ejpmr, 2016,3(8), 623-630

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

THE EFFECT OF PARTIAL PURIFIED PLANTARICIN AGAINST URINARY TRACT INFECTION (UTI) INDUCED BY *ESCHERICHIA COLI* AND *PROTEUS MIRABILIS* IN EXPERIMENTAL RAT MODEL

***[1]Rana Hussein Raheema and Abdul Karim Salim Mahood[1,2]**

[1]*Microbiology Department, College of Medicine, Wasit University, Iraq.
[2]Anatomy and Biology Department, College of Medicine, Wasit University, Iraq.

***Correspondence for Author: Rana Hussein Raheema**

Microbiology Department, College of Medicine, Wasit University, Iraq.

Article Received on 21/06/2016 **Article Revised on 11/07/2016** **Article Accepted on 31/07/2016**

ABSTRACT

The present study was purposed to assess the effect of plantaricin of *L. plantarum* in vivo by inducing uti in rats with *Escherichia coli* and *Proteus mirabilis* and to check out its effect on some biochemical and histopathological parameters. Fifty four rats were divided into five groups, Group (A): negative control (not infected group), Group (B): positive control (infected *E. coli, P. mirabilis* respectively and not treated group) for 2,5 and 10 days, Group (C): Animals were administrated orally 160 AU / ml of plantaricin for 14 days then infected with *E.coli* and *P. mirabilis* for 2,5 and 10 days, Group (D): infected with *E.coli, P. mirabilis* respectively and treated orally with 160 AU / ml of plantaricin for 14 days and Group (E): infected with *E.coli* and *P. mirabilis* respectively and treated with gentamicin 2 mg /ml. B.W.I.M for 14 day. At the end of experimental period, blood samples were taken to estimate the biochemical measurements such as serum creatinine and uric acid. Results showed an elevation of serum creatinine level in infected group with *Escherichia coli* and *P. mirabilis* compared with normal group. Result showed significant changes in serum uric acid and creatinine in infected and treated groups. Histological sections of kidney showed severe damage in infected groups compared with treated groups. It could be concluded that plantaricin of *L. plantarum* has potent protective role against UTI and appears by correction between biochemical parameters and histological studies.

KEYWORDS: *L. plantarum, E.coli, P. mirabilis.*

INTRODUCTION

Urinary tract infection (UTI) is the second most common type infectious presentation in community medical practice and in worldwide, about 150 million people are diagnosed with UTI each year (Stamm and Norrby, 2001). Urinary tract infection may involve only the lower urinary tract or both the upper and the lower tracts. Bacterial of urinary tract infection UTI are Enterobacteriaceae with a high predominance of *Escherichia coli* (Alain, 2003)(Po etal., 2002) *E. coli* is one of the most bacteria capable to attach to the bladder wall and form a biofilm that resists the body's immune response. (*Salvatore et al,.2011*). *E. coli* is the cause of 80–85% of urinary tract infections Nicolle LE, 2008) with *Staphylococcus saprophyticus* being the cause in 5–10%. Other member of enterobacteracaea is *Proteus mirabilis* which often associated with urinary stones (Po etal., 2002*) (*Brooks etal., 2001) *Proteus mirabilis* is one of the most common Gram-negative bacteria that infect the upper urinary tract. It is found in the kidney more often than *E. coli* and other bacteria Because of the production of urease and other virulence factors (Johanson etal., 1993). It can cause earnest renal damage, such as acute pyelonephritis, bladder or renal stone, fever and bacteriemia (Jansen etal., 2003). It is also the major cause of complicated urinary tract infection (UTI), especially infects patients with long-term indwelling urinary catheters more than 30 days (Pearson and Mobley, 2007) Individuals suffering from urinary tract infections caused by *P. mirabilis* often develop bacteruria, cystitis, acute pyelonephritis, and kidney and bladder stones. (Burall etal., 2004) (Wang etal., 2006). *P. mirabilis* is able to enter the blood stream inducing a systemic inflammatory response syndrome (SIRS), which as a mortality rate of 20%-50%. (Esposito *etal*., 1980). Urinary tract infections are among the most common infections with an increasing resistance to antimicrobial agents (Samra *et al.*, 2005). Lactic acid bacteria are capable to generate diverse antimicrobial composites in the course of lactic acid fermentation as bacteriocins, hydrogen peroxide (H2O2), organic acids and diacetyl, (Oyetayo etal.,2003). Bacteriocins are helpful in treatment without raising the antibiotic resistance level (Stiles, 1996). Bacteriocins are ribosomally synthesized, biologically active proteins or protein complexes that display antimicrobial action

towards usually closely related species (Sanchez *et al*.,2008).

