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STUDIES ON THE PERSISTENCE AND SAFETY OF A NEW *Lactobacillus plantarum* RECOMBINANT IN VIVO

BY

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ABSTRACT

Supplementation of male albino rats with *L. plantarum* ($\approx 4x10^9$ cfu. g^{-1}) and *L. plantarum* recombinant ($\approx 4x10^9$, $8x10^9$, and $2x10^9$ cfu. g^{-1}) significantly increased the total number of lactobacilli population with an increment percentage by 31.67%, 50.96, 55.79, and 39.71%, respectively after 4 weeks of administration when compared with the control. The *enterobacteriaceae* and aerobic counts in faeces of male albino rats fed *L. plantarum* recombinant at all levels decreased with the increasing of the time of treatment. Serum cholesterol, triglycerides and alanine aminotransferase of hyperlipaemic albino rats were reduced by the use of *L. plantarum* and *L. plantarum* recombinant. *L. plantarum* and *L. plantarum* recombinant exhibited significant decreased in the frequencies of micronucleated polychromatic erythrocytes than negative control. Meanwhile, there were no differences in the frequencies of chromosomal aberrations between *L. plantarum* feeding groups and the negative control so that they may have antimutagenic activity in bone marrow cells of rats. In conclusion the strains *L. plantarum* and *L. plantarum* recombinant can persist in the GIT of rats and also are non-toxic for rats so that they are therefore likely to be safe for human use in food and drug.

Key words: Probiotics, Safety, Genetic modification, *Lactobacillus plantarum*.

INTRODUCTION

Probiotics can be defined as a food or drug containing live microbes that, when ingested, is expected to confer beneficial physiologic effects to the host animal through microbial actions (Fuller, 1989). The last definition used at present was proposed by FAO in 2002 is that probiotics are live microorganisms, which confer a health benefit on the host when administerated in adequate amounts (Hamm and Hertel, 2002). Probiotics have been used for many years to aid in restoring and maintaining a healthy intestinal balance in favor of healthful bacteria, which is essential in maintaining good health. Probiotics are organisms or supportive substances that improve intestinal microbial balance (Percival, 1997). Among the numerous intestinal microbes, those that are expected to

beneficially affect the host by improving the intestinal microbial balance, and hence are selected as probiotics, include species of the genera *Lactobacillus, Bifidobacterium*, and *Enterococcus* (Fuller , 1991; Gordin and Gorbach, 1992). The representative species include *Lactobacillus acidophilus, Lactobacillus johnsonii, Lactobacillus gasseri, Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus plantarum, Bifidobacterium longum, Bifidobacterium breve, Bifidobacterium bifidum, Bifidobacterium infantis, Enterococcus faecalis*, and *Enterocuccus faecium. Bifidobacterium* species that specifically inhabit the intestinal tracts of animals, such as *Bifidobacterium thermophilum* and *Bifidobacterium pseudolongum*, are used in animal probiotics (Abe *et al.*, 1995). Strains of *Lactobacillus plantarum* form a group of industrially important lactic acid bacteria that are widely used as starters to stimulate malolactic fermentation in wine and lactic acid fermentation in meat and vegetables (Kiatpapan *et al.*, 2001). For many years, it has been recognized that elevated serum cholesterol is a risk factor associated with atherosclerosis and coronary heart disease, the latter being a major cause of death in Western countries. Numerous drugs that lower cholesterol have been used to treat hypercholesterolemia individuals. However, the undesirable side effects of these compounds can cause concern through their therapeutic use. Ingestion of lactic acid bacteria (LAB) would possibly be a more natural method to decrease serum cholesterol in humans. Cholesterol-lowering effect of lactic acid bacteria (LAB: *Streptococcus, Lactobacillus* and *Bifidobacterium*) is well-known (Lim *et al.*, 2004). Naruszewicz *et al.* (2002) proved that *Lactobacillus plantarum* (299v) administration leads to a reduction in cardiovascular disease risk factors, by lowering the concentrations of LDL cholesterol and fibrinogen and could be useful as a protective agent in the primary prevention of atherosclerosis in smokers. Previous studies have demonstrated that *Lactobacillus reuteri* administered in low doses has a hypocholesterolemic effect both therapeutically and preventively (Pereira *et al.*, 2003; Taranto *et al.*, 2004). Liong and Shah (2005) suggest that strains of lactobacilli could remove cholesterol via various mechanisms and may be promising candidates for use as a dietary adjunct to lower serum cholesterol in vivo.

