

## The Effect of Two Strains of LAB (*P. acidilactaci* and *L. lactis*) In Laboratory Rats.

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### ABSTRACT

The purpose of this study was to evaluate the impacts of two Lactic Acid Bacteria strains (LAB) *Pediococcus acidilactaci* (*P. acidilactaci*) and *Lactococcus Lactis* (*L. Lactis*) isolated from fermented dairy products. In this study, 24 female rats were divided into three groups (8 rats per each group): (1) untreated group, (2) *P. acidilactaci* group: rats fed orally with 10<sup>8</sup> CFU /ml skim milk, (3) *L. Lactis* group: rats fed orally with 10<sup>8</sup> CFU /ml skim milk for 6 weeks. Bodyweight gain, daily feed intake, relative weight organs, hematological parameters, ALT, AST, protein, albumin, globulin, urea, creatinine, and histopathological examinations of liver and kidney were determined at the end of the experiment. Results: In this analysis, statistical significance was found for certain parameters e.g. organ weights, hematology, and clinical chemistry; none of these improvements were expected due to the treatment since the changes remained close to controls, also, histological studies of the liver and kidney observed no and mild morphology changes in the liver or kidney. The current study indicates that administration of *P. acidilactaci* and *L. Lactis* strains of LAB didn't harm laboratory rats and safe for animal or human consumption.

**KEYWORDS:** LAB, *P. acidilactaci*, *L. lactis*, Hematological parameters, Histological studies

### 1. INTRODUCTION

Due to their long history of use, many strains of lactic acid bacteria (LAB) are commonly considered stable and their status is widely accepted as safe (Donohue, 2006). Probiotics are 'live' microbes that provide their hosts with essential health benefits (Dicks and Botes, 2010 and Hardy *et al.*, 2013). Protection features must be addressed in addition to showing the effectiveness of probiotics in improving human health (Donohue, 2006 and Salminen *et al.*, 1998)

As probiotics, several bacterial species can be used, especially *Lactobacillus*, *Bifidobacterium*, *Pediococcus*, *Lactococcus*, and *Leuconostoc* species, although it is believed that *Lactobacillus casei*, *Lactobacillus acidophilus* and *Bifidobacterium* species are the most highly important species. These bacteria are part of the gastrointestinal microflora and are particularly useful for milk product digestion (Gareau *et al.*, 2010 and Ashraf and Shah, 2014). Several clinical studies have confirmed that the daily ingestion of probiotics or their associated products, especially *Lactobacillus* and *Bifidobacterium*, dramatically enhances human health through several consequences, including vitamin K biosynthesis (Bentley and Meganathan, 1982), indigestible dietary fibers metabolic fermentation (Nilsson *et al.*, 2008), competition with pathogenic microbes for mucosal epithelial cell-binding sites (Candela *et al.*,

2008), and modulation, regulation, and enhancement of the host immune system answer (Leroy and De Vuyst, 2004). The medicinal impact of LAB used of different fermented foods on human health has been enhanced and their use has contributed to increases in liver disease, allergies, asthma, cancer, blood cholesterol, and hyperlipidemia (Parvez *et al.*, 2006 and Tsai *et al.*, 2014). Rackayová *et al.* (2021) used behavioral experiments and ultra-high-field *in vivo* 1H MRS to see whether a multistrain probiotic mixture had a beneficial effect on behavior and neurometabolic improvements in rats after long-term treatment. This probiotic formulation had also increased gut Bifidobacteria and attenuated changes in locomotor activity and neurometabolic profile in rats when given prophylactically.

*Lactococcus lactis* (*L. lactis*) belongs to the LAB group and is a Gram-positive bacterium. Lactic acid is typically the primary fermentation product, and glycolysis is the main energy source, *L. Lactis* is mostly recognized for its immense significance in the dairy industry, where this bacterium inoculates more than 100 million tonnes of milk each year for the processing of different fermented dairy products Teuber, (1995). In addition, the many years of safe use have created *L. Lactis* is also a preferred candidate for other significant uses, such as as a delivery vehicle for human vaccines and therapeutic peptides and as a development tool for various

compounds. (Hugenholtz *et al.*, 2000 and Solem *et al.*, 2013).