MATERIAL AND METHODS

Bacterial isolate

The bacterial isolate used for plantaricin production was *Lactobacillus plantarum* which isolated from fermented vegetables, while *E. coli* and *P. mirabilis* were used as indicator bacteria which obtained from uti patients come to Al-Zahra hospital in kut, Iraq, Clean-catch midstream urine was collected in a sterile tube then loop full of urine samples were cultured on MacConky agar, nutrient agar and blood agar base, the isolates were identified by bacteriological methods such as gram stain, colony morphology and biochemical tests. Antibiotic susceptibility testing (AST) for the *E. coli* and *P. mirabilis* bacteria were done using the Vitek 2 systems AST-GN 69.

Plantaricin assay

plantaricin activity was done by serial two fold dilutions of cell-free supernatant (crude plantaricin)(Pilasombut etal., 2005). Dilutions were used to check the antibacterial activity of plantaricin against indicator bacteria using the well diffusion method as described by (Franz et al., 1996). The AU was defined as the reciprocal of the highest dilution producing a clear zone of growth inhibition of the indicator bacteria, AU was calculated as:

(1000 / 100) × D, where 1000: constant, 100: volume of supernatant in a well(µl) and D: the dilution factor. It was calculated by described method (Parente et al., 1995), plantaricin concentration was determined according to (Lowery *et al*., 1951).

Extraction and partial Purification of Plantaricin

Basal Growth Medium was inoculated with *Lactobacillus plantarum* and incubated at 12 hours under anaerobic conditions (AL-Gharbawee, 2012). Cells were harvested by centrifugation at 6000 rpm for 15 minutes. The cell-free supernatant that was referred to as crude plantaricin extract (CPE) was heated at 80°C for 10 minutes, then cooled and centrifuged at 6000 rpm for 15minutes (Powell *et al*.,2007).

The supernatant was mixed with n-butanol at a ratio 1:1. The mixture was centrifuged at 4000 rpm for 10 minutes to achieve phase separation. The organic phase was evaporated at 80°C by rotary evaporator, then the sediment was re-suspended in 20mM sodium citrate buffer (pH 5) and referred to as partial purified plantaricin (PPP) (Abo-Amer, 2007). The antimicrobial activity of plantaricin and protein concentration was determined.

Inducing Infection

Rats were obtained from the national center for drug control and research. Baghdad. Induction of acute urinary tract infection by injection rats with (2.6×10^6) CFU/ml of *E. coli* and (3×10^8) CFU/ml of *P. mirabilis* according to the method of (Al- Ani *etal*., 2011).

Experimental Design

Fifty four rats were divided equally into five groups, three rat in each group (treatment begin after 48 hrs after inducing infection).

Group (A): negative control (not infected group), Group (B): positive control (infected *E. coli*, *P. mirabilis* respectively and not treated group) for 2(B1),5(B2) and 10 (B3) days, Group (C): Animals were administrated orally 160 AU / ml of plantaricin for 14 days then infected with *E.coli* and *P. mirabilis* for 2(C1),5(C2) and 10 (C3)days ,Group (D): infected with *E.coli*, *P. mirabilis* respectively and treated orally with 160 AU / ml of plantaricin for 14days and Group (E): infected with *E.coli* and *P. mirabilis* respectively and treated with gentamicin 2 mg /ml . B.W.I.M for 14 day. At the end of experiment of period, blood samples were obtained via cardiac puncture technique from each anesthetized animals in all groups and put in plain centrifuge tube for obtained blood serum to estimate the biochemical measurement such as Serum creatinine (Bartels and Bohmer.,1971), Serum uric acid (Fossati *etal*., 1980) and rats were sacrificed to examine the histopathological changes and samples from the kidney were fixed in 10% neutral buffered formalin. (Luna, 1968).

Statistical analysis

Statistical analysis was performed using SAS (Statistical Analysis System - version 9.1). Two-way ANOVA with Least significant differences (LSD) post hoc test was performed to assess the significant differences among means ns. $P < 0.05$ was considered statistically significant. (SAS.2010).

RESULTS

During our study we observed that most of the urine samples were infected with *E.coli* and *P. mirabilis* So they are the major cause of urinary tract infection (UTI). Results showed that *Escherichia coli* gram negative bacteria lactose fermentation and Biochemical test were positive for catalase, indole and methyl red while negative results for oxidase, urease and voges-proskauer. Also results showed *Proteus mirabilis* is gram-negative inability to metabolize lactose, it shows swarming motility and Biochemical test were positive for catalase, urease and methyl red while negative results for oxidase, indole and voges-proskauer. The susceptibility of *E.coli* and *P. mirabilis* to antibiotics is shown in (Table,1,2).

