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Protective effect of chamomile recutita flowers extract against Urinary tract infection induced by *Pseudomonas aeruginosa* in Experimental mice models

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Abstract

This study was conducted to investigate the protective role of ethanolic extract of chamomile recutita flowers induced UTI in the mice by *Pseudomonas aeruginosa*,the flowers extracted by preparing ethanol extract .10^8 cfu /ml of *Pseudomonas aeruginosa* was used to induce UTI by intra-urinary bladder injection . Thirty mice were divided into three groups .Group I: negative control (normal). Group II: positive control, mice were infected by injection of *Pseudomonas aeruginosa* for 2, 7, 15, 21 and 30 days and left without treatment. Group III : mice infected and treated with ethanolic extract of of Chamomile (*Matricaria recutica*) flowers orally by stomach tube daily after 48 h of infection for 15 , 21 and 30 days at dose 300 mg/kg-BW. At the end of experimental period ,blood samples were taken from mice and measured some biochemical parameters such as serum Creatinine , Serum uric acid ,serum glucose and Serum total proteins .animals of all groups were killed to examine histopathological changes in urinary bladder and kidney .The result showed significant increased ($P< 0.05$) in serum creatinine level and serum uric acid in infected groups as compared with control .The treated group showed significant decreased ($P< 0.05$) in serum creatinine level and serum uric acid level while no significant change in serum glucose level and serum total protein in infected and treated groups. Histological sections of kidney appeared damage, necrosis of renal tubule and inflammatory cell infiltration in infected groups while animals treated with ethanolic extract of Chamomile recutica flowers showed look -like normal appearance in kidney. It could be concluded that Chamomile recutica flower extract has potent protective role against UTI and appears by correction between biochemical parameters and histological studies.

Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections affecting humans throughout their life span (Chang.,2006), (Kucheria etal.,2005) .Urinary tract infections are a serious health problem affecting millions of people every year, each year urinary tract infections account for about 8 million doctor visits (Gokulakrishnan etal.,2012). The urinary tract infection (UTI) caused by *P. aeruginosa* is a serious health problem affecting millions of people worldwide each year and catheterization of the urinary tract is one of the most common predisposing factors to such infections (Mittal etal.,2009). *Pseudomonas aeruginosa* is an opportunistic human pathogen (Vianelli etal.,2006).

Chamomile (*Matricaria recutica*) that have been used as herb medicine ,the flowers of chamomile recutita are active pharmaceutical ingredient of herbal teas because it contains substances which have anti-inflammatory, sedative, analgesic, antispasmodic, antioxidant and antimicrobial properties (Bisset and Wichtl.,2001),(McKay and Blumberg.,2006).

Material & Method

urine samples were collected from UTI patients in educational Al-karama hospital of Al-kut city, the mid-stream urine was collected aseptically, cultured on blood agar and MacConkey agar at 37C ° for 24 hours then on nutrient agar .Colonies were selected and identified depend on morphological characteristics, Gram staining ,biochemical tests and the diagnosis was confirmed by using API 20E System kit. Antibiotic susceptibility testing (AST) for the *Pseudomonas aeruginosa* bacteria were done using the Vitek 2 systems AST-GN69.

Flowers of chamomile recutita were purchased from local market in AL-Kut city. After cleaning flowers were dried in shade and powdered, 50g of the powder was dissolved in 500 ml of 70% ethanol and placed on magnetic stirrer for 72 hours then filtered was put in incubator at temperature of 40c ° until dryness and the extract was kept in dark glass container at 4 ℃. The preliminary phytochemical tests were performed for testing detect the presence of active materials such as tannins (Harbone, 2001), flavonoids (Harbone, 2001), (Sofowora, 2005), saponnins (Sofowora, 2005), alkaloid (Harbone ,1973) and carbohydrates. Agar well diffusion method was used to check the antibacterial activity of chamomile recutita flowers extract against *Pseudomonas aeruginosa* in vitro (Perez et al., 1990) after preparation of different concentrations of plant extract (100,200,300 mg /ml)