The effects of oral administration of two LAB strains (*P. acidilactaci* and *L. lactis*) on health, development, hematology, daily blood chemistry in rats, and histological analysis of the liver and kidneys were investigated in this study.

## 2. MATERIALS AND METHODS

### 2.1. Preparation of LAB strains:

Isolation of *P. acidilactis* and *L. lactis* ssp *lactis* from fermented dairy products (laban zeer, laban rayeb and karish cheese) was used to create LAB strains.

Biochemical analysis and the API process were used to identify the strains. These were grown for 72 hours at 37 °C in MRS broth for rods and M17 broth for cocci isolated (Bikheet *et al.*, 2015). To feed the rats, 1 ml of each inoculation was combined with a separate aliquot of 10 ml skim milk (108 CFU/mL skim milk) (Zagato *et al.*, 2014).

### 2.2. Animals and Experimental Design:

In the biological laboratory of the Chemistry Department, Faculty of Agriculture, Minia University, twenty-four Sprague-Dawley female rats (180±5 g) were housed. Rats were acclimatized for one week to the laboratory conditions. They were housed in special healthy plastic cages and held for water and normal diet ad libitum. The experiments were performed according to the animal care and use standards of the Institutional Animal Ethics Committee, Minia University, Egypt. The rats were assigned randomly to 3 classes on day zero (8 females per group). The control group 1 was fed on a regular chow diet and each rat was given 1 ml of oral milk with LAB for 6 weeks. Group 2 administered 1 ml of oral skim milk with *P. acidilactis* daily to each rat for 6 weeks. Group 3 Each rat got an oral 1ml dose of skim milk with *L. Lactis* for 6 weeks a day. Bodyweight was recorded at the beginning and throughout the experimental period. At the end of this experiment (6 weeks), blood samples were taken from the retro-orbital plexus of all rats in each group after anesthetization. All blood samples were centrifuged at 3000 rpm for 15 minutes. The serum samples collected were kept at -20°C until they were used for biochemical analysis. Animals were dissected as quickly as possible, and liver, kidney, lung, heart and brain were removed, washed in ice-cold saline, and wiped away.

### 2.3. Hematological parameters:

Blood samples were taken into tubes containing 10% EDTA and labeled to indicate the

treatment groups as well as the research intervals. Red blood cells (RBCs) and white blood cells (WBCs) were counted according to Dacie and Lewis (1984), while hemoglobin (Hb) was measured according to VanKampen and Zijlstra (1961). Centrifuging blood in heparinized microhematocrit tubes (capillary tubes 1mm internal diameter and 7.5 cm length) for 5 minutes at 15,000 r.p.m. was used to assess hematocrit (HCT percent) or packed cell volume (PCV) (Dacie and Lewis, 1991). According to Schalm *et al.* (1975), the percentage of each form of total leucocyte population concerning the total count of WBCs was calculated.

### 2.4. Clinical chemistry analysis:

AST, ALT, total protein, albumin, blood urea nitrogen (BUN), creatinine as renal functions, and globulin were performed using commercially available kits from Bio- Diagnostic Co., Egypt.

### 2.5. Histopathological studies:

Small sections of the kidneys and liver of each control animal and treated groups were fixed for twenty-four hours in a 10% formal saline solution. Then, samples were washed (for dehydration) with water and serial dilutions of absolute ethyl alcohol. Paraffin bees wax tissue blocks were prepared for sectioning at a thickness of 4 microns by sliding microtome after routine processing. For histopathological analysis using a light microscope, the tissue parts obtained were collected on glass slides, deparaffinized, and stained by hematoxylin and eosin stains (Banchroft *et al.*, 1996).

### 2.6. Statistical analysis:

The mean standard error (SE) of eight measurements was used to calculate the experimental effects. ANOVA methods were used to conduct the study of variance. GraphPad Prism® was used for statistical calculations by GraphPad Software, San Diego, CA, USA (Motulsky, 1999).