Table 1: Antibiotic susceptibility testing of *Escherichia coli* by vitek-2 method

Antimicrobial	MIC	Interpretation
Ampicillin	≥ 32	R
Amoxicillin/clavulanic acid	8	S
Ampicillin/sulbactam	8	S
Piperacillin /tazobactam	≤ 4	S
Cefazolin	≥ 64	R
Ceftazidime	4	*R
Ceftriaxone	≥ 64	R
Cefepime	2	*R
Ertapenem	≤ 0.5	S
Imipenem	≤ 0.25	S
Gentamicin	≤ 1	S
Tobramycin	≤ 1	S
Ciprofloxacin	≥ 4	R
Levofloxacin	≥ 8	R
Nitrofurantoin	≤ 16	S
Trimethoprim-sulfamethoxazole	≥ 320	R

Table 2: Antibiotic susceptibility testing of *Proteus mirabilis* by vitek-2 method

Antimicrobial	MIC	Interpretation
Ampicillin	≥ 32	R
Amoxicillin/clavulanic acid	8	S
Ampicillin/sulbactam	8	S
Piperacillin /tazobactam	8	S
Cefazolin	≥ 64	R
Ceftazidime	4	*R
Ceftriaxone	≥ 64	R
Cefepime	2	*R
Ertapenem	≤ 0.5	S
Imipenem	≤ 0.25	S
Gentamicin	≤ 1	S
Tobramycin	≤ 1	S
Ciprofloxacin	≥ 4	R
Levofloxacin	≥ 8	R
Nitrofurantoin	≤ 16	R
Trimethoprim-sulfamethoxazole	≥ 320	R

Partial purification of plantaricin was performed by extraction with n-butanol in a 1:1 ratio. Plantaricin was removed from the aqueous phase and could be recovered from the organic phase this suggests that at least part of the plantaricin molecule had a hydrophobic character and shares this property with other bacteriocins. (Sanni *et al*., 2003). The specific activity of plantaricin recorded 914.28 AU/mg protein with 4.114 purification folds and 40% yield as shown in table (3).

Table (3): Purification of plantaricin produced by *Lactobacillus Plantarum*

Purification steps	Volume (ml)	Activity (AU/ml)	Protein concentration (mg/ml)	aSpecific activity (AU/mg)	bTotal activity (AU)	C Purification fold	dYield (%)
Crude plantaricin extract (CPE)	250	160	0.72	222.22	4000	1	**100**
After heating (80°C / 10min)	250	160	0.66	242.42	4000	1.090	**100**
Extraction with butanol (1:1)	25	640	0.7	914.28	16000	4.114	**40**

aSpecific activity (AU/mg): represents plantaricin activity divided by protein concentration.
bTotal activity (AU): represents Activity (AU/ml) × Volume (ml).

cPurification fold: represents specific activity of purified fraction divided by specific activity of crude extract.
dYield (%): represents (total activity of purified fraction divided by total activity of crude extract) × 100 (Nissen – Meyer etal.,1993).

Results of antibacterial activity of *Lb.plantarum* and plantaricin against indicator bacteria showed that plantaricin had antibacterial activity against *E.coli* and *P. mirabilis* as shown in (Table, 4). This result came in agreement with (Anyogu *et al*., 2014; Omar *et al*., 2008) plantaricins produced by *L. plantarum* strains had broad spectra of inhibition activity against Gram-negative bacteria including *E. coli*. Chang *etal*., 2016 were found that LAB isolated from fermented vegetables have high potentials for production of antimicrobial substances. Morelli., 2000 revealed the probiotic play important role in enhance innate host defenses by production of antimicrobial substances and the growth inhibition and/or competitive exclusion of the enteric pathogens.

Table (4): Antibacterial activity of *Lb.plantarum* and plantaricin against indicator bacteria

Indicator bacteria	*Lb.plantarum*	Plantaricin
Escherichia coli	B 15.00±0.57[a]	A 25.00±0.28[a]
Proteus mirabilis	B 15.00±0.28[a]	A 30.00±0.58[a]
LSD= 1.4885		

Means with different small letter in the same column differ significantly (P<0.05).
Means with different capital letter in the same row differ significantly (P<0.05).

Results showed the effect of plantaricin on some biochemical parameters such as serum creatinine level and serum uric acid level. Table (5) illustrated the serum creatinine level was elevated in infected group with *Escherichia coli* and *P. mirabilis* compared with normal group, the increase in serum creatinine level which indicator of kidney damage leads to decrease in glomerular filtrate rate. The results of the current study were in agreement with Ibrahim *etal*., 2015 who showed an increased level of Serum creatinine in infected rats with *E. coli* O157:H7-induced urinary tract and found suffering from severe destruction in kidney. The serum creatinine level was decreased in treated groups with plantaricin while increased in group treated with gentamicin.

Table (5): Serum creatinine values (mg/dL) of rat in different groups

Bacteria	Group A	Group B (B3)	Group C (C3)	Group D	Group E
E. coli	C0.58±0.01[a]	A1.16±0.01[a]	B 0.83±0.01[a]	B 0.77±0.04[a]	A1.17±0.003[a]
P. mirabilis	C0.58±0.01[a]	A1.07±0.04[a]	B 0.78±0.11[a]	B0.85±0.02[a]	A1.16±0.01[a]
LSD=0.131					

Means with different small letter in the same column differ significantly (P<0.05).
Means with different capital letter in the same row differ significantly (P<0.05).

Table 6 showed that the level of serum uric was a significant increased (P < 0.05) in rat that infected with *Escherichia coli* and *P. mirabilis* compared with control group while treated group with plantaricin of *Lb.plantarum* caused a significant decreased in serum uric acid level (P<0.05) as compared with group treated with gentamicin.