Characterization of *L. plantarum* and genetically modified *L. plantarum* as a promising probiotic strains based, on the one hand, on their technological properties and, on the other hand, on their ability to survive passage through the human stomach (Pavvan *et al.*, 2002) and on bacterial translocation in acute liver injury. Therefore the aim of this work is to study the persistence in the GIT and safety of a new recombinant of *L. plantarum*.

MATERIALS AND METHODS

3. 1 Materials:

Bacterial strains.

A recipient strain *Lactobacillus plantarum* (DSMZ 20174) and a donor strain *Lactococcus lactis sup. lactis* (DSMZ 20729) were used.

Strains were obtained from the Egyptian Microbial Culture Collection (EMCC), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Vector and host strain:

E. coli DH5α strain was used for bacterial transformation. The PUC19 (Prommega) was used as vector.

Media:

MRS medium was used for cultivation and maintenance of *Lactobacillus plantarum* and to determined the number of lactic acid bacteria (Oxoid Manual, 1991). M17 Medium was used for cultivation and maintenance of *Lactococcus lactis sup. lactis* (O'sullivan and Klaenhammer 1993). LB medium was used for cultivation and maintenance of *E. coli* DH5α. Nutrient agar medium was used to enumerate total viable bacterial count of the different examined samples, MacConkey agar medium enumerate coliform bacteria, Bismuth sulphite agar medium to enumerate of *Salmonella sp* and Baird Parker agar medium was used to detect the count of *Staphylococcus sp* (Oxoid Manual, 1991).

Experimental animals:

Thirty male albino rats of an average weight 120– 140 g at the beginning of the experiment were used in this study. They were obtained from the farm of the General Organization of Serum and Vaccine (Helwan farm). These animals were clinically healthy and were acclimatized to the laboratory conditions before being used for two weeks.

Cholesterol was obtained from Winlab laboratory chemical, cholic acid was obtained from LOBA chemie.

Methods:

Survival at low pH:

Lactobacillus plantarum and *Lactobacillus plantarum* recombinant were studied for their ability to survive at low pH. Each strain was grown on MRS agar (pH5.6) by incubating for 3 days at 30° C and thereafter one colony forming unit was inoculated into 10 ml of sterile saline and stirred to become suspension. A 0.5 ml of bacterial suspension was inoculated into 10 ml of sterile phosphate-buffered saline [NaCl (9g/l), $Na_2HPO_4x\ 2H_2O$ (9g/l) and KH_2PO_4 (1.5 g/l) adjusted with 8M HCl] tubes with pH value 1, 2, 3, 4, 5 and 6. The tubes were incubated at 37 °C and the viable organisms were counted after exposure for 0, 0.5, 1, 2.5 and 4 hr on MRS agar (pH5.6) incubated at 30 °C for 2 days.

Survival in the presence of bile salts at different pH levels:

Lactobacillus plantarum and *Lactobacillus plantarum* recombinant were studied for their ability to survive at different pH values in the presence of bile salt. Each strain was grown on MRS agar (pH 5.6) by incubating for 3 days at 30° C and thereafter one colony forming unit was inoculated into 10 ml of sterile saline and stirred to become suspension. A 0.25 ml of bacterial suspension was inoculated into 5 ml sterile MRS broth tubes with pH values 1, 2, 3, 4, 5 and 6 (adjusted using 8 M HCl / NaOH). 0.15% or 0.3% standardized mixture of the salts of bile acids was also added to the tubes with pH values. The tubes were incubated at 37°C and the viable bacteria were counted after exposure for 0, 0.5, 1, 2.5 and 4 hr on MRS agar incubated at 30°C for 2 days.

Nutritional experiment design:

Rats were subjected to the induction of hypercholesterolemia by mixing the pure cholesterol and cholic acid at a ratio of 3 :1. Then, 10% of this mixture was prepared using few drops of tween 80 as emulsifying agent. This solution was mixed daily with the basal diet in quantities providing 0.5 gm / kg body weight (B.W.) daily for 10 weeks. Blood samples were collected every 2 weeks for the evaluation of total cholesterol. After the induction of hypercholesterolemia rats were divided to five groups were feeding as follows:

Negative control: rats were continued feeding on the basal diet for 4 weeks.