Thirty mice were obtained from the national center for drug control and research, baghdad. Induction of urinary tract infection by injection mice with 10^8cfu /ml of *Pseudomonas aeruginosa* intra-urinary bladder according to the method of (Abdul Ratha etal.,2014) .Group I: negative control (normal). Group II: positive control, mice were infected with injection of Pseudomonas aeruginosa for 2, 7, 15, 21 and 30 days and left without treatment. Group III : mice infected and treated with ethanolic extract of of Chamomile (*Matricaria recutica*) flowers orally by stomach tube daily after 48 h of infection for 15 , 21 and 30 days at dose 300 mg/kg-BW. At the end of experimental period ,blood samples were taken from anaesthetized mice then the mice were killed for histopathological examination and measured the following parameters: serum creatinine (Bartels and Bohmer 1971), Serum uric acid (Fossati etal., 1980), serum glucose concentration using (Trinder, 1969) and (Tietz, 1995) and Serum total proteins (Tietz, 1999). Samples from the kidney and urinary bladder were fixed in 10% neutral buffered formalin. (Luna, 1968**) .** Statistical analysis was performed using SPSS-21 (Statistical Packages for Social Sciences- version 21). One Way Analysis of Variance (ANOVA) and Least significant differences (LSD) post hoc test was performed (multiple comparisons), to assess significant difference among means. $P<0.05$ was considered statistically significant as described by (Released, 2012)**.**

Results & Discussion

Results showed that the *Pseudomonas aeruginosa* isolates, gram negative, circular mucoid smooth colonies, grape-like odor , grows on MacConkey agar without ferment lactose and produce pigment from yellowish green to bluish green on nutrient agar ,the biochemical test were positive result for catalase ,oxidase and urease test while negative result for indole test.

The susceptibility of isolated *Pseudomonas aeruginosa* to antibiotics is shown in (Table,1) . Urinary tract infections are common conditions worldwide and the pattern of antimicrobial resistance varies in different regions. (Amin etal.,2009) and antimicrobial resistance is an increasing public health threat which leads to increased morbidity and mortality (Cosgrove,2006). *Pseudomonas aeruginosa* resistance to the antibiotics used in the treatment of UTI especially in hospital acquired infection (Salih etal.,2011) and excessive use of broad-spectrum antibiotics in hospitals has led to the emergence of highly resistant strains of *P. aeruginosa* (Al-Grawi, 2011).

Table 1: Antibiotic susceptibility testing of *Pseudomonas aeruginosa* by vitek-2 method

Antimicrobial	MIC	Interpretation
Ampicillin	≤ 2	S
Amoxicillin/clavulanic acid	≤ 2	S
Ampicillin/sulbactam	≤ 2	S
Cefazolin	32	I
Ceftazidime	≤ 1	S
Ceftriaxone	≤ 1	S
Cefepime	≤ 1	S
Imipenem	1	S
Gentamicin	≤ 1	S
Tobramycin	≤ 1	S
Ciprofloxacin	≤ 0.25	S
Levofloxacin	≤ 0.12	S
Nitrofurantoin	256	R
Trimethoprim-sulfamethoxazole	80	R

S = sensitive , R = resistant , I = intermediate resistant

Photochemical screening of chamomile recutita flowers extract revealed the presence tannins, flavonoids, saponins, alkaloids and carbohydrates as shown in (Table,2) . (Srivastava etal.,2010) reported that chamomile

has been used as an herbal medication because it contains various bioactive photochemical that could provide therapeutic effects.

Table 2: Results of phytochemical analysis of chamomile recutita flowers extract.

