

Histopathological Effect of Lactobacilli on Murine Spleen

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ABSTRACT:

BACKGROUND:

Lactobacilli are ubiquitous in the environment and in human body. They widely used as probiotic therapeutic agents. However, several authors reported that this bacteria may cause several serious infections in various parts of human body.

OBJECTIVE:

This work is aimed to illustrate the histopathological effect of lactobacilli in murine spleen which play a major role in the immune system.

MATERIALS AND METHODS:

Three *Lactobacillus* isolates (namely, *L. bulgaricus*, *L. plantarum* and *L. acidophilus*) were isolated from yogurt, vinegar and vagina, respectively. These isolates were injected intraperitoneally in mice in a dose 1.5×10^8 CFU/ml.

RESULTS:

Several histopathological changes were caused by lactobacilli represented by degeneration, necrosis, presence of haemosidrin and macrophages and lymphoblasts.

CONCLUSION:

This non-pathogenic bacteria was able to cause severe damage to the spleen of mice.

KEYWORDS: lactobacilli, spleen, red pulp, white pulp

INTRODUCTION:

Lactobacilli belong to a diverse group of bacteria that are gram-positive, facultatively anaerobic and non-spore forming. They produce lactic acid as a major product of carbohydrate fermentation. These bacteria are ubiquitous in the environment and propagate in ecological niches⁽¹⁾. In humans they colonize the oral cavity, gastrointestinal tract, and vagina⁽²⁾. In general, lactobacilli are currently proposed as probiotic agents in several dietary products^(3,4).

In blood cultures, they are usually considered as contaminants, but in recent years they have been recognized as causal infectious agents of endocarditis, urinary tract infections, meningitis, intra-abdominal infections and bacteraemia⁽⁵⁾. Land and his coworkers reported that the molecular DNA fingerprinting analysis showed that the *Lactobacillus* strain isolated from blood samples was indistinguishable from the probiotic strain ingested by the patients. This report indicates that invasive disease can be associated with probiotic lactobacilli⁽⁶⁾.

Slover and Danziger⁽⁷⁾ stated that more research is needed to expand our basic understanding of the

conditions under which lactobacilli cause infection, especially with the mounting interest in using *Lactobacillus* sp. products as "natural forms" of disease treatment.

Due to the presence of B and T lymphocytes, the immunotoxic effect of xenobiotics or their metabolites on these cell populations may be reflected in the spleen. Hence, it is one of the recommended organs to evaluate for enhanced histopathology of the immune system⁽⁸⁾

The aim of this study is to evaluate the histopathological effect of lactobacilli on murine spleen.

MATERIALS AND METHODS:

Laboratory animals

Twelve female mice weighed 22 – 26 grams were distributed into four groups (A, B, C and D), three animals per group. Each animal was put in a single cage; however, all animals were fed the same food and water.

Isolation and identification

Lactobacilli were isolated from three different samples of vinegar, yoghurt (purchased from local markets) and vaginal secretion. One milliliter of each sample (vinegar and yoghurt) and a vaginal swab taken from a 37 yrs old woman were spread onto De Mann-Rogosa-Sharpe agar (MRSA) (pH 5.5, Himedia, India) plates and incubated

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anaerobically at 37 °C for 48 h. The lactobacilli were initially identified by their ability to grow on the selective MRSA, Purified cultures were streaked onto CaCO₃-agar plates gram-positive staining, rod shape, and catalase-negative phenotype. Biochemical analyses, including sugar fermentation profile and gas production in MRS broth (pH 5.5, Himedia, India), were conducted as described in (9,10).

McFarland turbidity standard tube no. 0.5 was employed to adjust the lactobacilli concentration to 1.5×10^8 CFU/ml, while conformation was achieved using the spread plate technique (11).

Lipase activity assay

Lipase activity was detected according to Lopes *et al.* (12) using olive oil as substrate.

Inoculation protocol

All animals were anaesthetized with sodium pentobarbital (25 mg / kg) (12). Abdomen and surrounding area were sterilized with 75 % ethanol then 50 µl of the bacterial suspension was injected intraperitoneally by aid of 1 ml syringe (13).

Group A was injected with *L. bulgaricus* (isolated from yogurt), group B was inoculated with *L.*

plantarum (isolated from vinegar), group C was inoculated with *L. acidophilus* which has been isolated from the vagina, while group D was inoculated with normal saline to be considered as a control group.

