal saline and serially diluted. The diluted homogenates (0.1 ml) were plated on MRS agar for the enumeration of lactobacilli and on MacConkey agar for the enumeration of enterobacteria, especially *E. coli*. The plates were incubated at 37°C for 24 h and colony forming units on the plates were recorded.

### Histopathological analysis

The small intestine of the rats were removed. The organs were fixed in 10% formalin, dehydrated in increasing percentages of alcohol, cleared in xylene for 2 h for embedding. The embedded organs were sectioned using microtome and stained with haematoxylin-eosin (Silva et al., 1999).

### Statistical analysis

The data gathered from toxicological assay and faecal flora assay were processed using one way Analysis of Variance (ANOVA), SPSS 10.0. The level of significance was set at P < 0.05. Means were compared by Duncan T- tests.

## RESULTS AND DISCUSSION

The aspartate aminotransferase (AST) activity of rats treated with *L. casei* and challenged with *E. coli* (CC) was highest and significantly different (P < 0.05) from the control (C) (Table 1). AST is an enzyme that increases in activity in diseases such as severe bacterial infections, malaria, pneumonia, pulmonary infarcts, and tumours of organs such as heart and muscle (Cheesbrough, 1991). *Lactobacilli* can translocate (Berg, 1983) and survive in

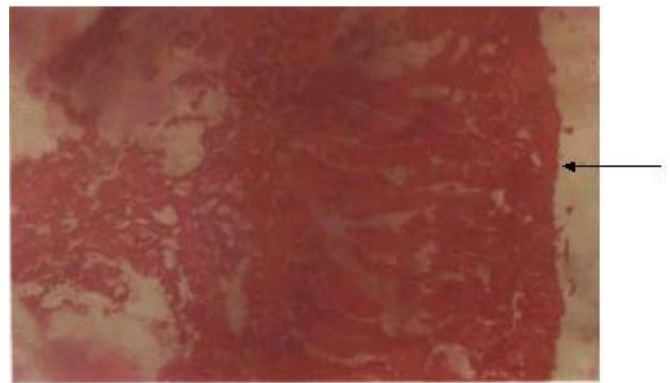
**Table 1.** Serum biochemical markers in rats after *in vivo* feeding trials.

| Group | AST (iu/l)    | ALT (iu/l)   | ALP (iu/l)     | Cholesterol (mg/dl) |
|-------|---------------|--------------|----------------|---------------------|
| C     | 93.47±20.91   | 31.43±1.96   | 32.90±0.00     | 109.67±7.09         |
| SA    | 133.33±6.81   | 18.27±1.80   | 32.60±0.52     | 104.67±9.87         |
| SC    | 139.67±32.86  | 15.50±5.37   | 32.30±0.52     | 98.33±0.58          |
| CA    | 149.83±92.98  | 64.57*±29.78 | 948.33*±8.26   | 116.00±11.27        |
| CC    | 459.00*±96.26 | 68.77*±19.93 | 1560.00*±29.13 | 108.33±3.51         |
| CT    | 86.40±11.14   | 39.07±4.33   | 512.00*±11.81  | 109.67±2.52         |

Values are means ± SD of 4 replicates per group. \*Values along column are significantly different ( $P < 0.05$ ) from the control (C). C: rats placed on basal feed alone. SA: rats placed on basal feed and dosed with *L. acidophilus*. SC: rats placed on basal feed and dosed with *L. casei*. CA: rats placed on basal feed, dosed with *L. acidophilus* and challenged with *E. coli*. CC: rats placed on basal feed, dosed with *L. casei* and challenged with *E. coli*. CT: rats placed on basal diet and challenged with *E. coli*.

the spleen, liver, and lungs (Bloksma, 1981). In the course of their translocation, they can cause cellular injury that may increase AST level in the serum. This may account for increase in AST observed for rats in groups SA and SC when compared with the control (C). The higher AST level observed in groups CA and CC may be due to the combine activities of *Lactobacillus* and *E. coli* in the GIT. In their study, Silva et al. (1999) reported that to obtain protective effect in animals, treatment with a probiotic agent had to be initiated 10 days before challenge with a pathogen. But, in this report orogastric dosing with *Lactobacillus* and challenge with *E. coli* was simultaneous. Moreover, the Alanine aminotransferase (ALT) activities of the serum in the rats also reveal that groups CA and CC dosed with *Lactobacillus* and simultaneously challenged with *E. coli*, were significantly higher ( $P < 0.05$ ) than the control (C). Alanine aminotransferase (ALT) is principally found in the liver and is regarded as being more specific than AST for detecting liver cell damage (Johnston, 1991; Cheesbrough, 1991). The implication of this result is that, there is a pronounced toxicological effect in rats of groups CT, CA, and CC. The lower ALT values in rats treated with *Lactobacillus* alone (SA and SC) compared to the control indicate liver function improvement brought about by the *Lactobacillus*. Hepatocytes play a major role in absorbing and metabolising many toxic chemicals (Eka et al., 1994). They are therefore liable to injury by various chemicals, including food.