Positive control: animals were injected IP by single dose of 25mg/kg cyclophosphamide and use as positive control.

Group 1: rates were feeding on the basal diet containing $\approx 4x10^9 cfu.g^{-1}$ diet of *L. plantarum* (Lp).

Group 2: rats were feeding on the basal diet containing $\approx 4x10^9 cfu.g^{-1}$ diet of *L. plantarum* recombinant (TLp).

Group 3: rats were feeding on the basal diet containing $\approx 8x10^9 cfu.g^{-1}$ diet of *L. plantarum* recombinant (2TLp) (about twice volume of group 3).

Group 4: rats were feeding on the basal diet containing $\approx 2x10^9 cfu.g^{-1}$ diet of *L. plantarum* recombinant (0.5TLp) (about half volume of group 3).

Genetic modification procedures:

Plasmid was extracted according to (O'sullivan and Klaenhammer, 1993). The cloning procedure was carried out according to Sambrook et al. (1989) and using PUC19 vector (prommega) and *E. coli* DH5α as host strain. Transformation procedure was carried out. The colony that transformed was isolated from MRS plates which containing ampicillin.

Cytogenetic techniques

Micronucleus Analysis

Twenty-four hours after the last administration, five animals of each group were sacrificed by cervical dislocation. Slides were prepared according to Salamone *et al.* (1980). The bone marrow of the femur was flushed with fetal bovine serum. Smears were fixed with methanol and stained with Giemsa. The slides were coded and micronucleated polychromatic erythrocyte (MNPCE) frequencies among 2000 polychromatic erythrocytes per animal were estimated for each individual using a 100x magnification.

Chromosomal Aberrations:

Animals were injected IP with colchicine solution. Two hour later, animals were sacrificed by cervical dislocation and chromosomes of bone marrow cells were prepared according to Yosida and Amano (1965). The slides were stained with Giemsa and 100 good metaphase spreads of each group were examined microscopically to analyze the different types of chromosomal aberrations.

Statistical analysis:

Student's paired (t) –test was used to detect statistical significance of MNPCEs among the different groups. Whereas, analysis of variance test (ANOVA) was used to analyze the statistical significance of chromosomal aberrations between the different groups (SAS,1996).

RESULTS AND DISCUSSION

Persistence of *Lactobacillus plantarum* recombinant:

L. plantarum and *L. plantarum* recombinant were evaluated for their ability to persist in GIT of rats after 4 weeks of oral administration. Results recorded in Table 1 clearly show that *L. plantarum* supplementation ($\approx 4x10^9$ cfu.g^{-1}) significantly increased the total number of lactobacilli population with an increment percentage by 31.67% after 4 weeks of inoculation when compared with the control. Same trend was noticed when *L. plantarum* recombinant was inoculated either with the same inoculation, twice or half the inoculation count ($\approx 4x10^9$, $8x10^9$, and $2x10^9$ cfu. g^{-1}, respectively). The increment percentage reached 50.96, 55.79, and 39.71%, respectively. Data from the same table showed that *L. plantarum* and *L. plantarum* recombinant are able to persist for 10 days after the last dose (4 weeks), with bacterial levels ranging from 5.99 to7.59 log cfu. g^{-1} of faeces. It is noteworthy that half the inoculation content of *L. plantarum* recombinant ($\approx 2x10^9$ cfu. g^{-1}) was still found in the faeces 10 days after the last administration and maintained it self at levels of approximately 6. 19 log cfu. $g^{-1}$of faeces greater than the twice content of *L. plantarum* when inoculated ($\approx 4x10^9$ cfu. g^{-1}). Foo *et al.* (2003) and Pavan (2002) came to the same conclusion.

The faecal enterobacteriaceae counts for the control and the groups containing *L. plantarum* ($4x10^9$ cfu. g^{-1}) and *L. plantarum* recombinant ($4x10^9$ cfu. g^{-1}, $8x10^9$ cfu. g^{-1}, and $2x10^9$ cfu. g^{-1}) were shown in Table (2). The enterobacteriaceae counts for the control rats increased consistently throughout the experiment also after the last dose by 10 days. In contrast, the enterobacteriaceae counts of rats fed *L. plantarum* recombinant at all levels decreased with the increasing of the time of treatment. When *L. plantarum* was used, the enterobacteriaceae counts of rats were lower than the control while it was higher than that of *L. plantarum* recombinant at all inoculation levels. Same trend was noticed after 10 days of the last dose. In agreement with Kasravi *et al.* (1997) we showed that oral administration of 10 ml skim milk containing of *L. plantarum* or *L. plantarum* recombinant at concentration of $\approx 4x10^9$ cfu. g^{-1} for 4 weeks significantly increases the lactobacilli count in the faeces in the experimental groups. This is accompanied by a decrease in the number of enterobacteriaceae. There was no difference between the human and rat strains in this respect.