Table (6): Serum uric values (mg/dL) of rat in different groups

Bacteria	Group A	Group B (B3)	Group C (C3)	Group D	Group E
E. coli	E 4.34±0.25[a]	A7.93±0.06[a]	D 5.75±0.08[a]	C 6.39±0.13[a]	A8.23±0.32[a]
P. mirabilis	D4.33±0.31[a]	B7.33±0.26[a]	C5.66±0.12[a]	C6.12±0.11[a]	A7.93±0.04[a]
LSD=0.5879					

Means with different small letter in the same column differ significantly (P<0.05).
Means with different capital letter in the same row differ significantly (P<0.05).

Histopathology results of the kidney in the second group showed glomerular shrinkage after 2nd day of infection with *E.coli* (Figure,1), the hollow area in Bowman's capsule represents the space between the outer envelope of Bowman's capsule and the capillary network of glomerular lobules. This space is usually filled with urine formed from the renal blood flow by filtration across the glomerular capillary wall (Shimizu etal., 1999). Therefore, the evanescence of this space suggests an enlargement of globular lobules and the shrinkage of the outer envelope of Bowman's capsule. (Shimizu etal., 1999) demonstrated an increase of mesangial cells in the glomerular lobules, which perhaps causes the enlargement of the lobules and the collapse of the capillary lumen and suggests a marked decrease in the renal blood flow. Other sections showed *congestion of*

blood vessels (Figure, 2) and necrotic proximal tubules. Also showed lymphocyte infiltration (Figure, 3).

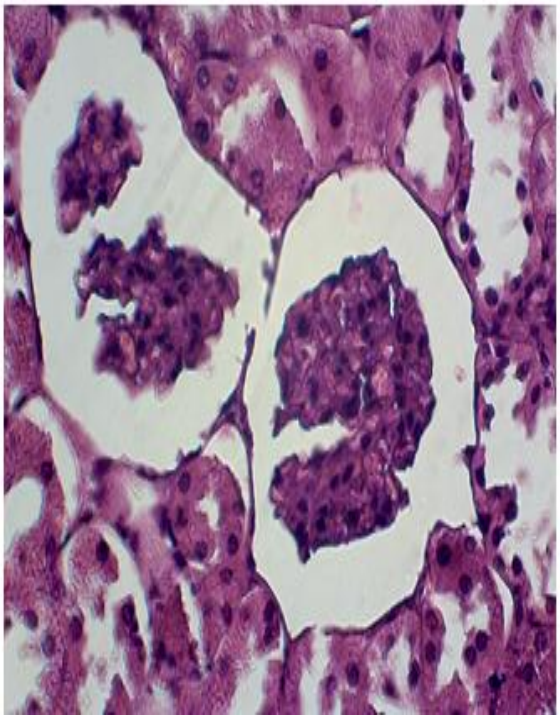
The histological changes in kidney increased after 5 days of infection with *Escherichia coli* and *Proteus mirabilis* and this result came in agreement with **(**Al-Ani *etal*., 2011) who revealed that histologic changes in the infected kidneys with *Proteus mirabilis* which progressed over the 7-day will increased throughout the period of the experiment.

Histopathology of kidney revealed a hypertrophy in blood capillary, congestion of blood and necrosis in tubules after 5 days of infection with *Escherichia coli* (Figure, 4). In addition to a hyalinization of the *renal* artery (Figure, 5), also congestion of blood vessel, *necrosis in convoluted tubules* and hypertrophy (Figure, 6) and lymphocyte infiltration after 5 days of infection with *Proteus mirabilis* (Figure, 7). (Reid *et al*. 2003) suggested that the probiotic of *Lactobacillus* spp increasing the phagocytosis as well as increasing the protection of T-lymphocytes and natural killer cells, an observation thought to be in part due to the regulation of cytokine function. Some authors have reported that the immune system stimulation exerted by the LAB used in yoghurt production would allow the maintenance of an improved resistant against pathogens (Erickson and Hubbard., 2000).

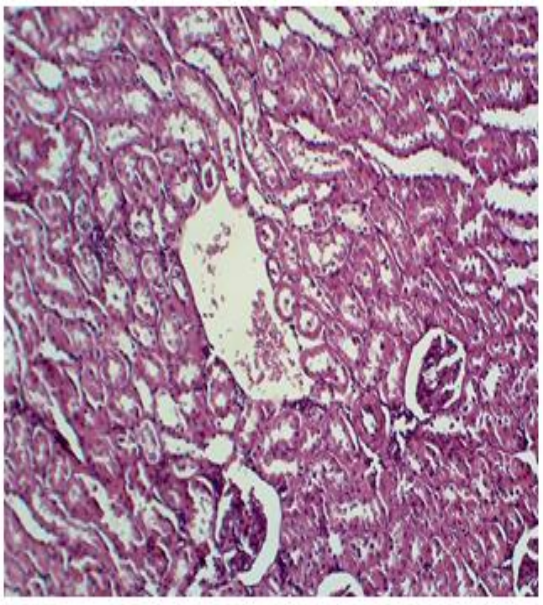
Proteus mirabilis which have many virulence factors, such as adhesion, swarming, urease, hemolysin and protease production that caused urinary tract infection (Mobley and Belas, 1995).