Chemical compounds	Ethanolic extract
Tannins	+
Flavonoids	+
Saponnins	-
Alkaloid	±
Carbohydrates (Benedict's test)	+

(+) indicates positive results; (−) indicates negative results

Results of antibacterial activity showed the ethanolic extract of chamomile recutita flowers exhibited antibacterial activity against *pseudomonas aeruginosa* as shown in (Table ,3). (Gosztola etal., 2010) have shown that Chamomile species have strong antibacterial, antifungal, antiviral, antiparasitic, spasmolytic and antioxidant activities . (Al-Ismail and Talal.,2003) has been reported that generally the aqueous chamomile extract was more effective against moulds and yeast, while the alcoholic ones inhibited more the bacteria

Table 3 : Antibacterial activity of ethanolic extract of chamomile recutita flowers against *pseudomonas aeruginosa*

Concentration mg /ml	Mean ± SE
100	15.66±0.33 c
200	18.00±0.57 b
300	21.33±0.33 a

Means with different letters differ significantly ($P < 0.05$)

This study showed the effect of chamomile recutita flowers extract on some biochemical parameters, kidney functions was assayed by the determination the levels of serum creatinine and uric acid .The serum creatinine level (Figure,1) ,(Table,4) in infected group with *pseudomonas aeruginosa* was increased in 7days and decreased serum creatinine level in 15 ,21 and 30 days , but did not reach to the normal level as compare with the G1 (normal control group). The elevation in serum creatinine within days or weeks is produced by kidney damage, which leads to a decrease in glomerular filtration rate. (Bilal etal.,2015) showed that the elevated levels of serum urea and creatinine are usually considered as biochemical indicators of chronic renal failure i.e. serum urea >50gm/dl and creatinine greater than 1.2 gm/dl. The end-stage renal failure patients usually show the serum creatinine around 10gm/dl and serum urea levels around 100 gm/dl. (National Kidney Foundation, 2002) reported that end-stage renal failure (ESRF) is caused death without renal replacement therapy. Treatment with ethanolic extract of Chamomile recutita flowers caused highly significant reduction in serum creatinine level in mice infected with *pseudomonas aeruginosa*

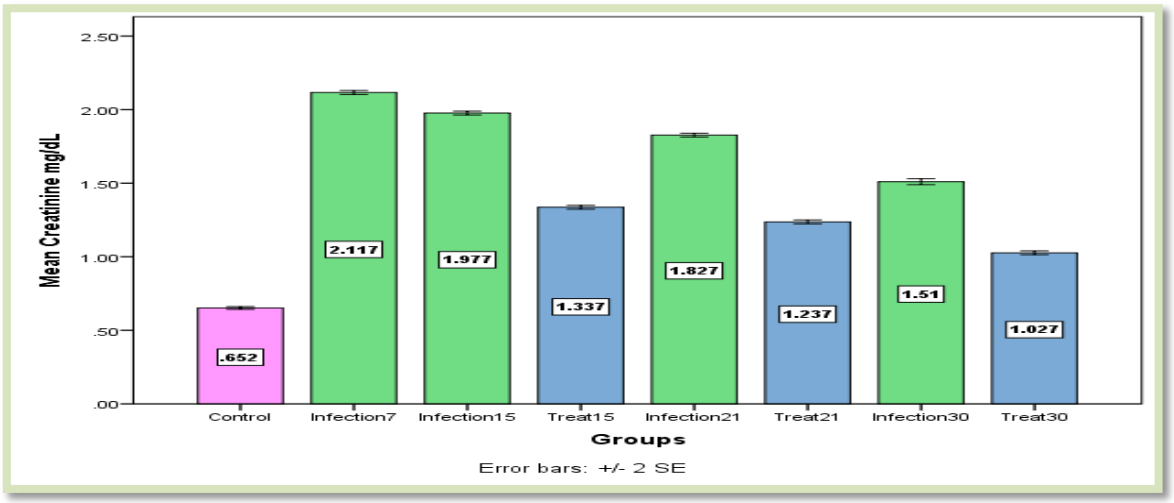


Figure (1): comparison of serum creatinine levels (mg/dl) of the experimental mice groups

Table (4): Mean values ± SE of serum creatinine levels (mg/dl) of the experimental

Groups	Mean ± SE
Control	0.65 ±0.004 h
Infection after 7 days	2.11 ±0.006 a
Infection after 15 days	1.97 ±0.006 b
Treated after 15 days	1.33 ±0.006 e
Infection after 21 days	1.82 ±0.006 c
Treated after 21 days	1.23 ±0.006 f
Infection after 30 days	1.51 ±0.01 d
Treated after 30 days	1.02 ±0.006 g