Lactobacillus is an important group of intestinal microflora, and plays a great part in colonization resistance (Kasravi *et al.*, 1997). It has previously been shown that certain diseases are associated with reduction of the total intestinal lactobacillus population and supplementation of these bacteria improves the overall condition, both experimentally and clinically (Fabia *et al.*, 1993). In this experiment, oral supplementation with either ($\approx 4x10^9$ cfu. g^{-1}) *L. plantarum* or ($\approx 4x10^9$, $8x10^9$, and $2x10^9$ cfu. g^{-1}) *L. plantarum* recombinant reduced the rate of aerobic bacteria even after 10 days after the last dose as shown in Table 3. Kasravi *et al.* (1996) came to the same conclusion.

Table (1): Effect of inoculum's concentration of *L. plantarum* and *L. plantarum* recombinant on the Lactobacilli count (log cfu. g^{-1}) faeces during feeding trials.

Treatment	Zero time	2 weeks	4 Weeks	After 10 days from stopping treatment
Control	5. 39ab	5. 99c	6. 22d	6. 49c
Lp	5. 49a	7. 82b	8. 19c	5. 99d
TLp	5. 29ab	8. 72a	9. 39a	7. 09a
2 TLp	5. 24ab	8. 91a	9. 69a	7. 59a
0. 5 TLp	5. 19b	8. 19b	8. 69b	6. 19cd

Table (2): Effect of inoculum's concentration of *L. plantarum* and *L. plantarum* recombinant on the enterobacterial count (log cfu.g^{-1}) faeces during feeding trials.

Treatment	Zero time	2 weeks	4 Weeks	After 10 days from stopping treatment
Control	5. 67ab	7. 24a	7. 66a	7. 72a
Lp	5. 39c	5. 28b	5. 65b	6. 36b
TLp	5. 48bc	5. 20b	4. 86c	5. 55d
2 TLp	5. 76a	5. 26b	4. 37c	5. 16e
0. 5 TLp	5. 27c	5. 26b	5. 06c	6. 05c

Table (3): Effect of inoculum's concentration of *L. plantarum* and *L. plantarum* recombinant on the aerobic viable count (log cfu. g^{-1}) faeces during feeding trials.

Treatment	Zerotime	2 weeks	4 Weeks	After 10 days from stopping treatment
Control	5. 49a	7. 13a	7. 91a	8. 09a
Lp	5. 11a	5. 78c	5. 98c	7. 23ab
TLp	5. 40a	6. 56abc	6. 75bc	6. 27c
2 TLp	5. 6a	6. 94ab	7. 23ab	6. 75bc
0. 5 TLp	5. 20a	6. 07bc	6. 56bc	6. 01c

Data recorded in Table 4 showed that pH level decreased (as expected) during growth of LAB due to the production of organic acid and by bile salt hydrolysis (Pereira *et al.*, 2003) conjugated bile salt analysis of the strains at regular time intervals showed a marked decrease in pH level, in this case after 4 weeks of feeding on *L. plantarum* which reached 5.7. The other strain yielded similar expect that they reached to the same level of pH after just 2 weeks of the experiment on *L. plantarum* recombinant at any levels of feeding. Data at the same Table show that there was a slight increase of pH level 10 days after the last dose either with *L. plantarum* recombinant or *L. plantarum*.

One beneficial effect that has been suggested to result from human consumption of LAB is a reduction in serum cholesterol levels, as suggested by the results of several human and animal studies (Pereira and Gibson, 2002). The

effect of *L. plantarum* and *L. plantarum* recombinant at different ($\approx 4x10^9$, $8x10^9$, and $2x10^9$ cfu. g^{-1}) on the serum cholesterol, triglycerides and alanine aminotransferase reduction of hyperlipaemic albino rats was studied.

