

***In vivo and in vitro Study of Probiotic Effect of Lactobacillus acidophilus on Pathogenecity of Proteus mirabilis isolated from Urinary Tract Infection (UTI)***

دراسة تأثير الراشح الطبيعي المستخلص من بكتيريا اللبن على امراضية بكتيريا المتقلبات المعزولة من التهاب المجاري البولية

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**Abstract**

The aim of the study was to treat the pathogenecity and adhesion property of *Proteus mirabilis* isolate, obtained from urinary tract infection (UTI) patients. After the identification of *Proteus mirabilis* it was found that the pathogenic bacteria possessed the adhesion ability both *in vivo* and *in vitro* bioassays, with *Lactobacillus acidophilus* filtrate as Probiotic. *Proteus mirabilis* was subjected to different concentrations of *Lactobacillus acidophilus* filtrate to investigate its adhesion property and pathogenecity. Three concentrations (25, 50,100) % of *Lactobacillus acidophilus* concentrated filtrate were used *in vitro* bioassays against pathogenic bacteria. Results showed that the third fold 25% was the most effective concentration in reducing the adhesion intensity of the bacteria. This concentration was selected to be used *in vivo* for detection of infectivity to the animal tissues (Kidney and liver) and also the effect of *Proteus mirabilis* and Probiotic on the level of hormones in liver; Serum glutamate pyruvate transaminase, Serum glutamate oxaloacetate transaminase and Alkaline phosphatases (GPT, GOT , ALP) and kidney enzyme (Urea) in serum of mice was investigated. It was found that the levels of liver hormones and kidney enzyme increased at the time of infection, and these levels returned to normal or decrease after treating the animals with 25% of concentrated filtrate of Probiotic.

**المستخلص**

يهدف البحث الى معالجة امراضية وصفة الالتصاق لبكتيريا المتقلبات والتي تم الحصول عليها من مرضى يعانون من التهاب المجاري البولية . بعد تشخيص البكتيريا الممرضة وجد بانها تمتلك قابلية الالتصاق وذلك من خلال الاختبارات الحيوية خارج وداخل الجسم الحي باستعمال الراشح الطبيعي المستخلص من بكتيريا اللبن , اخضعت البكتيريا الممرضة لثلاث تراكيز مختلفه من الراشح الطبيعي لبكتيريا اللبن وذلك لدراسة تأثير الراشح على صفة الالتصاق والامراضية . استعملت ثلاث تراكيز ( 25 ، 50 ، 100 ) % من الراشح الطبيعي المركز لبكتيريا اللبن في الاختبارات الحيوية خارج الجسم الحي . اوضحت النتائج بانه التركيز الثالث 25% كان التركيز الاكثر تأثيرا في خفض شدة التصاق البكتيريا الممرضة . انتخب هذا التركيز 25% في الاختبارات الحيوية داخل الجسم الحي وذلك لدراسة تأثيره على خفض امراضية البكتيريا الممرضة في الانسجة الحيوانية (الكبد والكلية) وعلى مستوى الهرمونات في الكبد وانزيم الكلية (اليوريا) في داخل جسم الحيوان (الفار) . اوضحت النتائج ارتفاع مستويات الهرمونات في الكبد واليوريا في الكلية عندما حققت الفئران بالبكتيريا الممرضة وعادت الى مستوياتها الطبيعية او انخفضت قليلا عندما عولجت بالتركيز المثبط الثالث 25% لبكتيريا اللبن .

## Introduction

Studies on the pathogenicity of UTI demonstrated that the ability of *P. mirabilis* to adhere to epithelium is of prime important in the initiation of infection *in vitro* study, shown a protein from pathogenic bacteria *P. mirabilis* that adhere to human epithelial cells. This protein was purified and found to be organized as flexible rods (Fimbriae), so they suggest that these fimbriae are the adherence elements responsible for binding pathogenic *P. mirabilis* to tissue, different strains of bacteria within a species may vary widely in their hydrophobic surface properties and ability to adhere to host cells, the adherence of bacteria to biological surface is a complex process which often involve lock and key type interaction between bacterial attachment fimbria and specific complex carbohydrate structures of the host cell membranes receptor [1].