Means with different letters differ significantly (P< 0.05)

(Figure ,2),(Table 5) illustrated that the result of serum uric level revealed a significant increased (P < 0.05) in mice that infected with *pseudomonas aeruginosa* as compared with control group while treated group with ethanolic extract of chamomile recutita flowers caused a significant decreased in serum uric acid level (P<0.05) as compared with infected and control groups. Impaired renal excretion of uric acid rather than uric acid overproduction was considered to be the major cause of hyperuricemia (Hikita etal.,2011) and elevated serum uric acid levels are seen in patients with reduced glomerular iltration rate (GFR) ,however, in recent years, it has been proposed that uric acid itself plays a causal role in the pathophysiology of chronic kidney disease and possibly in acute kidney injury(Giordano etal.,2015) .(Najla etal.,2012) revealed that the treatment of diabetic animals with 100 mg/kg/day chamomile extracts significantly inhibited increase of serum uric acid.

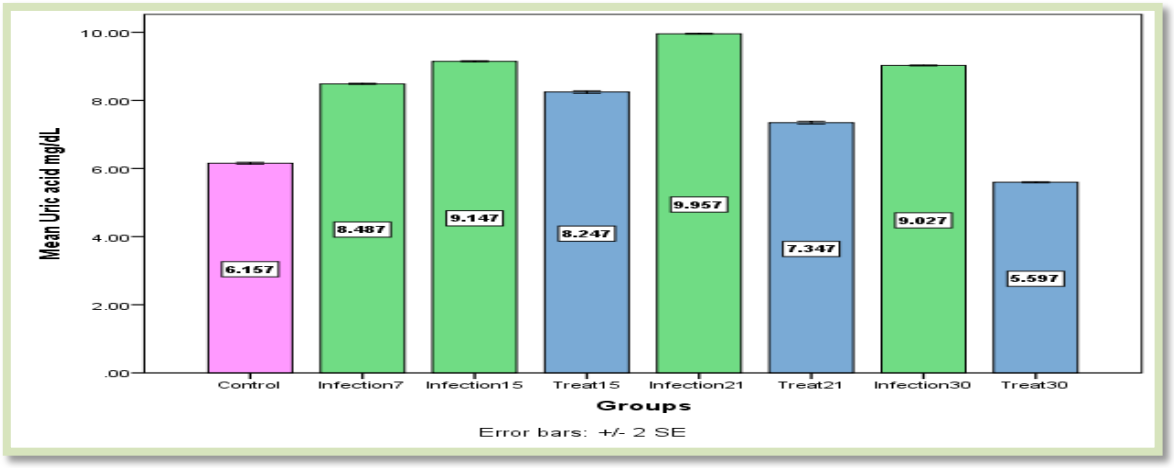


Figure (2): comparison of serum uric acid levels (mg/dl) of the experimental mice groups

Table (5): Mean values ± SE of serum uric acid levels (mg/dl) of the experimental mice groups.

Groups	Mean ± SE
Control	6.15 ±0.01 g
Infection after 7 days	8.48 ±0.008 d
Infection after 15 days	9.14 ±0.008 b
Treated after 15 days	8.24 ±0.01e
Infection after 21 days	9.95 ±0.006 a
Treated after 21 days	7.34 ±0.02f
Infection after 30 days	9.02 ±0.006 c
Treated after 30 days	5.59 ±0.008 h

Means with different letters differ significantly (P< 0.05)

Results illustrated in (Figure, 3), (Table, 6) and (Table, 7), (Figure,4) respectively showed that the mean values of serum glucose level and serum total protein level were no significant changes and within the normal reference ranges . After 15, 21 and 30 days of administration of chamomile recutita flowers extract, they gradually decreased in serum glucose level. (Najla etal.,2012) showed the hypoglycemic properties of chamomile extract are reported to be due to their higher contents of flavonoides and different bioactive compounds. (Egoro etal.,2015) revealed that there was no statistical significant differences (p>0.05) in the mean values of serum total protein in the premenopausal and postmenopausal patients infected with *Escherichia coli* isolated from urinary tract .