The histopathlogical changes in kidney showed that the changes in mice treated with plantaricin then infected with bacteria were simple sings in compare with untreated mice, section in kidney showing as normal with mild congestion (Figure, 8), others showed normal structure (Figure, 9) and normal glomerulus (Figure, 10).

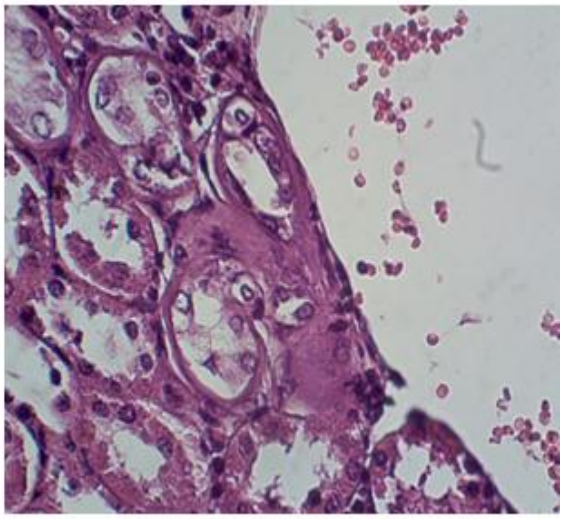
Alwan (2011), showed that kidney of treated mice with *Lactobacillus acidophilus* against urinary tract infections caused by *Proteus mirabilis* gave normal appearance of the histological structure of the glomeruli and renal tubules compared with normal tissue of the control group mice. Also this study came in agreement with (Breul., 1998) who showed Probiotics are live microorganisms when administered in appropriate doses result in significant beneficial effects on health of human beings and animals which have been well documented through an array of scientific research and include prevention or treatment of urogenital tract disorders, urinary tract infections, cancer and immunomodulation. Hudault *etal*., 1997 Probiotics control different pathogens by production of antibacterial compounds, increased antibody levels and increased macrophage activity.



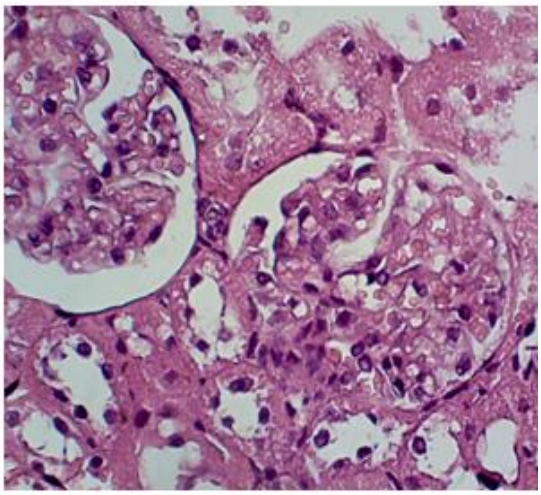
Figure, (1): section in kidney showing glomerular shrinkage (H&E 400 X).



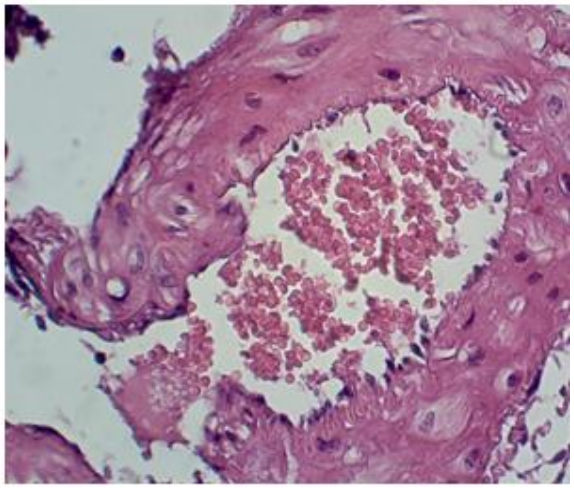
Figure, (2): section in kidney showing *congestion of* blood vessels. (H&E 100 X).



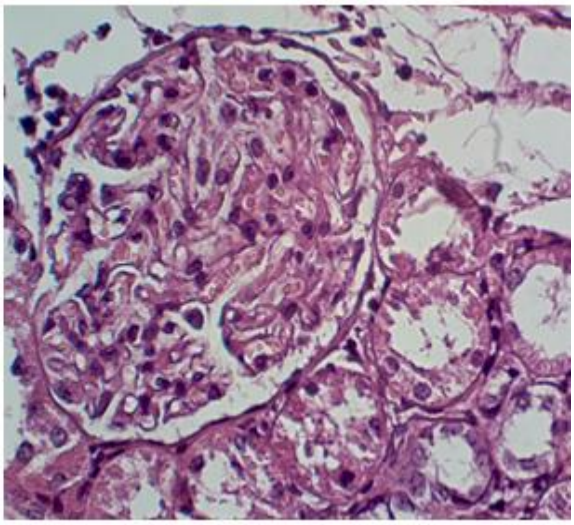
Figure, (3): section in kidney showing necrotic proximal tubules (H&E 400 X).