Probiotic is defined as " living organisms which upon ingestion in certain numbers exert health benefits beyond inherent base nutrition" one of the most significant groups of probiotic organisms are the lactic acid bacteria, these bacteria have a long history safe use in food [2].Gastrointestinal and renal infections originating from bacteria are generally due to a restricted number of species of bacteria belonging to *Escherichia*, *Shigella*, *Salmonella*, *Proteus* and *Staphylococcus*, lactic acid bacteria adhesion have a role in microbial of the urogenital and intestinal tract and disease control, *L. acidophilus* appears to be beneficially involved in the interference with establishment of pathogens in the gastro-intestinal tract showed that mechanism of action are believed to involve competitive exclusion and production of inhibitory substance, including bacteriocins [3]. Researchers suggested that the minimum concentration from LAB in the product using in therapy should be ( $1 \times 10^5$ ) bacteria/ml or 1g like *L. acidophilus*, which are widely used in the industry (food preservation) and in the therapy, probiotic are extremely safe and are not associated with any significant or detrimental side effects [4]. *Lactobacillus* therapy seems to reduce the recurrence rate of uncomplicated lower urinary tract infections in women, so it is used against urinary tract infections and gut disorder. *L. acidophilus* has a superior capability of producing lactic acid, which is an antimicrobial and helps the body protection from harmful bacteria adhering to the intestinal mucosa [5]. *Lactobacillus* spp. inhibits the activities and proliferation of pathogenic bacteria by several ways such as production of lactic acid, production of antibiotics. *L. acidophilus* produces acidophilin that have action and inhibited several bacterial like *E. coli*, *Helicobacter pylori*, *Proteus*, so *lactobacillus* strain showed to competitively inhibit adhesion of enteropathogenic bacteria. There is an upsurge in interest in these species as research is beginning to reveal the many possible health benefits associated with lactic acid bacteria [6]. Study was conducted to evaluate the effect of *Lactobacillus acidophilus* on *Proteus mirabilis* pathogenicity.

## Materials and Methods

### Urine Sample Collection

Mid-stream urine specimen samples were collected in sterile tubes from patients in Al-Karkh and Al-Yarmoq hospitals in Baghdad. A total of 50 samples were aseptically collected and transferred to the laboratory at the time of collection in sterile tubes.

### Isolation and Identification of Urine Samples

Primary isolation was conducted by taking a full loop of undiluted urine samples and spread on blood agar to examine swarming activity and non-lactose ferment on MacConkey agar plates, and microscopical examination was carried out to examine cell shape, grouping and non-spore forming. Further identification of *Proteus* isolate was made by using Api 20E Kit according to [7].

#### *Lactobacillus* isolates

*Lactobacillus acidophilus* isolate was obtained from Biotechnology Department–College of Science/Al-Nahrian University.

#### Bacterial Adhesion test

The method of [8] was used to examine the adhesion property of *Proteus mirabilis* isolate.

#### Inhibitory Effect of LAB (*Lactobacillus acidophilus*):

##### A- In Vitro Assay

##### 1. On Solid Medium (MRS Agar)

Culture of LAB previously cultivated in MRS broth was streaked on MRS agar, and then incubated under anaerobic conditions at 37°C for 24 hr. [9]. After incubation a cork pore (5mm) was used to make discs of LAB growth and put on surface of the nutrient agar that was previously inoculated with 0.1 ml of pathogenic bacteria. After incubation at 37°C for 24 hr, the inhibition zone around the discs was estimated in (mm). Same procedure was repeated by using different incubation times of LAB (18, 24, 48) hr to determine the optimum incubation period that given the highest inhibition effect.