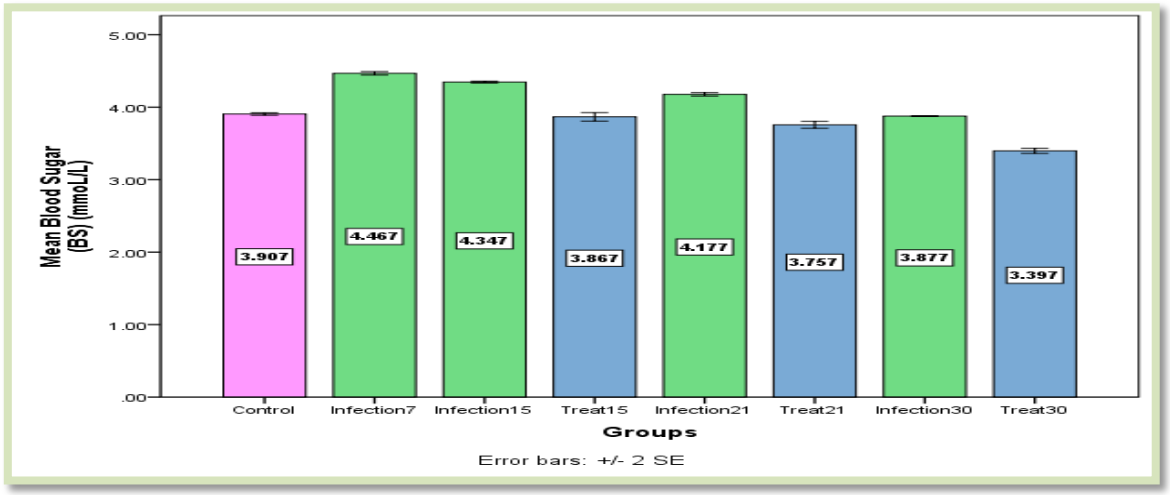


Figure (3): comparison of serum glucose levels (mmoL/L) of the experimental mice groups

Table (6): Mean values ± SE of serum glucose levels (mmoL/L) of the experimental mice groups

Groups	Mean ± SE
Control	3.90 ±0.008 c
Infection after 7 days	4.46 ±0.01 a
Infection after 15 days	4.34 ±0.006 b
Treated after 15 days	3.86 ±0.03c
Infection after 21 days	4.17 ±0.01c
Treated after 21 days	3.75 ±0.02 e
Infection after 30 days	3.87 ±0.003 c
Treated after 30 days	3.39 ±0.01 f