Three days later, animals were sacrificed; spleen was taken for histopathological study (14).

RESULTS AND DISCUSSION:

Isolation and identification

Results revealed that *L. bulgaricus*, *L. plantarum* and *L. acidophilus* were isolated from yogurt, vinegar the vagina, respectively; all of them were proved to be lipase positive.

Histopathological study

Spleen of control group showed normal texture (figure 1). All lactobacilli isolates were able to cause histopathological damage to murine spleen tissues represented by degenerative effect of certain parenchymal cells with necrosis (figure 2) as a wide spread effect with haemolysis of RBCs which lead to haemosidrin pigment deposit (figurer 3) especially in red pulp and in between splenic sinusoid with congestion

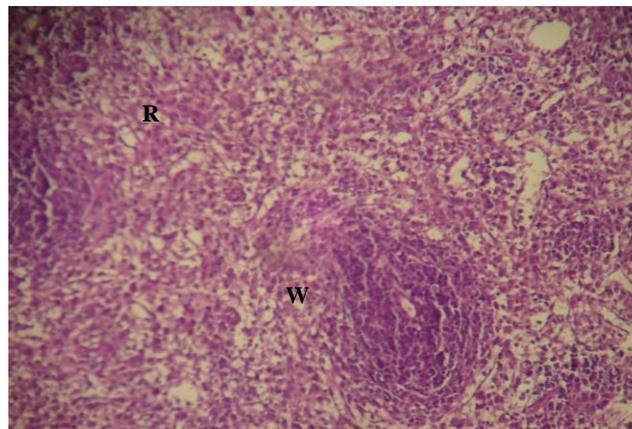


Figure 1: Normal murine spleen structure which consists of white pulp (W) and red pulp (R). 100X. H&E

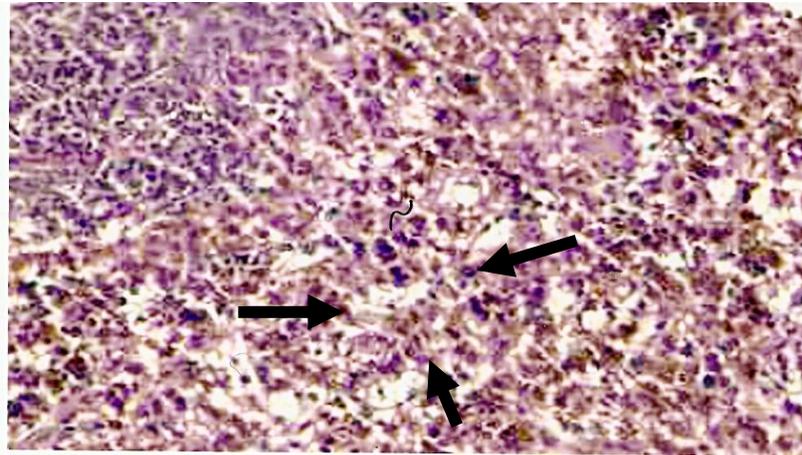


Figure 2: Section in murine spleen shows necrosis (arrows). 40X. H&E

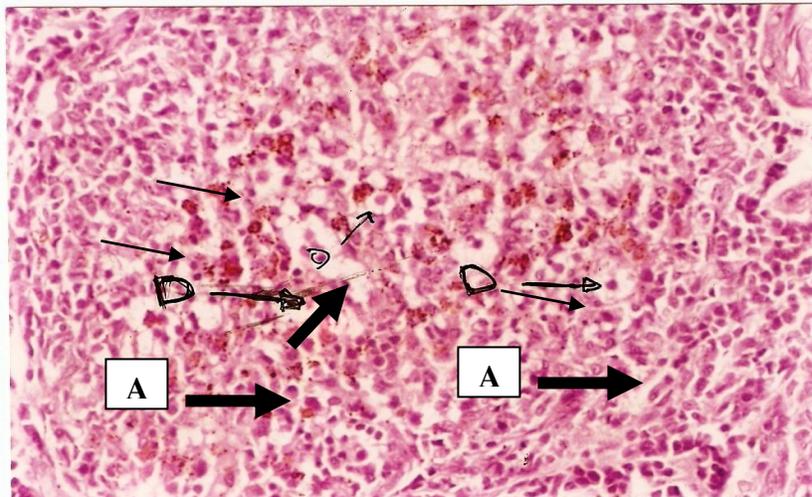


Figure 3: Section in murine spleen shows Apoptosis (A) and hemosiderin (thin arrows) in red pulp. 100X. H&E

Macrophages with cytoplasmic engulfed apoptotic debris can be seen as well (figure 4). Additionally figure 4 shows a presence of lymphoblasts (B cells).