##### 2. In Liquid Medium (MRS Broth)

MRS broth was inoculated by 1% of LAB culture, then incubated anaerobically at 37°C for different period of times (18, 24, 48) hr. After incubation the culture was centrifuged at 6000 rpm for 15 minutes and the supernatant was obtained. After adjusting the pH of the filtrate to 6.5 by using NaOH (1ml), it was filtered through Millipore filter unit 0.22 µm. Then well diffusion method that mentioned by [10] was used. Nutrient agar plates was inoculated with 0.1ml of pathogenic bacteria by a spreader, and incubated, so then 5mm wells were made by a cork pore. Each well was filled with *L. acidophilus* filtrate, and then incubated at 37°C for (18, 24, 48) hr. The inhibition zone around wells were measured by (mm) and compared with that of the control which contained MRS broth without bacteria. The filtrate was concentrated by incubator at 40°C for three times, one fold refer to first concentration 100%, two fold refer to second concentration 50% and three fold refer to third concentration 25% and the well diffusion method was repeated to detect the effect of each concentrated filtrate against the *P. mirabilis*.

##### B- In Vivo Assay

##### Histopathology assay of bacteria and probiotic (LAB filtrate) on mice

##### Laboratory animals

Thirty two bulb/C mice were obtained from Biotechnology Research Center/Al-Nahrain University/Baghdad, age (8–12) weeks, weigh (23–25) g and were divided into eight groups, each group was isolated in a separated plastic cage, in room with temperature (23–25) C .

**Organs isolation**

Mice of each group above were dissected and the belly opened vertically, two kidneys and liver were taken from mice for histopathological examination placed in Petri dishes contained physiological salty solution to remove fatty tissues and sticky bundles. lastly the samples were kept in cans containing 40% formalin.

**Histopathological examination**

Diagnosed according to the [11] under control of histopathological examiner.

**Experimental Model**

Experiment was carried out for histopathological study to clarify the effect of *L. acidophilus* filtrate on the pathogenicity of *P. mirabilis* by injecting 0.1 ml ( $1.5 \times 10^8$  cfu/ml) of *P. mirabilis* for all experimental group except the control group and after 24 hour the *L. acidophilus* filtrate was administrated in the same dosage, after another 24 hour the animals were dissected, two kidneys and livers from each mice were taken aseptically to determine the infection by pathogenic bacteria. Organ sections were stained using haematoxylin stain and then examined under microscope at 100x lens. The groups of experimental mice were classified into eight groups and each group contains three mice as follow:

**Group A:** mice were injected with *P. mirabilis*, and left for 24 hr. After 24 hr incubation time mice were dissected. Liver and kidney organ were removed for histopath.

**Group B:** mice were injected with *P. mirabilis*, and left for 24 hr. After 24 hr incubation time mice were injected with *L. acidophilus* three fold concentrated filtrate (25%) and dissected after 24 hr. liver and kidney organ were removed for histopath.

**Group C:** the procedure carried out in group A above was repeated in group C but dissected after 48 hr. liver and kidney organ were removed for histopath.

**Group D:** the procedure carried out in group B above was repeated in group D but dissected after 48 hr. Liver and kidney organ were removed for histopath.

**Group E:** mice were injected with *P. mirabilis*. and left for 24 hr. After 24 hr incubation time mice were dissected. Blood was pulled using cardiac puncture for enzymatic analysis.

**Group F:** mice were injected with *P. mirabilis* .and left for 24 hr. After 24 hr incubation time mice were injected with *L. acidophilus* three fold concentrated filtrate 25% and dissected after 24 hr. Blood was pulled using cardiac puncture for enzymatic analysis.

**Group G:** the procedure carried out in group F above was repeated in group G but dissected after 48 hr. Blood was pulled using cardiac puncture for enzymatic analysis.

**Group H:** mice treated orally with 0.1 ml PBS (control).

**Biochemical examination**

According to biomere company, blood was collected from mice by heart puncture, the serum was separated by centrifugation at 5000 rpm for 10 min. and treated as follows: Each enzymes (GPT, GOT, AL. ph and Urea) two test tubes were used for each samples one contained blank reagent and second contained the sample.