The ALP activities of rats treated with *Lactobacillus* and *E. coli* (CT, CA, and CC) were also significantly higher ( $P < 0.05$ ) than those recorded in those treated with *Lactobacillus* only (SA and SC). A rise in ALP activities has been linked with an increased osteoblastic activity (Baron et al. 1994) and lack of bile flow (cholestasis). Only a slight anticholesterolaemic effect was also observed in rats treated with *Lactobacillus*. *Lactobacilli* had been found to have direct effect on cholesterol levels by assimilation and removal from the growth medium.



**Figure 1.** Histopathological section of small intestine of rat dosed with *L. acidophilus* isolated from fresh cow milk. Arrow shows intact intestinal villus pattern (X 100).

This had been demonstrated in pigs (Gilliland et al., 1985) and in rats (Bertazzoni et al., 2001). Serum ALT and ALP levels had been reported to increase with increase in serum cholesterol (Johnston, 1999).

The result of the histopathological analysis confirmed the protective effect of the *lactobacillus* alone. The protection of the GIT was observed in rats treated with *Lactobacillus* (SA and SC), where the villus patterns of the small intestine of the rats were well preserved (Figure 1). In rats treated with *Lactobacillus* and *E. coli* (CA and CC), there was partial protection of the villus pattern (Figure 2) while the intestinal villus pattern of group treated with *E. coli* only (CT) was markedly eroded (Figure 3). The mucus is as a result of wearing off of the intestinal epithelial cells.

The ability of the isolates to protect the GIT against pathogens can be confirmed by monitoring the count of enterobacteria, especially *E. coli* and beneficial bacteria e.g. *Lactobacilli*, in rat faeces (Mitsuoka, 1992). There was an increase in faecal *Lactobacilli* count in rats

**Table 2.** Total count of faecal bacteria during feeding trials.

| Group | Enterobacteria count |             | <i>Lactobacilli</i> count |            |
|-------|----------------------|-------------|---------------------------|------------|
|       | Day 0                | Day 3       | Day 0                     | Day 3      |
| C     | 5.62±0.08            | 6.49±0.43   | 6.00±0.45                 | 7.21±0.17  |
| SA    | 5.43±0.73            | 5.19±0.23   | 5.30±0.53                 | 8.24*±0.10 |
| SC    | 6.13±0.31            | 5.26±0.03   | 5.37±0.36                 | 8.32*±0.68 |
| CA    | 5.98±0.30            | 5.78±0.44   | 5.43±0.46                 | 7.47±0.77  |
| CC    | 5.96±0.47            | 5.49±0.39   | 5.81±0.23                 | 8.07±0.22  |
| CT    | 5.58±0.35            | 7.01±0.43** | 5.25±0.06                 | 6.24±0.25  |

**Figure 2.** Histopathological section of the small intestine of rat dosed with *L. acidophilus* and challenged with *E. coli*. Arrow shows area where the intestinal villus pattern was slightly eroded (X 100).**Figure 3.** Histopathological section of the small intestine of rat challenged with *E. coli*. Arrow shows area where the intestinal villus pattern was markedly eroded (X 200).

treated with *Lactobacilli* (Table 2). A slight decrease in enterobacteria count was also observed in most of the rats. There was increase in the *enterobacteria* and *Lactobacilli* count from day zero (0) to day 3 in both controls (C and CT). In a similar study, Chang et al. (2001) reported an increase in the *Lactobacilli* count in faeces of rat that was fed basal diet devoid of probiotic agent. The high *Lactobacilli* count in groups treated with *Lactobacilli* and *E. coli* (CA and CC) may be responsible for the partial protection of the GIT of rats in these groups.

Earlier report showed that a selected probiotic strain *L. reuteri* and *L. acidophilus* showed increasing effect on numbers of enterobacteria in piglets (Ratcliffe et al. 1986). The ability of the *lactobacilli* to produce toxic metabolites such as lactic acid, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and bacteriocin has been suggested as being responsible for their ability to inhibit other bacteria (Juven et al. 1992). Other factors such as host immunomodulation (Hatcher and Lambrecht, 1983) also play a prominent role. The report presented here showed that *L. casei* has a better probiotic effect than *L. acidophilus* in terms of liver function improvement, anticholesterolaemic property, and protection of the GIT from infection.

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