## 3. RESULTS AND DISCUSSION

### 3.1. Changes in body weight and consumption of food:

All animals did not show abnormal physical, behavioral changes or unexpected deaths throughout the experiment. Data in Table (1) shows a significant elevation in body weight gain and feed efficiency ratio of about 29% and 24.3%, respectively at rats received *L. lactis*. Although *P. acidilactaci* increase these parameters but not significant compared with control.

**Table 1. Effect of *P.acidilactaci* and *L. lactis* on body weight gain and Feed intake of rat.**

Groups	Final weight(g)	Bodyweight gain (g)	Daily intake (g)	feed FER (%)
Control	230.0 ±0.4	54.0±0.6	14.0	10.71±0.6
<i>P.acidilactaci</i>	238.0± 2.6	58.0±1.0	14.5	11.73±0.2
<i>L. lactis</i>	254.7±6.8 <sup>a</sup>	69.7±3.9 <sup>a</sup>	15.0	13.30±0.7 <sup>a</sup>

.Data represent the mean ± SE of observations from 8 rats. <sup>a</sup> significant difference from the control group at P < 0.05. FER= Feed Efficiency Ratio

The result was in the same line with Zhao et al (2020) which reported that the weight gain of rats treated with a high dose of Lactic acid bacteria (*L. plantarum* ATCC8014 (1×10<sup>9</sup> CFU/ml) was the highest – significantly higher than the weight gain of the control group of rats.

Moreover, the ability of the probiotic to utilize additional nutrients present in milk such as calcium, vitamins, carbohydrates, and proteins led to the proliferation of a greater number of LAB in the bodies of LAB treated animals in milk (Gasmalla et al., 2013).

In addition, the LAB and prebiotics effects on cholesterol metabolism (Mandal et al., 2009), While LAB alone significantly increases hypocholesterolemic in vivo (Bukowska et al., 1998

and Xie et al., 2011). Liu et al. (2019) showed that some LAB strains (*Lactococcus lactis*) can be used to manufacture long-chain vitamin K2 efficiently and developing the processes of vitamin K2 biotechnological production

### 3.2. Changes in relative weight organs:

The results from Table 2 show that no major variations in relative organs weight were found (liver, kidney, heart) between two strains and the control, except for lung decrease and brain increase in *P.acidilactaci* group. This indicated that the *L. lactis* did not cause any adverse effects on many organs.

**Table 2. Effect of *P.acidilactaci* and *L. lactis* on relative weight of organs:**

Group	Liver	Kidney	Heart	lung	Brain
Control	3.7±0.1	0.62±0.06	0.39±0.0	0.82±0.07	0.68±0.006
<i>P.acidilactaci</i>	3.7±0.03	0.63±0.02	0.36±0.003	0.67±0.013	0.76±0.009 <sup>a</sup>
<i>L. lactis</i>	3.8±0.06	0.67±0.02	0.34±0.023	0.76±0.04	0.73±0.03

.Data is presented as means ± SE of observations from 8 rats. <sup>a</sup> (significantly different from the control group at P < 0.05.

Several scientific studies have indicated that daily intake of probiotics, especially *Lactobacillus* and *Bifidobacterium*, dramatically improves human health through a variety of effects, including vitamin K biosynthesis (Bentley and Meganathan, 1982).

The influence on the liver of feeding separate doses of mixed LAB strains, kidney, and spleen are measured by Tian et al., (2017) and Shokryazdan et al., (2016), and the organ to body ratio is determined. In the different dose classes and there were no major variations in the relative heart, kidney, testicle, or ovary weight of the rats.

Tsai et al., (2014) proved that rats fed on strains of LAB had not significant differences in the weight of the major organs, as well as the rate of daily food and water intake was similar to the control group. In this research, histopathological analyses of the organs which did not show any defects or histopathological changes in the organs confirmed our findings.

### 3.3. Haematological Parameters:

The hematological parameters such as RBCs, WBCs, Hct, MCV, and MCH were not significantly changed in rats received *L. lactis* sp

*lactis*. However, a significant increase in these parameters was noticed in rats treated with *P. acidilactaci* (Table 3). Oral administration of *L. lactis* did not cause any significant effect on differential leucocytes to count in the female albino rat. Whereas, *P.acidilactaci* caused a significant increase in these parameters comparing control in the same Table.