Table (1) : GPT and GOT test

Reagents	GPT	GOT
Reagent 1	1ml	-
Reagent 2	-	1ml
Incubate for 5 min at 37°C	-	-
serum	0.2 ml	0.2 ml
Mix and incubate at 37°C	-	-
Reagent 3	1ml	1ml
Mix let stand for 20min at room temp.	-	-
NaOH 0.4N	10ml	10ml

The solution was mixed with the sample well for 5 min. then measured by spectrophotometer (wave length: 505nm).

Activities of these two enzymes in serum were estimated from the activity table attached with kit of each enzyme.

**AL. Ph. (alkaline phosphates):** Samples used in this test were the same serum sample used for GPT and GOT test to estimate the activity of Al. ph. enzyme procedure as bellow.

Table (2): AL. Ph. (alkaline phosphates) procedure

Reagent	Serum sample	Serum bank	standard	reagent
Reagent1	2ml	2ml	2ml	2ml
Incubate for 5 minutes at 37° C	-	-	-	-
Serum	50 µl	-	-	-
Reagent 2	-	-	50 µl	-
Incubate for exactly 15 min at 37°C	-	-	-	-
Reagent 3	0.5 ml	0.5 ml	0.5ml	0.5ml
Mixed well or preferably vortex	-	-	-	-
Reagent 4	0.5 ml	0.5ml	0.5ml	0.5ml
Serum	-	50 µl	-	-
Distilled water	-	-	-	50 µl
Mix. Let stand for 10 minutes in the dark	-	-	-	-
Measurement				

Calculation =  $\text{OD serum sample} - \text{OD serum blank} \times n / \text{OD Standard}$

**Blood urea:** for estimate the activity of blood urea we use the following procedures.

Table ( 3): Blood urea procedure

Reagent	Serum sample	Serum bank
Reagent1	10 µl	1ml
Mixing for 3 minutes at 37° C	-	-
Reagent 2	-	0.2ml
Mixing for 5 minutes at 37° C	-	-

**Reading result on (580nm) and calculation by following equation:**

Bl. Urea:  $\text{OD serum sample} - \text{OD serum blank} / \text{OD standard} \times \text{standard concentration}$ .

**Statistical analysis**

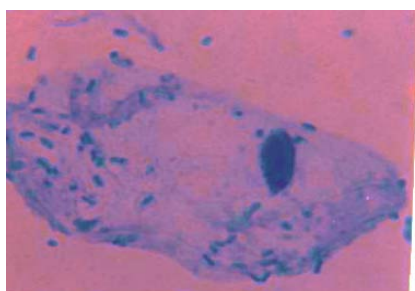
The values of the investigated parameters were given in terms of mean  $\pm$  standard error, and differences between means were assessed by analysis of variance (ANOVA) and Duncan test, using the computer program SPSS version 7.5. The difference was considered significant when the probability value was equal or less than 0.05 [12].

**Results and Discussion****Isolation and Identification of *P. mirabilis***

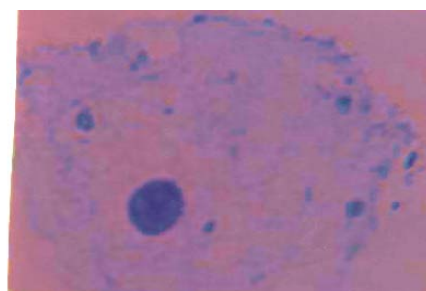
The colonies were grown on MacConkey agar for 24 hr appeared pale (non-lactose ferment) and on blood agar showed its swarming motility which gave the characteristic of *Proteus* which subjected to more test for identify the species. Microscopical examination revealed that cells of the suspected isolate (*Proteus*) appeared as gram negative, rods (purple) and non-spore forming. Further identification of *Proteus* isolate was done by using Api 20 E kit to differentiate *P. mirabilis* which is unable to make indole ring and is the major property distinguishing from another *proteus* species.