Results recorded in Table 5 clearly exhibited a positive relation between the following daily administration of viable *L. plantarum* recombinant orally at any concentration and the reduction of serum cholesterol of albino rats till it reached the end of the experiments. Four weeks of feeding on $\approx 8x10^9$ cfu. g^{-1} *L. plantarum* recombinant gave the higher reduction of serum cholesterol being 61. 50 mg. dl^{-1} with a decrement percentage being 48. 13 % when compared with the initial concentration. On the other hand, it was noticed that there was slightly change in serum cholesterol level at the presence of *L. plantarum* when compared with the control. The percentage of reduction reached 34. 39 and 30. 61 respectively at the end of the experiment. From the foregoing results, it could be stated that *L. plantarum* recombinant had a higher reduction in serum cholesterol level by about 14 % when compared with *L. plantarum* these results are in accordance with Nakajima *et al.* (1992) and Kitazawa *et al.* (1993) who stated that EPS (exopolysaccharides) produced by LAB may confer health benefits so that studies with mouse models indicate that EPS have immunostimulatory, antitumoral, or cholesterol-lowering activity.

Table (4): Effect of inoculum's concentration of *L. plantarum* and *L. plantarum* recombinant on the pH of faecal bacteria faeces during feeding trials.

Treatment	Zerotime	2 weeks	4 Weeks	After 10 days from stopping treatment
Control	6. 7a	6. 1a	6. 5a	6. 41a
Lp	6. 8a	6. 0a	5. 7b	6. 12ab
TLp	6. 6a	5. 8ab	5. 3c	5. 72cd
2 TLp	6. 5a	5. 5b	5. 3c	5. 52d
0. 5 TLp	6. 4a	5. 6b	5. 6bc	6. 02bc

Table (5): Effect of inoculum's concentration of *L. plantarum* and *L. plantarum* recombinant on serum cholesterol concentration ($mg.dl^{-1}$) in hyperlipaemic albino rats.

Animal group	Zero time	2 weeks	4 Weeks
Control*	$101. 07^{d}$	$75. 67^{a}$	$70. 13^{a}$
Lp	$102. 27^{d}$	$71. 23^{b}$	$67. 10^{b}$
TLp	$123. 20^{a}$	$69. 53^{b}$	$65. 10^{c}$
2 TLp	$118. 57^{b}$	$64. 46^{c}$	$61. 50^{d}$
0. 5 TLp	$110. 43^{c}$	$70. 30^{b}$	$65. 67^{bc}$

* = Diet without any supplementation of LAB strains

The level of serum triglycerides in albino rats fed on different concentration $\approx 4x10^9$, $8x10^9$, and $2x10^9$ cfu. g^{-1} of *L. plantarum* recombinant diets were significantly lower than in those fed on diet free from the strain (control) or fed on diet supplemented with $\approx 4x10^9$ cfu. g^{-1} *L. plantarum*. The

reduction in triglycerides levels with the above mentioned groups was 78. 2%, 80. 82% and 70. 27%, respectively (Table 6). The level of serum triglycerides in the group fed on diet supplemented with *L. plantarum* was also lower than that in the control, but higher than the group fed on diet supplemented with *L. plantarum* recombinant at any concentration. It is noteworthy from the data in Table 6 that the *L. plantarum* recombinant at any concentration were more effective in lowering serum triglycerides than the *L. plantarum*. Schaarmann *et al.* (2001) reported that consumption of probiotic strain lower triglycerides in hypercholesterolemic women from 114 mg. $100ml^{-1}$ to 41mg. 100 ml^{-1} after 153 days.

Table (6): Effect of inoculum's concentration of *L. plantarum* and *L. plantarum* recombinant on serum triglycerides concentration (mg. dl^{-1}) in hyperlipaemic albino rats.

Animal group	Zero time	2 weeks	4 Weeks
Control	213.67^{c}	143.23^{a}	98.32^{a}
Lp	205.00^{d}	114.27^{b}	70.95^{b}
TLp	233.87^{a}	86.20^{d}	50.96^{d}
2 TLp	241.67^{a}	82.37^{e}	46.35^{e}
0. 5 TLp	205.00^{d}	107.60^{c}	60.93^{c}