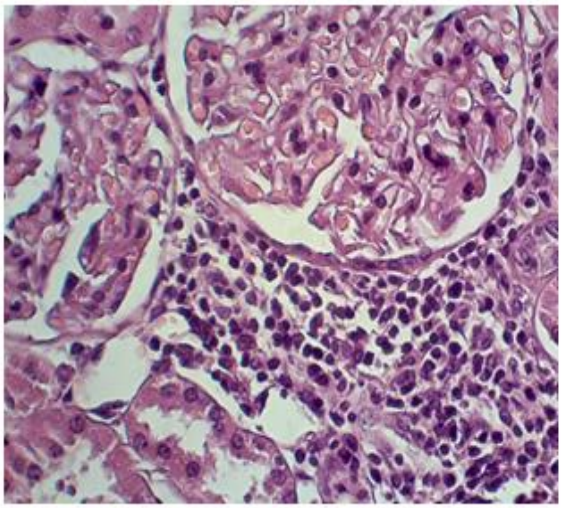
Figure, (4): section in kidney showing hypertrophy in blood capillary, congestion of blood and necrosis in tubules (H&E 400 X).



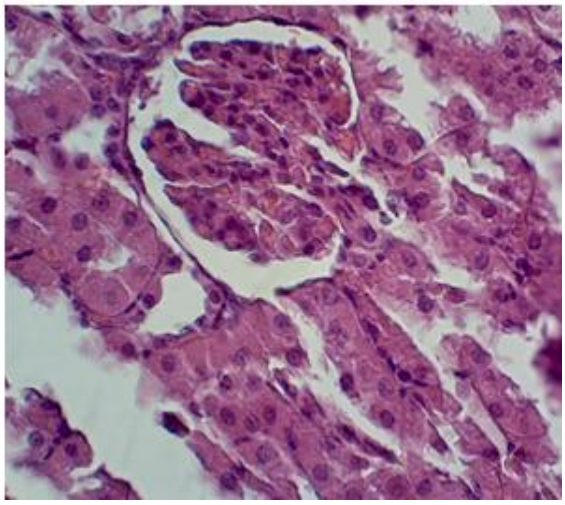
Figure, (5): section in kidney showing hyalinization of the *renal* artery. (H&E 400 X).



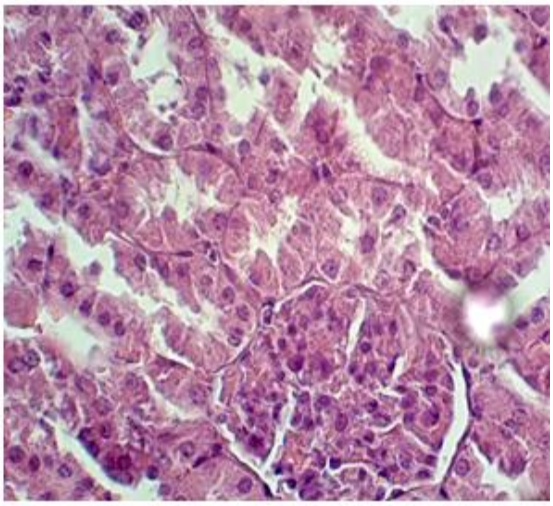
Figure, (6): section in kidney showing congestion of blood vessel, necrosis in convoluted tubules and hypertrophy. (H&E 400 X).



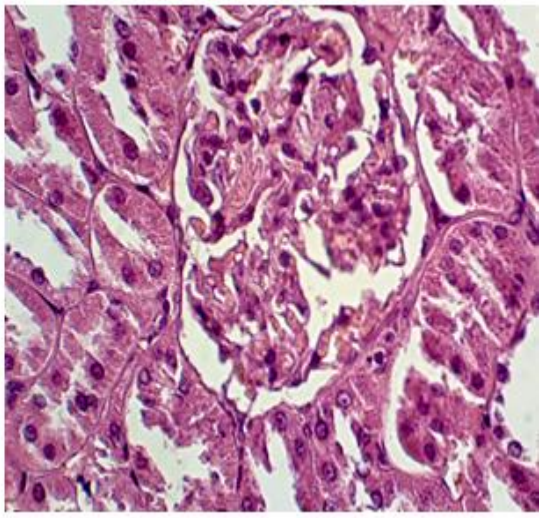
Figure, (7): section in kidney showing lymphocyte infiltration. (H&E 400 X).



Figure, (8): section in kidney showing as normal with mild congestion. (H&E 400 X).