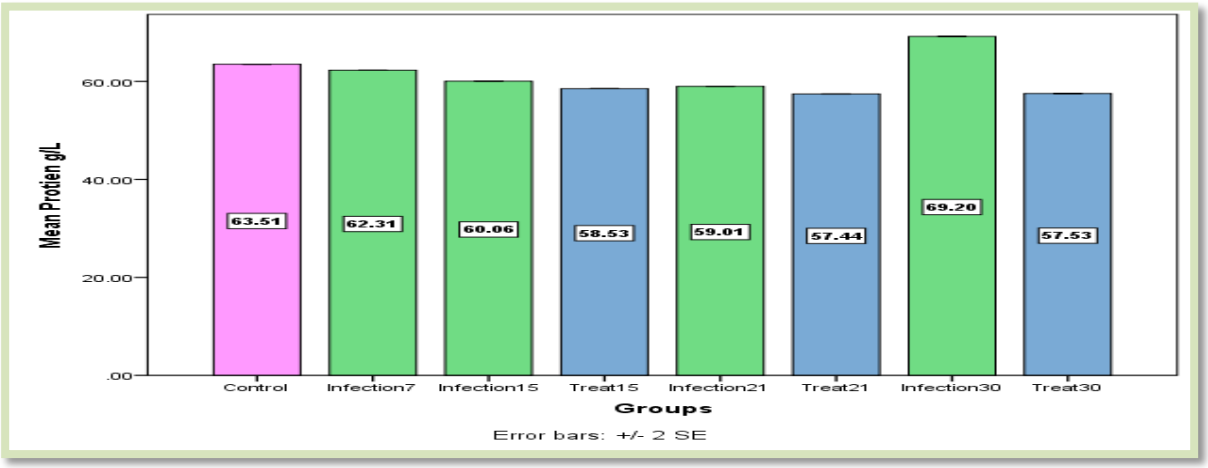
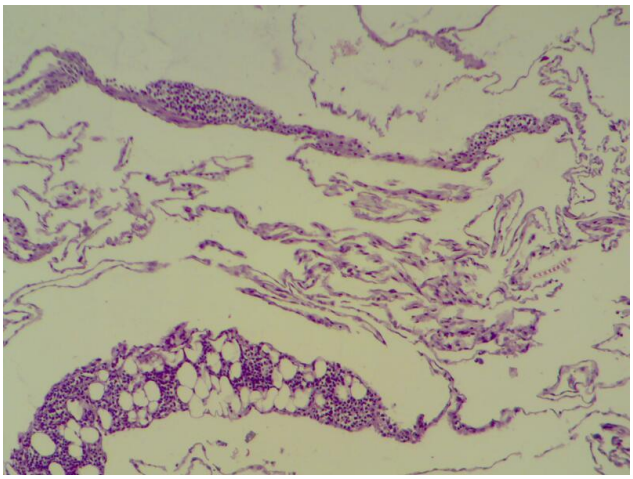


Figure (4): comparison of serum total protein levels (g/L) of the experimental mice groups

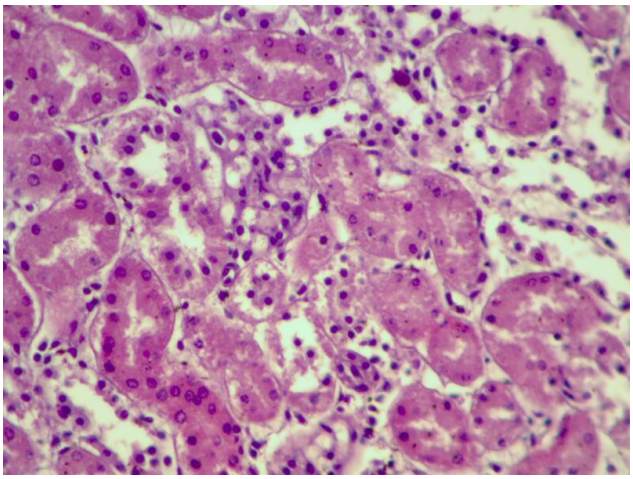
Table (7): Mean values ± SE of serum total protein levels (g/L)) of the experimental mice groups

Groups	Mean ± SE
Control	63.51 ±0.003 b
Infection after 7 days	62.31 ±0.01c
Infection after 15 days	60.06 ±0.006 d
Treated after 15 days	58.53 ±0.01f
Infection after 21 days	59.01 ±0.006 e
Treated after 21 days	57.44 ±0.01 h
Infection after 30 days	69.20 ±0.006 a
Treated after 30 days	57.53 ±0.008 g