Septic complication represents frequent causes of morbidity in liver diseases and following hepatic operations. Most infections are caused by the individual own intestinal microflora. The intestinal microflora composition is important in physiological and healthophosiological processes in the human GIT, but their influence on liver in different situation is unclear. We therefore studied the effect of following daily administration of viable *L. plantarum* and *L. plantarum* recombinant orally at different concentration for four weeks on the level of ALT. Administration of different bacterial strains in liver rat model has shown different effect on liver indicated by the release of the liver enzymes. The administrated LAB (Table 7) showed different effect on hepatocellular damage indicated by the release of liver enzymes. *L. plantarum* recombinant at $\approx 4x10^{9}$ cfu. g^{-1} and $\approx 8x10^{9}$ cfu. g^{-1} decreased hepatocellular damage indicated by decreasing the release of the enzyme ALT compared to the control by 14. 89% and 15. 96% respectively. On the other hand, the *L. plantarum* group $\approx 4x10^{9}$ cfu. g^{-1} and $\approx 2x10^{9}$ cfu. g^{-1} *L. plantarum* recombinant showed no difference in the release of ALT and it was only 8. 88% and 8. 83% respectively. While it showed higher values compared to the control group with a significant difference by about 6%. We used enzyme ALT because it is more specific to the liver injury than aspartate aminotransferase (AST) (Adawi *et al.*, 2001). From the previous results it could be concluded that feeding strains of lactobacilli that survive in the GIT reduces liver injury in the rat. It seems that maintaining the intestinal barrier functions of decreasing the number of the translocated bacteria could be one of the factors counteracting the liver injury (Galberg *et al.*, 1999). It seems that LAB species/strains could differ in their effect on liver. Knowing, characterization, definition and understanding of the most beneficial strains and their mechanisms are of almost importance.

Table (7): Effect of inoculum's concentration of *L. plantarum* and *L. plantarum* recombinant on liver ALT concentration (mg. dl^{-1}) in hyperlipaemic albino rats.

Animal group	Zero time	2 weeks	4 Weeks
Control	49. 10^{b}	51. 03^{a}	47. 7^{b}
Lp	50. 07b	50. 77^{a}	45. 74^{c}
TLp	52. 72^{a}	49. 63^{ab}	44. 87^{cd}
2 TLp	52. 63^{a}	48. 13^{b}	44. 23^{d}
0. 5 TLp	53. 67^{a}	51. 20^{a}	48. 93^{a}

Micronucleated Polychromatic Erythrocytes:

The mean frequencies of micronucleated polychromatic erythrocytes in the different experimental groups are presented in Figure 1. The result showed that animals of the first group induced non significantly decrease in the frequency of micronucleated polychromatic erythrocytes (11. 80 ± 0. 84) in bone marrow cells when compared with negative control (12. 20 ± 0. 84). While, the other three groups exhibited significant decreased ($p \leq 0.01$) in the frequencies of micronucleated polychromatic erythrocytes (8. 20 ± 0. 84, 7. 80 ± 0. 83, 6. 20 ± 0. 84) respectively, as compared with negative control (12. 20 ± 0. 84). In relation to the comparison to the positive control. Also the results showed that there are statistically significant differences ($p<0.01$) between the frequencies of micronucleated polychromatic erythrocytes in all treated groups (8. 20 ± 0. 84, 7. 80 ± 0. 83, 6. 20 ± 0. 84, respectively) and positive control (90. 20 ± 6. 06).

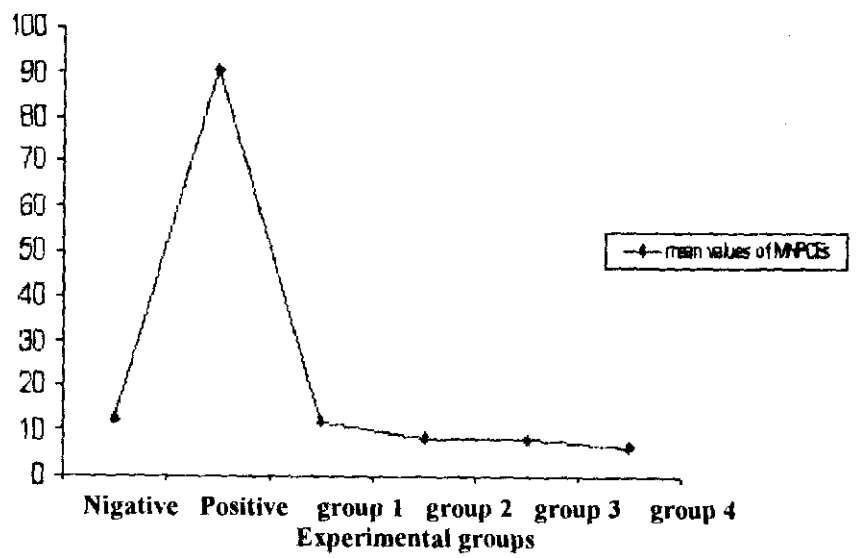

Fig (1): Main values of MNPCEs in bone marrow cells of treated male rats in all experimental groups.