**Bacterial adhesion of *Proteus mirabilis* In vitro**

The initial step in the infection of *P. mirabilis* to the host cell is the adhesion. The adherence of *P. mirabilis* was viewed under microscope using uroepithelium of healthy female which was infected with *P. mirabilis*, Figure (1) A refer that the highest numbers of pathogenic bacteria adhered to the UEPCs (ranging 48–57 bacteria/cell) and that is agreed with [13] who is stated that the adherence range of *P. mirabilis* to UEPCs ranged between (45-55) bac./cell. Interference with adhesion process caused prevention of infection, so when the third fold of concentrated filtrate 25% was used by using same method of [9] we observed minimizing of bacteria adhesion (ranging 2–4 bacteria/cell) which shown in Figure (1)B . This may relate to the effect of inhibitory substances that released from *L. acidophilus* such as acidophiline, bacteriocine and others, which effect the growth and adhesion of several gram negative bacteria by altering some surface structures like pili (which is the adherence element in *P. mirabilis*) or by the acidic PH of LAB [14].



A. Infected with *P. mirabilis*



B. Treated with (25%) con. filt .*L.A.*

Fig (1) : uroepithelial cell

## Inhibitory Effect of LAB *L. acidophilus*

### A- *In vitro*

#### 1-On solid medium

In this study MRS agar medium was used for testing the ability of LAB isolate for the production of inhibitory materials when grown under anaerobic condition. The result showed that *L. acidophilus* isolate was effective against *P. mirabilis* after (24, 48, 72) hr. incubation time. The incubation period of 24 hr was more significant than the others and gave zone of inhibition on *P. mirabilis* growth reached to (18 mm) this may be due to acidophillin production by *L. acidophilus*, moreover the variability in inhibition of LAB against pathogenic bacteria may relate to the type of pathogenic bacteria, type of inhibitory substances and its quantity and ability for distribution in media [15] as shown in Figure (2).



Fig (2) Inhibitory Effect of *L.A* on solid medium

#### 2. In liquid media

Inhibitory effect of filtrate obtained from LAB grown in MRS broth was also measured by using well diffusion method which used to determine the inhibition activity of *L. acidophilus* filtrate grown at different incubation period (24, 48, 72) hr. against *P. mirabilis* isolate. The filtrate of *L. acidophilus* gave optimum inhibitory effect after 24 hr incubation time when the inhibition zone reached to (22 mm) in diameter as shown in Figure (1-3A), while incubation for (48, 72) hr showed lower inhibitory effect. It was clear from the result that MRS broth was better stimulator for inhibitory product than on MRS agar and that agree with the explanation by [16] who recorded that the MRS broth stimulated inhibitory effect against gram negative bacteria (*E. coli*, *Klebsiella* spp and *Proteus* spp.). The effect of concentrated filtered (one fold 100%, two fold 50% and three fold 25%) also determined by using well diffusion method and result showed that in diameter of inhibition zone reached to (27 mm) as shown in Figure (3)B. This indicates the three fold concentrated filtrate have the highest inhibitory effect because the inhibitory substances increased with concentration and death of tested bacteria increased with the increasing of inhibitory substances like acidophillin and bacteriocine [17]. In this research it was clear that the incubation time of 24 hr was the best time for production of inhibitory substance and the third fold 25% of concentrated filtrate has the best inhibitory effect against *P. mirabilis* so this research depended on it in *in vivo* experiments.