Figure, (9): section in kidney showing normal structure (H&E 400 X).



Figure, (10): section in kidney showing Normal glomerulus. (H&E 200 X).

REFERENCE

1. Stamm WE, Norrby SR. Urinary tract infections: disease panorama and challenges. *J Infect Dis.* 2001; 183: Suppl 1: S1-S4.
2. Alain, M., (2003). Urinary tract infections. Mosby. Inc. USA.
3. Po, R. H.; Mei- Ling, C. C.; Wen- Hwei, C.; Hui-Jupan, L. S.Y.; Shan- Chang, S.W.; Chin-Yu, L.; Wei- Chuan, H.; and Kwen-Tay, L. Anti Microbial Drug resistance in pathogens: causing Nosocomial. Infection at a university Hospital in Taiwan, Emerging. Infectious Diseases. 2002; 8: 1.
4. Salvatore, S; Salvatore, S, Cattoni, E, Siesto, G, Serati, M, Sorice, P, Torella, M "Urinary tract infections in women.". *European journal of obstetrics, gynecology and reproductive biology*, 2011 Jun; 156(2): 131–6.
5. Nicolle LE Urinary tract infection: traditional pharmacologic therapies. Dis Mon. 2003; 49: 111–28.
6. Brooks, G. F.; Butel, J. S.; Morse, S. A. C. (2001). Medical Microbiology. 26/E. Lange. Medical book. Me Graw: Hill. VSA.
7. Johanson D E, Russel D M, Lockatell CV, Zulty JC, Warren JW and Mobley HLT. Contribution of *Proteus mirabilis* urease to persistence, urolithiais and acute pyelonephritis in a mouse model of ascending Proteus mirabilis. Infect Immun, 1993; 61: 2748-2754.
8. Jansen AM, Lockatell VC, Johnson DE and Mobley HLT. Visualization of *Proteus mirabilis* morphotype in urinary tract: The elongated swarmer cells in rarely observed in ascending urinary tract infection. Infect Immun, 2003; 71: 3607-3613.
9. Pearson MM and Mobley HLT. The type III secretion system of Proteus mirabilis HI4320 does not con-tribute to virulence in the mouse model of ascending urinary tract infection. J Med Microbiol, 2007; 56: 1277-1283.
10. Burall LS, Harro J M, Li X, Lockatell C V, Himpsl S D, Hebel J R, Johnson D E and Mobley H LT. *Pro-teus mirabilis* genes that contribute to pathogenesis of urinary tract infection: Identification of 25 signa-ture-tagged mutants attenuated at least 100-fold. Infect Immun, 2004; 72: 2922–2938.
11. Wang WB, Lai H C, Hsueh PR, Chiou YY, Lin S B and Liaw S J. Inhibitio swarming and virulence factor expression in Proteus mirabilis by resveratrol. J tor expression in Proteus mirabilis by resveratrol. J M Microbiol, 2006; 55: 1313-1321.
12. Esposito, A. L., R. A. Gleckman, S. Cram, M. Crowley, F. McCabe and M. S. Drapkin. Community-acquired bacteremia in the elderly: analysis of one hundred consecutive episodes. J. Am. Geriatr. Soc., 1980; 28: 315-319.
13. Samra KSS, Cenk S, Horu G, Mehmet G, Beril O (2005) Increasing antimicrobial resistance of *Escherichia coli* isolates from community acquired UTI during 1998-2003 in Manisa.
14. Oyetayo VO, Adetuyi FC and Akinyosoye FA Safety and protective effect of Lactobacillus acidophilus and *Lactobacillus casei* used as probiotic agent in vivo, *Afr. J. Biotechnol.* 2003; 2: 448-452.
15. Stiles M, E, Biopreservation by lactic acid bacteria, *Antonie van Leuwenhoek*, 1996; 70: 331-345.
16. Sánchez, J., Borrero, J., Gó Sala, B., Basanta, A., Herranz, C., Cintas, L.M. & Hernández, P.E. "Cloning and heterologous production of hiracin JM79, a sec – dependent bacteriocin produced by Enterococcus hirae DCH5, in lactic acid bacteria and Pichia pastoris", Appl. Environ. Microbiol. 2008; 74(8): 2471 – 2479.
17. Pilasombut, K.; Sakpuaram, T.; Wajjwalku, W.; Nitisinprasert, S.; Swetwiwathana, A.; Zendo, T.; Fujita, K.; Nakayama, J. and Sonomoto, K. Purification and amino acid sequence of a bacteriocins produced by *Lactobacillus salivarius* K7 isolated from chicken intestine. Songklanakarin J. Sci. Technol., 2005; 28(1): 121-131.