Chromosomal Aberrations:

The present study indicated that the male rats feeding with four different doses of *L. plantarum* for two months not induced mutagenic effect in bone marrow cells of male rats. Table 8 illustrates the mean values of different individuals and total chromosomal aberrations in bone marrow cells of control and *L. plantarum* feeding male rats. Structural chromosomal aberrations types observed were of chromatid gaps and breaks, fragments and centromeric attenuations. Numerical chromosomal aberrations types were of hypoploids and polyploidy. Data of the experiment indicated that there are no significant differences in the frequencies of both individuals and total chromosomal aberrations between the *L. plantarum* feeding animals and the negative control. However, at the comparison between the frequencies of both individuals and total chromosomal aberrations in male rats bone marrow cells of positive control and those of different doses bacteria feeding groups, results showed that there are significant differences ($p<0.01$) between *L. plantarum* feeding animal groups and the positive control. This means that *L. plantarum* and *L. plantarum* recombinant not induce mutagenic effect in the male rat bone marrow cell after two monthes of feeding.

Functional foods or supplements may be used in the context of a healthy lifestyle or as a mean to compensate for an unhealthy lifestyle. Adverse long-term or cumulative effects of functional food or supplement intake are of public health concern (de Jong *et al.*, 2003). In this century various health benefits have been purported to result from consumption of foods containing live microorganisms, particularly lactic acid bacteria. Probiotics can provide relief for lactose intolerant individuals and reduce bouts of diarrhea. Evidence for other claims such as lowering serum cholesterol, suppressing cancer and stimulating the immune system remains to be clearly established by conducting well-controlled, statistically-valid clinical trials (Scheinbach, 1998).

In the present study, cytogenetic results indicated that *L. plantarum* exhibited significant decreased in the frequencies of micronucleated polychromatic erythrocytes than negative control. Meanwhile, there were no differences in the frequencies of chromosomal aberrations between *L. plantarum* feeding groups and the negative control. This means that LAB bacteria may have antimutagenic activity in bone marrow cells of rats. This result was supported with Burns and Rowland (2000). They reported that the various LAB can inhibit genotoxicity of dietary carcinogens in vitro and the degree of inhibition was strongly species dependent. For example, Renner and Munzner (1991) and Pool-Zobel *et al.* (1993) demonstrated that *Lactobacillus casei* and *L. lactis* inhibited the mutagenic activity of nitrosated beef by over 85%, whereas *Lactobacillus confusus* (*Weisella confusa*) and *Lactobacillus sake* had no effect. It seems likely that these results together with similar results by other workers are a consequence of binding of the mutagens by the LAB (Zhang and Ohta, 1991and Bolognani *et al.*, 1997). Both the chromosome aberrations and the micronucleus test in bone marrow cells, showed that *Lactobacillus casei* exhibit strong anticlastogenic action. Pool-Zobel *et al.* (1996) investigated the ability of range of species of LAB to inhibit DNA damage in the colon mucosa of rats treated with the

Table (8): Frequencies of different types of chromosome aberrations in male rat bone marrow cells of all experimental groups.