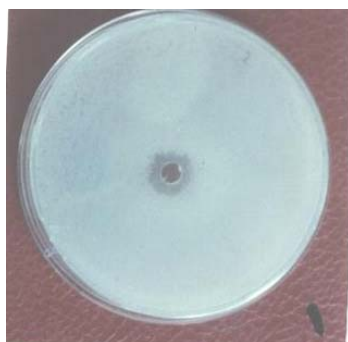
A. *L.acidophilus* filtrateB. *L.acidophilus* (25%)con.filtrate

Fig (3): Inhibitory Effect of *L.acidophilus* filtrate and three fold (25%) concentrated filtrate in liquid medium

### B- In Vivo Assay

#### Histopathological examination

Liver sections taken from group (A) which treated with *P. mirabilis* only showed degenerative changes and hyperplasia (increase in the number of the cells) of the hepatocytes and kuffer cells, while the study of the specimens of groups (B and D) which treated with *P. mirabilis* and *L acidophilus* and dissected after 24 and 48 hr were shown normal appearance in the hepatocyte but chronic inflammatory cells in the portal area, as shown in figure (4). Kidney sections were taken from group (A) which treated with *P. mirabilis* only displayed bleeding with focal or mild tubular degeneration and rear glomeruli showed degenerative changes. The result agrees with [18] who they stated that major cause of UTI especially in kidney is a gram negative bacteria which belongs to the enterbacteriaceae family. Group (B and D) which treated with *P. mirabilis* and *L acidophilus* and dissected after (24 , 48) hr, kidney sections revealed normal organ with mild degenerative changes in the renal tubules appeared in kidney sections and this may be due to the effect of probiotic bacteria which protects the kidney from *P. mirabilis* as shown in Figure (5), that result agree with [19] who they showed that using *Lactobacillus* therapy seems to reduce the recurrence rate of uncomplicated urinary tract infection.

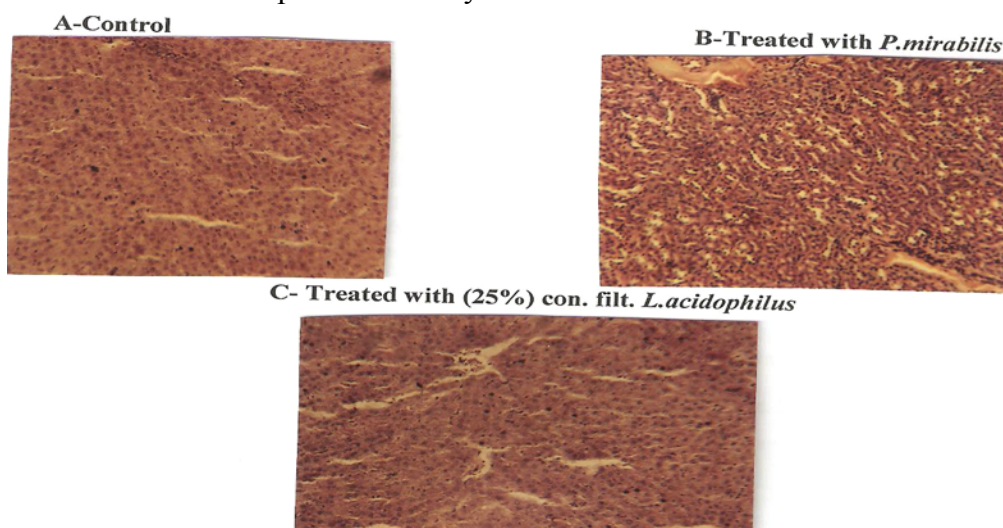


Fig (4): Histopathological section of liver.