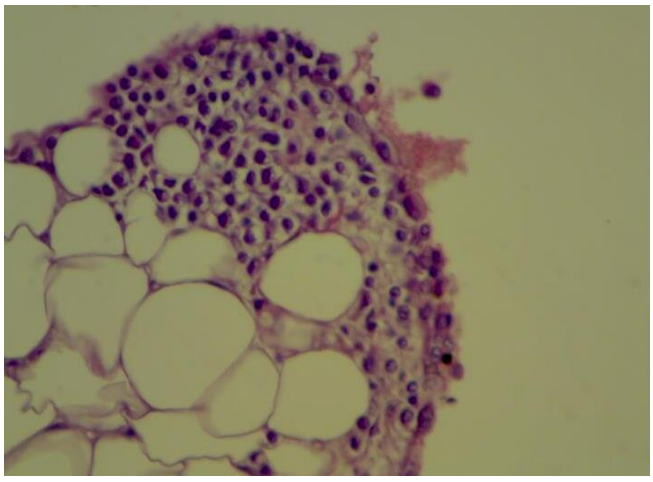
Histopathological results of second group, the urinary bladder revealed damage of epithelium with inflammatory cell infiltration in 2nd days of urinary tract infection (Figure. 5) .kidney was showed necrosis of renal tubule and inflammatory cell infiltration in 2nd days of urinary tract infection (Figure , 6), the most common cause of acute tubular necrosis is a lack of oxygen, when blood cannot reach the tissues and cells of the kidneys due to a blockage or restriction, the kidneys can be damaged or destroyed . In urinary bladder histopathology showed damaged of epithelium, congestion of blood vessels and inflammatory cell infiltration after 1st week of urinary tract infection (Figure 7, 8).



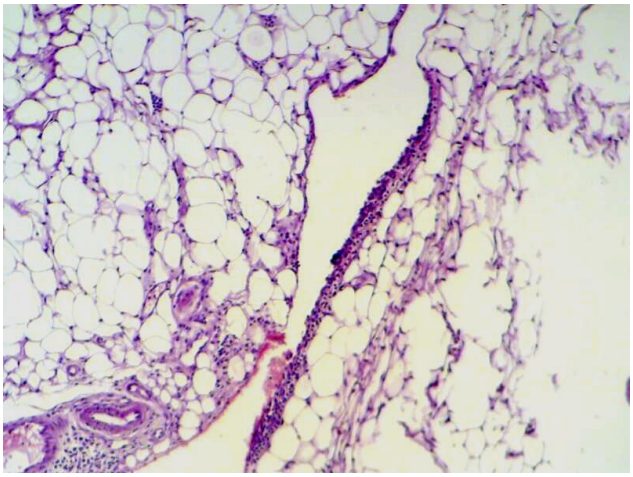
Figure, (5): Section in urinary bladder (positive control) shows damage of epithelium with inflammatory cell infiltration (H&E 200 X).



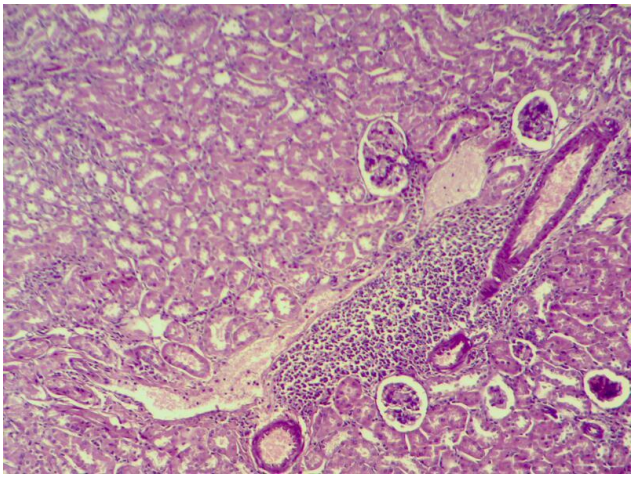
Figure, (6): section in kidney (positive control) shows necrosis of renal tubule and inflammatory cell infiltration (H&E 400 X).



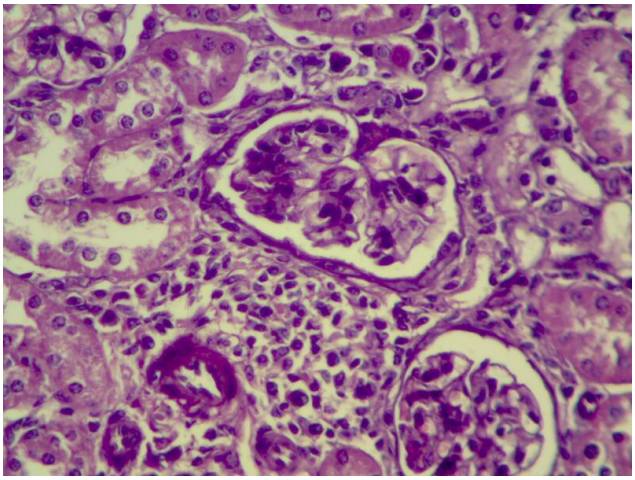
Figure, (7): Section in urinary bladder epithelium (positive control) shows damage of epithelium and inflammatory cell infiltration (H&E 400 X).