Szabo et al., (2011) and Tsai et al., (2014) found that treatment with LAB strains had a statistical significance on parameters of hematology, none of these improvements are hypothesized to be attributable to the treatment as the changes continued in the historical controls. Also, hematological parameters were in the normal physiological range when mice fed LAB strains at different doses (Tian et al., 2017).

### 3.4. Clinical chemistry analysis:

Serum hepatic markers (ALT, AST) and concentration of serum proteins, Albumin, Globulin, urea, and creatinine results are presented in Table 4. At the terminal sampling time, clinical chemistry values for the rats treated with *P.acidilactaci* and *L.*

**Table 3. Haematological findings in rats that were treated orally with *P.acidilactaci* , *L. lactis* for 6 weeks.**

Parameters Groups	Control	<i>P.acidilactaci</i>	<i>L. lactis</i>
RBCs (Cell/ $\mu$ L)	7.14 $\pm$ 0.09	7.55 $\pm$ 0.05 <sup>a</sup>	7.43 $\pm$ 0.08
WBCs ( $10^3$ /mm)	10.13 $\pm$ 2.3	15.60 $\pm$ 0.5 <sup>a</sup>	10.97 $\pm$ 0.23
Hct (%)	50.5 $\pm$ 0.2	53.3 $\pm$ 0.4 <sup>a</sup>	50.6 $\pm$ 0.8
Hgb (g/dL)	15.43 $\pm$ 0.3	16.17 $\pm$ 0.1	15.33 $\pm$ 0.2
MCV (FL/cell)	70 $\pm$ 0.3	71 $\pm$ 0.7 <sup>a</sup>	70 $\pm$ 0.7
MCH (Pg/cell)	20.8 $\pm$ 0.4	22.20 $\pm$ 0.2 <sup>a</sup>	20.63 $\pm$ 0.1
Neut (%)	28 $\pm$ 0.6	37 $\pm$ 0.3 <sup>a</sup>	28.67 $\pm$ 0.3
Lym (%)	65 $\pm$ 0.5	52 $\pm$ 0.5 <sup>a</sup>	66 $\pm$ 0.5
Mon (%)	3 $\pm$ 0.6	5.7 $\pm$ 0.7 <sup>a</sup>	3.3 $\pm$ 0.3
Eosi (%)	3.0 $\pm$ 0.5	2.0 $\pm$ 0.3	3.0 $\pm$ 0.3
Baso (%)	0	0	0

Data are presented as means  $\pm$  SE of observations from 8 rats. <sup>a</sup> (significantly different from control group at  $P < 0.05$ . <sup>b</sup> significantly different from *P.acidilactaci* group at  $P < 0.05$

**Table 4. Blood chemistry in rats that were treated orally with *P.acidilactaci* , *L. lactis* for 6 weeks.**

Parameters Groups	Control	<i>P.acidilactaci</i>	<i>L. lactis</i>
ALT (U/L)	24 $\pm$ 0.6	25.33 $\pm$ 1.2	22 $\pm$ 0.6 <sup>b</sup>
AST (U/L)	32 $\pm$ 0.6	36 $\pm$ 0.6	29 $\pm$ 0.6
Proteins	8.55 $\pm$ 0.5	8.05 $\pm$ 0.4	9.02 $\pm$ 0.1
Albumin	2.58 $\pm$ 0.3	1.67 $\pm$ 0.02 <sup>a</sup>	2.90 $\pm$ 0.04
Globulin	5.97 $\pm$ 0.046	6.37 $\pm$ 0.41	6.12 $\pm$ 0.029
Albumin/Globulin	0.43	0.26	0.47
Urea	9.9 $\pm$ 0.6	11.3 $\pm$ 0.8 <sup>a</sup>	9.0 $\pm$ 0.4
Creatinine	0.9 $\pm$ 0.01	1.3 $\pm$ 0.1 <sup>a</sup>	1.0 $\pm$ 0.01

Data are presented as means  $\pm$  SE of observations from 8 rats. <sup>a</sup> (significantly different from the control group at  $P < 0.05$ . <sup>b</sup> significantly different from *P.acidilactaci* group at  $P < 0.05$

*lactis* for the control group was noticed. Statistically significant variations in the serum activity of ALT, AST, and concentration of protein and globulin were obtained, while significant increases in urea and creatinine in *P.acidilactaci* group. Rats of *L. lactis* group also, had no variations in AST, protein, albumin, globulin, urea, and creatinine. However, a significant decreases in ALT comparing with *P.acidilactaci* group were observed.