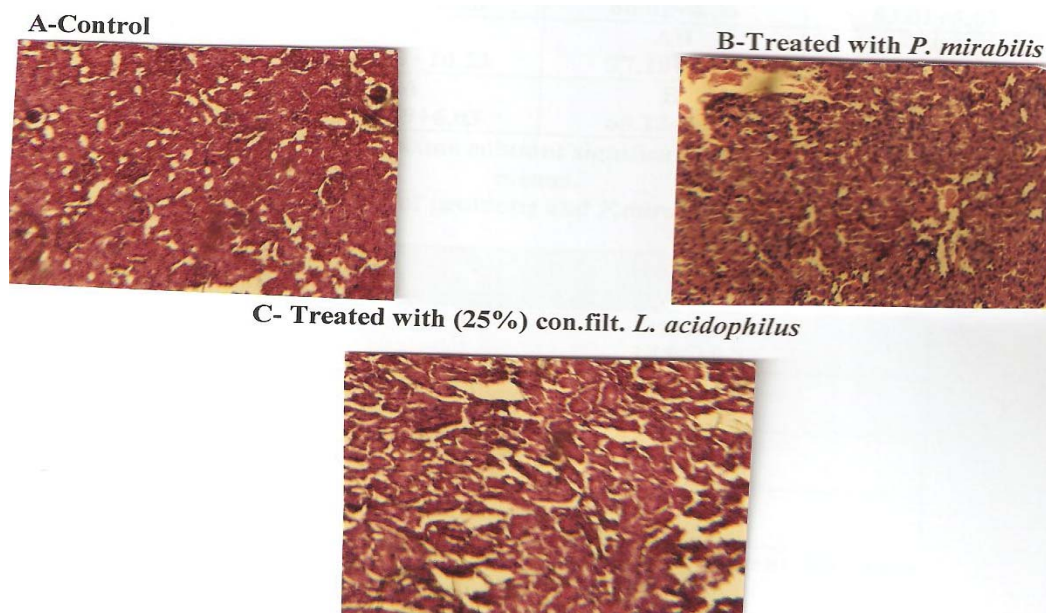


Fig (5): Histopathological section of kidney.

### Biochemical examination

In this research the effect of *P. mirabilis* and *L. acidophilus* on GOT, GPT, Alp and Urea levels have been investigated. The results in Table(4, 5) showed that significant increase in GOT, GPT, ALP and Urea levels when treated with *P. mirabilis* in comparison with the control, whereas when treated with 25% probiotic the result showed that there was no significant differences among enzymes for both incubation periods (24 , 48) hr as compared with the control. After mice were treated with *L.acidophilus* (probiotic bacteria) levels of each enzymes were returned to normal or decreased slightly in group (F and G) when compared to that of the negative control .Significant increase ( $P \leq 0.05$  ) on GPT and other enzymes was shown after *P. mirabilis* treatment to in Group (E) in comparison with the negative control. On the other hand, there was no significant differences in GPT, GOT, Alp and urea level ( $P \leq 0.05$ ) in group (F and G) which treated with *L. acidophilus* after effected by pathogenic bacteria, this result may explain that the effect of probiotic as therapeutic agent because it is therapeutically used in modulate immunity, lower liver enzyme, lower cholesterol, improve lactose intolerance and prevent cancer [20].

Table (4): The effect of probiotic and *P. mirabilis* on liver enzyme

Groups	GOT IU/ml $\mu \pm SD$	GPT IU/ml $\mu \pm SD$	ALP IU/ml $\mu \pm SD$
Control	A 181.33+7.16	A 68.74+6.50	A 62.21+2.96
Treated with <i>P.m</i>	B 205.23+8.08	B 86.01+4.86	B 83.01+5.07
Treated after 24 h with <i>L.A</i>	A 185.11+10.33	AB 77.18+0.62	A 70.16+1.51
Treated after 48 h with <i>L.A</i>	A 183.49+6.03	B 68.13+0.29	A 71.62+2.24

Different capital letters in the same column: significant difference ( $P \leq 0.05$ ) between means.

**Table (5): The effect of probiotic and *P. mirabilis* on kidney enzyme**

Groups	Urea mg/dl $\mu \pm SD$
Control	A 14.96 $\pm$ 0.22
Treated with <i>P.m</i>	B 23.71 $\pm$ 1.13
Treated after 24 h with <i>L.A</i>	A 17.13 $\pm$ 1.06
Treated after 48 h with <i>L.A</i>	A 16.50 $\pm$ 1.32

Different capital letters in the same column: significant difference ( $P \leq 0.05$ ) between means.