18. Franz CMAP, Schillinger U, Holzapfel WH. Production and characterization of enterocin 900, a bacteriocin produced by *Enterococcusfaecium* BFEE 900 from black olives. *Int J Food Microbiol.* 1996; 29: 255-270.
19. Parente, E.; Brienza, C.; Moles, M. and Ricciardi, A. A comparison of methods for the measurement of bacteriocin activity. J. Microbiol. Methods, 1995; 22: 95-108.
20. Lowry, O.H.; Rosebrough, N.J.; Farr, A.L. and Randall, R.J. (1951). Protein 12. measurement with the folin phenol reagent. J Biol Chem, 1951; *193*: 265-75.
21. Al – Gharabawee, R. H. R. (2012). The effect of purified bactriocin and ethanolic extract of *Plantago lanceolata* and *Quercus infectoria* on enerropathogenic *E. coli*. PhD thesis, Baghdad University, Collegeof Vererinary Medicine.
22. Powell, J. E.; Witthuhn, R.C.; Todorov, S.D. and Dicks, L.M.T. Characterization of bacteriocin ST8KF produced by a kefir isolate *Lactobacillus plantarum* ST8KF. Int. Dairy J., 2007; 17: 190–198.
23. Al-Ani IM, Al-Murayati HY, Salman MO, Ghani MM Histological findings of ascending urinary tract infections in experimental rats caused by Proteus mirabilis. Biomed. Res. 2011; 22(4): 425-430.
24. Bartels, H. and Bohmer, M. Eine Mikromethodzur Kreatinine Test Immung. Clinical Chimica Acta, 1971; 32: 81-85.
25. Fossati, P., Precscipe, L, Berti, G. Use of 3,5-dichloro-2-hydroxybenzene Suiphoric acid 14-aminophenazone chromagenic' sy in direct enzymatic assay of uric acid in sert andurine Clin. Chem. 1980; 26: 227-230.
26. Luna, L.G. (1968). Manual of histological staining methods of the armed forces institute of pathology. 3rd. ed., Mcgrow-Hill Book Company New York.
27. SAS.2010.SAS/STAT Users Guide for Personal Computer. Release 9.1. SAS Institute, Inc., Cary, N.C., USA.
28. Sanni, A.I.; ogunbanwo, S.T. and Onilude, A.A. Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1. Afr. J. Biotechnol., 2003a; 2(8): 219–227.
29. Anyogu A, Awamaria B, Sutherland JP, Ouob LII Molecular characterisation and antimicrobial activity of bacteria associated with submerged lactic acid cassava fermentation. Food Chem. 2014; 39: 119-127.
30. Omar NB, Abriouel H, Keleke S, Valenzuela AS, Martínez-Cañamero M, López RL, Ortega E, Gálvez A Bacteriocin-producing Lactobacillus strains isolated from poto poto, a Congolese fermented maize product and genetic fingerprinting of their plantaricin operons. Intern. J. Food Microbiol. 2008; 127: 18-25.
31. Chang, M.H, Hong, S.F, Chen, J.H, Lin, M.F, Chen, C, S and Wang, S.C. Antibacterial activity Lactobacillus plantarum isolated from fermented vegetables and investigation of the plantaricin genes. African Journal of Microbiology Research. 2016; 10(22): 796-803.
32. Morelli, L. In vitro selection of probiotic *lactobacilli*: A critical appraisal, *Current Issues in Intestinal Microbiology*, 2000; 1: 59-67.
33. Ibrahim*, O.M.S., Sarhan, S.R. and Hameed, A.A. In Vivo and In Vitro Antibacterial Activities of Cranberry Extract against E. coli O157:H7 in Urinary Tract Infected Rats. Advances in Animal and Veterinary Sciences. 2015; 3(4): 233-244.
34. Shimizu, k. Aiba, y. Tanaka, k. Akatsuka, A. Endoh, M and Koga, Y. Induction of Glomerular Lesions in the Kidneys of Mice Infected with Vero Toxin–Producing Escherichia coli by Lipopolysaccharide Injection. The Journal of Infectious Diseases, 1999; 180: 1374–7.
35. Reid G, Jass J, Sebulsky MT and McCormick JK "Potential uses of probiotics in clinical practice. Clin. Microbiol. Rev., October 2003; 16(4): 658–72.
36. Erickson KL and Hubbard NE. Probiotic immunomodulation in health and disease. J Nutr, 2000; 130(suppl): 403S–9S.
37. Mobley, H. L. T. and Belas, R. Swarming and pathogenicity of Proteus mirabilis in the urinary tract. Tends in Microbiol. 1995; 3(7): 280-284.
38. Alwan, A.H. The protective effect of probiotics (*Lactobacillus acidophilus*) against urinary tract infections caused by Proteus mirabilis in vitro. Magazin of al-kufa university for biology. 2011; 3: 2. ISSN: 2073-8854.
39. Breul S, Les. probiotiques en alimentation animale. Med Chir Dig, 1998; 27: 89-91.
40. Hudault, S. Lievin, V. Bernet-Camard, M. F and Servin, A. L. Antagonistic activity exerted *in vitro* and *in vivo* by *Lactobacillus casei* (strain GG) against *Salmonella typhimurium* C5 infection, *Appl. Environ. Microbiol.*, 1997; 63: 513-518.