Moon *et al.*, (2014) showed that rats treated with *P. acidilactaci* had ALT and AST closer to the control group, and lower than the high-fat diet group. No significant differences were also observed in serum albumin, urea nitrogen, total protein in a group treated with LAB strains (Tian *et al.*, 2017). LAB strains have shown that they do not cause oral toxicity in animals (Jones *et al.*, 2012). Suzuki *et al.*, (2020) are ingesting *L. Lactis* significantly improved the condition of atopic dermatitis.

### 3.5. Histopathological Examinations:

The effects of *P. acidilactaci* and *L.lactis spp lactis* on the rats were evaluated by histological

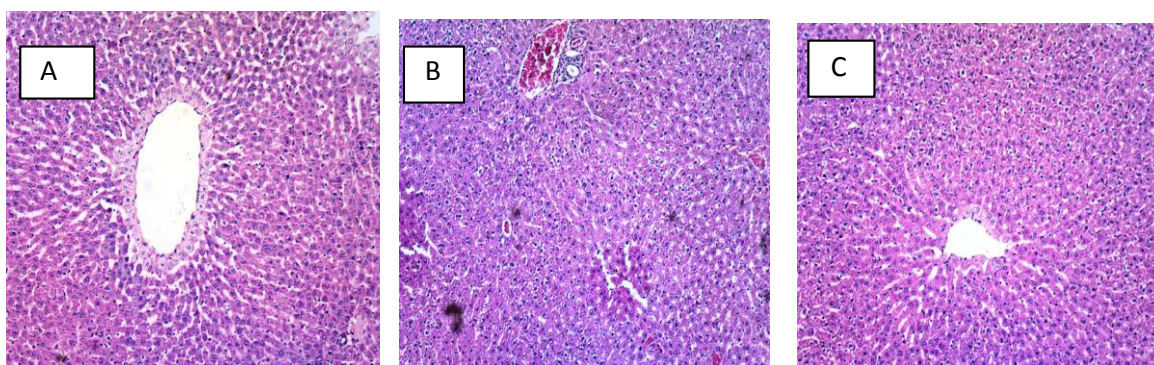
studies of the liver and kidney observed mild to moderate and no changes in the liver or kidney morphology resulting from the 6 weeks treatment with the test strains *in vivo* (Figs.1 and 2).

Tsai *et al.*, (2014) and Shokryazdan *et al.* (2016) proved no macroscopic changes in the morphology of the liver and kidney in animals treated with the test strains of LAB.

## 4. CONCLUSION

*P. acidilactaci* and *L. Lactis ssp lactis* isolated from fermented dairy products (laban zeer, laban rayeb, and Karish cheese) were found to be healthy at a dose of 108 CFU/ml skim milk for 6 weeks of care in this study. Both strains were well tolerated, with no adverse effects observed on development, feed consumption. There were no macroscopic improvements or noticeable variations in the relative weights and morphology of major organs. In conclusion, LAB strains (*P. acidilactaci* and *L. lactis ssp lactis*) are non-pathogenic and healthy to eat by humans and animals.