### Reference

1. Jawetz, E.; Melnick, J. L. and Adelberg, E. A. (1998). "Medical Microbiology". Middle east edition appleton and hongenor walk Connecticut / San Matea. California.
2. Matsumoto, T. (1998). Virulence factors and host response in UTI Sangyoika. Daigiaku. Zasshi. B: 45-60. (Abst)
3. Reid, G.; Tieszer, C. and Lam, D. (1995). Influence of *Lactobacilli* on the adhesion of *Staphylococcus aureus* and *Candida albicans* to fibers and epithelial cells. J. Ind. Microbiol: 15: 248-253.
4. McFarland, L. V., Elmer, G. W. (1995). Biotherapeutic agents: Past, present and future, Microecol. Ther.; 23: 46-73.
5. Donohue, D. C. and Salminen, S. (1996). Safety of probiotic bacteria. Asia Pacific J. Clin. Nutr., 5: 25-28.
7. Hirayama, K. and Rafter, J. (1999). The role of lactic acid bacteria in colon cancer prevention: Mechanistic considerations. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology. 56 (1): 391-394. (Abst).
8. Atlas, R. M.; Brown, A. E.; Parks, L. C. (1995). Laboratory Manual of Experimental Microbiology. (1<sup>st</sup> ed). Mosby. Inc. Missouri.
9. Iwahi, T., Abc, Y. and Tsuchiya, K. (1982). Virulence of *E. coli* in a sending urinary tract infection in mice. J. Med. Microbiol. 15: 303-316.
10. Silva, M.; Jacobus, N. V.; Deneke, C. and Gorbach, S. L. (1987). Antibacterial substances from a human *Lactobacillus* strain. Antimicrob. Agent and Chemother.; 31 (8): 1231-1233.
11. Vignolo, G. M.; Suriani, F., Halgado, A. P. R. and Oliver, G. (1993). Antibacterial activity of *Lactobacillus* strains isolated from dry fermented sausages. J. Appl. Bacteriol., 75: 344-349.
12. Bancroft, I., and Stevens, A. (1982). Enzyme histochemistry. In theory and practice of histological techniques Bancroft and Steven A (eds) 2<sup>nd</sup> edition. Churchill Livingstone, London, 374-405.
13. Perez-Serrano, J.; Denegri, G.; Casado, N. and Fodriguez-Cabaeiro (1997) *In vivo* effect of oral albendazole and albendazole sulphoxide on development of secondary echinococcosis in mice. *International Journal for Parasitology*, 27: 1341- 1345.

14. Mobley, H. L. T. and Chippendale, G. R. (1990). Hemagglutinin urease and hemolysin production by *Proteus mirabilis* from clinical sources. J. Infec. Dis. 161: 525-530.
15. Ramphal, R.; Sadoff, J. C.; Pyle, M. and Silipigni, J. D. (1984). Role of pilin in the adherence of *P. aeruginosa* to injured tracheal epithelium. Infec. Immun. 44: 38-40.
16. Egorov, N. S. (1985). Antibiotics a scientific approach. Mir Publishers, Moscow.
17. Gupta, V.; Radramma; Rati, E. R. and Joseph, R. (1998). Nutritional quality of lactic acid fermented bitter melon and fenugreek leaves. International Journal of Food Science and Nutrition. 49 (2): 101-108.
18. Barefoot, S. F. and Kaenhammer, T. R. (1983). Detection and activity of Lactocin B. A bacteriocin produced by *Lactobacillus acidophilus*. Appl. Environ. Microbiol. , 45 (6): 1808-1815.
19. Gyi, D.; Rahman, M.; Lai, H.; Carlson, R.; Guard, Pettier J. and Hughes, C. (1995). A cell surface polysaccharide that facilitated rapid population by differentiation swarm cells of *Proteus mirabilis* Mol. Microbiology. 17:1167-1175.
20. -Crociani, J.; Grill, J.; Huppert, M. and Ballngue, J. (1995). Adhesion of different bifidobacteria Caco\_2 cells and comparison with *in vivo* study. Lett. Appl. Microbiol. 21:146-149.
21. Fergus M. C. (1999). *Probiotic Spectra of Lactic Acid Bacteria (LAB)*. Food Sci. and Nut. 39 (1): 17-43.