	No. of examined animals		Numerical aberrations		Structural aberrations				Total chromosomal aberrations
			hypoploids	polyploidy	Chromatid gaps	Chromatid breaks	fragments	Centromeric attenuations	
Negative control	5	M ± SD	0. 60 a ± 0. 55	0. 00 a ± 0. 00	1. 40 b ± 0. 55	0. 40 b ± 0. 55	0. 00 b ± 0. 00	2. 80 b ± 0. 84	5. 20 b ± 1. 30
Positive control	5	M ± SD	1. 60 a ± 1. 14	0. 00 a ± 0. 00	5. 20 a ± 1. 30	5. 20 a ± 1. 30	3. 20 a ± 1. 10	5. 80 a ± 1. 48	21. 00 a ± 2. 74
Group1	5	M ± SD	0. 60 a ± 0. 89	0. 00 a ± 0. 00	1. 80 b ± 0. 45	0. 60 b ± 0. 55	0. 40 b ± 0. 55	3. 40 b ± 0. 55	7. 20 b ± 1. 30
Group2	5	M ± SD	0. 60 a ± 0. 55	0. 00 a ± 0. 00	1. 20 b ± 0. 84	0. 60 b ± 0. 55	0. 40 b ± 0. 55	3. 20 b ± 0. 84	6. 00 b ± 1. 00
Group3	5	M ± SD	0. 80 a ± 0. 84	0. 40 a ± 0. 55	1. 20 b ± 0. 45	0. 60 b ± 0. 55	0. 00 b ± 0. 00	3. 40 b ± 0. 55	6. 40 b ± 1. 14
Group4	5	M ± SD	1. 00 a ± 0. 71	0. 40 a ± 0. 55	080 b ± 0. 45	0. 00 b ± 0. 00	0. 00 b ± 0. 00	3. 80 b ± 0. 84	6. 00 b ± 1. 22

Each value represents the mean and standard deviations of five animals.
M = Mean SD = Standard deviations.

carcinogens MNNG or 1, 2-dimethylhydrazine (DMH). They found that *L. acidophilus* (isolated from a yoghurt), *Lactobacillus gasseri*, *L. confusus*, *B. breve* and *Bifidobacterium longum*, prevented MNNG-induced DNA damage when given at a dose of 10^{10} cells/kg body weight, 8 hours before treating with the carcinogen. In most cases the DNA damage was reduced to a level similar to that in untreated rats. Also, Burns and Rowland (2000) and Marotta *et al.* (2003) found that the effective probiotics and to a lesser extent prebiotics have the potential to exert significant antimutagenic properties and reduction of colon cancer risk. Zhou *et al.* (2000) results demonstrated that 4 weeks consumption of LAB strains had no adverse effects on animals' general health status, haematology, blood biochemistry, gut mucosal histology parameters, or the incidence of bacterial translocation. The results obtained in this study suggest that the potentially probiotic strains *L. plantarum* and *L. plantarum* recombinant are non-toxic for mice and are therefore likely to be safe for human use in food and drug.

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دراسات على البقاء فى القناة المعويه والمميزات الفسيولوجية والأمان لسلالة جديده معدلة من الـ *Lactobacillus plantarum* recombinant

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اضافة *L. plantarum* بتركيز $4x10^9$ cfu. g^{-1} والسلالة المعدلةمن الـ *L. plantarum* إلى عليقة الفئران الألبينو ($\approx 4x10^9$, $8x10^9$, and $2x10^9$ cfu. g^{-1}) يزيد بدرجة معنوية أعداد عصويات حمض اللاكتيك بمقدار ٣١,٦٧ ، ٥٠,٩٦ ، ٥٥,٧٩ ، ٣٩,٧١ % على التتابع بعد أربعة أسابيع من التغذية عند المقارنة مع الكنترول. أعداد الـ *enterobacteriaceae* والبكتريا الهوائية فى براز الفئران التى تتغذى على *L. plantarum* والولسلالة المعدلة من الـ *L. plantarum* عند كل التركيزات حدث لها انخفاض بزيادة مدة المعاملة . كما حدث انخفاض فى كوليسترول الدم والجلسريدات الثلاثية وكذلك انزيم الـ alanine aminotransferase . الـ *L. plantarum* والسلالة المعدلة من الـ *L. plantarum* أدت الى خفض معنوى فى تكرارية كرات الدم الحمراء التى حدث لها تلون نووى micronucleated polychromatic erythrocytes بالمقارنة بالكنترول السالب. وفى نفس الوقت لم يوجد أى اختلاف فى تكرارية الاضطراب الكروموزومى chromosomal aberrations بين المجاميع التى تتغذى على *L. plantarum* والسلالة المعدلة من الـ *L. plantarum* وبين الكنترول السالب مما يدل على احتمال تمتعهم بنشاط مضاد لتطفر خلايا النخاع العظمى للفئران. والخلاصة أن سلالات الـ *L. plantarum* والسلالة المعدلة من الـ *L. plantarum* يمكن أن تبقى فى القناة المعوية كما أنها غير سامة للفئران مما يدل على أنها غالبا امنة للاستخدام الآدمي.