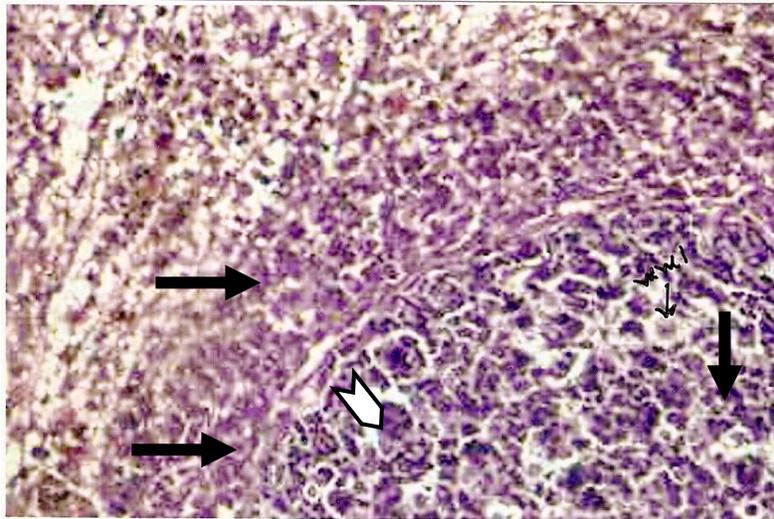


Figure 4: Section in murine spleen shows macrophages with engulfed apoptotic debris () and lymphoblast (). 100X. H&E.

Elmore ⁽⁸⁾ stated that an acute immune response to antigens may result in increased cellularity in the B cell areas and an increase in secondary follicles with prominent germinal centers. Immature B cells, or lymphoblasts, will proliferate in response to antigenic stimuli.

Typical histological changes that can be observed after exposure to lactobacilli are pathological alteration in red and white pulps, hence, we can confirm that these changes highly suggestive of the pathogenicity of lactobacilli. However, such changes could be attributed to the effect of cellular component such peptidoglycan, DNA, RNA, cytoplasmic membrane of lactobacilli ⁽¹⁵⁾.

Lipotechoic acid and peptidoglycan are major components of the cell wall of the gram-positive bacteria, which can be regarded as the gram positive analogues of lipopolysaccharide can activate polyclonal proliferation of lymphocytes including macrophage and B cells ⁽¹⁶⁾.

Veckman *et al.* ⁽¹⁷⁾ suggested that direct as well as indirect cytokine-mediated mechanisms are involved in the regulation of chemokine gene expression by Gram-positive bacteria. They demonstrated that *L. rhamnosus* stimulate Th1 cell chemotaxis. Also the ability of this bacteria to induce IL-12, IL-18, and IFN α/β production suggests that nonpathogenic *L. rhamnosus* are able to stimulate Th1 immune responses.

Brisbin *et al.* ⁽¹⁸⁾ reported that *Lactobacillus*

acidophilus, *Lactobacillus reuteri*, and *Lactobacillus salivarius* induced significantly more interleukin 1 β (IL-1 β) expression in spleen parenchymal cells than in cecal tonsil cells, indicating a more inflammatory response in the spleen than in cecal tonsils.

Although *Lactobacillus* spp. had traditionally been considered to be relatively avirulent, they could cause lifethreatening infections with serious outcomes. Several cases of liver abscess due to *Lactobacillus* spp. (*L. rhamnosus*, *Lactobacillus acidophilus*, and *Lactobacillus paracasei*) were reported. Moreover, bacteremia due to anaerobic, nonsporulating, Gram-positive bacilli, 25% of the isolates were identified as *Lactobacillus* spp. by 16S rRNA gene sequencing, and the isolation of *Lactobacillus* spp. was associated with clinically significant bacteremia ⁽¹⁹⁾.

CONCLUSION:

As a conclusion, the so called non pathogenic lactobacilli caused histopathological changes in spleen of mice when injected intraperitoneally.

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