**Histopathological liver:**



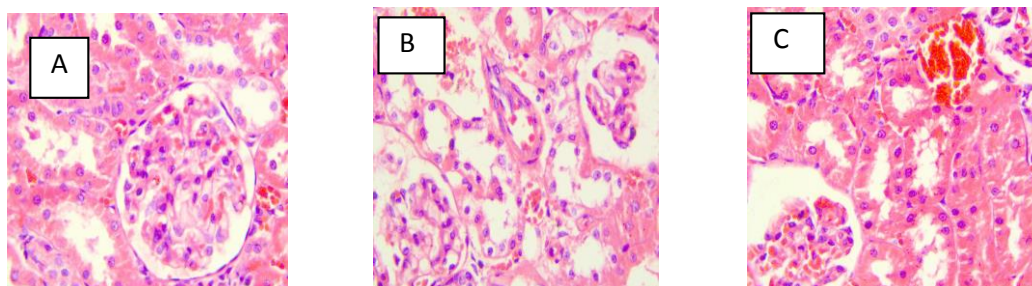
**Fig 1.** photomicroscograph sections of liver group of control (A), *P. acidilactaci* (B) and *L. lactis ssp lactis* (C) (H&E, scale bar=200µm).

**Table 5.** Histopathological notes on liver tissue of rats treated with *P. acidilactaci* (B) and *L. lactis ssp lactis*(C).

	control	<i>P. acidilactaci</i> (B)	<i>L. lactis ssp lactis</i> (C)
Loss architecture	-	-	-
Congested veine	-	+	-
Hepatocyte damage	-	+	+

(-) no, (+) mild histopathological change

**Kidney Histopathological:**



**Fig 2.** photomicroscograph sections of kidney group of control (A), *P. acidilactaci* (B) and *L. lactis ssp lactis* (C). (H&E, scale bar=50µm).

**Table 6.** Histopathological notes on kidney tissue of rats treated with *P. acidilactaci* (B) and *L. lactis ssp lactis*(C)

	Control	<i>P. acidilactaci</i> (B)	<i>L. lactis ssp lactis</i> (C)
Glomerular structure damage	-	+	+
Congested blood vessel	-	+	++
Dilated tubules & damage epithelium	-	+	-

(-) no, (+) mild, (++) moderate hispathological changes

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## الملخص العربي

### تأثير سلالتين من بكتريا حمض اللاكتيك *P. acidilactaci* و *L. lactis* في فئران التجارب

مها محمود بخيت<sup>١</sup> ، ماجدة عويس محمود<sup>٢</sup> ، هناء محمد حسن<sup>٢</sup> ، إيمان الحسيني ياسين<sup>٢</sup>.

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الغرض من هذه الدراسة هو تقييم تأثير سلالتين من بكتيريا حمض اللاكتيك (*P. acidilactaci*) و *Pediococcus acidilactaci* (*P. acidilactaci*) و *Lactococcus Lactis* (*L. Lactis*) المعزولين من منتجات الألبان المخمرة ، في هذه الدراسة ، تم تقسيم ٢٤ أنثى من الجرذان. إلى ثلاث مجموعات (٨ جرذان لكل مجموعة): (١) مجموعة غير معالجة ، (٢) مجموعة *P. acidilactaci*: جرذان تم تغذيتها عن طريق الفم بـ ١٠٨ CFU / ml لبن منزوع الدسم لمدة ٦ أسابيع. تم تقدير الزيادة وزن الفئران ، المأكول يوميا من العلف ، والوزن النسبي للأعضاء ، وتم عمل صورته دم كامله ، وتقدير ALT ، و AST ، والبروتين ، والألبومين ، والجلوبيولين ، واليوريا ، والكرياتينين ، والفحص المرضي للكبد والكلى في نهاية التجربة. النتائج: في هذه التحليلات، تم العثور على دلالة إحصائية لبعض المعلمات مثل أوزان الأعضاء وأمراض الدم والكيمياء السريرية ؛ لم يكن من المتوقع أن يكون أي من هذه التحسينات ناتجا عن العلاج نظرا لأن التغييرات ظلت قريبة من المجموعه الضابطه ، كما أن الدراسات النسيجية للكبد والكلى لاحظت عدم وجود تغييرات شكلية خفيفة في الكبد أو الكلى. تشير الدراسة الحالية إلى أن إعطاء سلالات *P. acidilactaci* و *L. Lactis* من بكتريا حمض اللاكتيك لم يكن له تأثير سلبي على فئران التجارب وهو آمن للاستهلاك الحيواني أو البشري.

الكلمات الدالة: - LAB ، *P. acidilactaci* ، *L. lactis* ، تقديرات الدم ، الدراسات النسيجية.