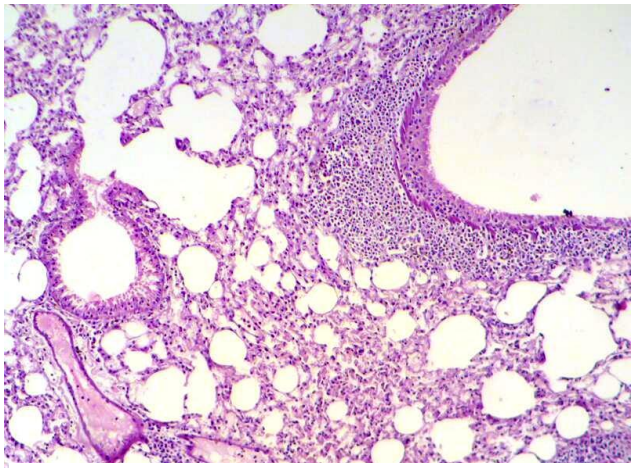
Figure, (8): Section in urinary bladder (positive control) shows damage of epithelium, congestion of blood vessels and inflammatory cell infiltration (H&E 400 X).



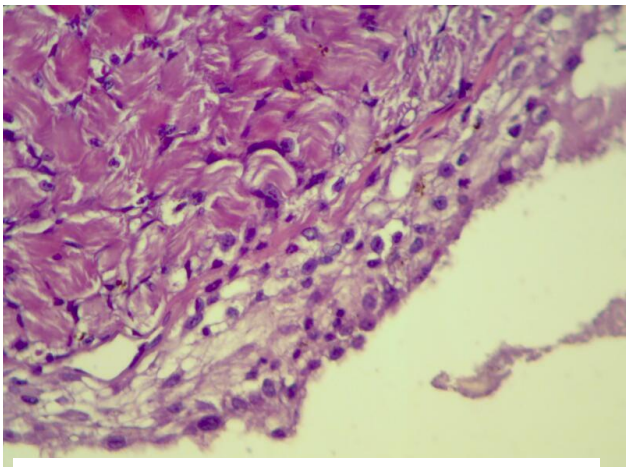
Figure, (9): Section in kidney (positive control) shows damage , necrosis of renal tubule and inflammatory cell infiltration (H&E 200 X).



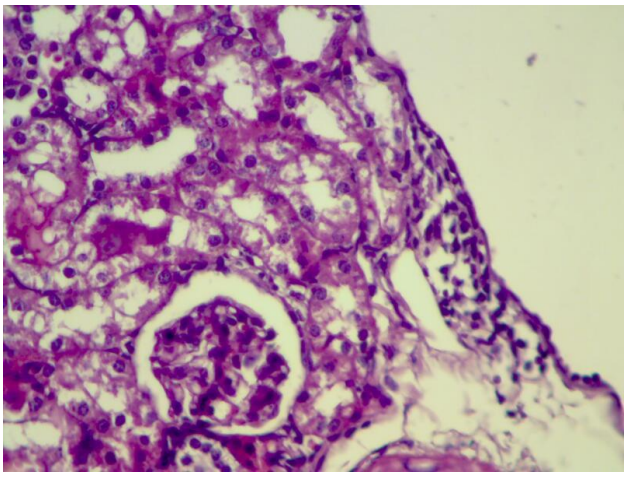
Figure, (10): Section in kidney (positive control) shows damage, necrosis of renal tubule and inflammatory cell infiltration (H&E 400 X).



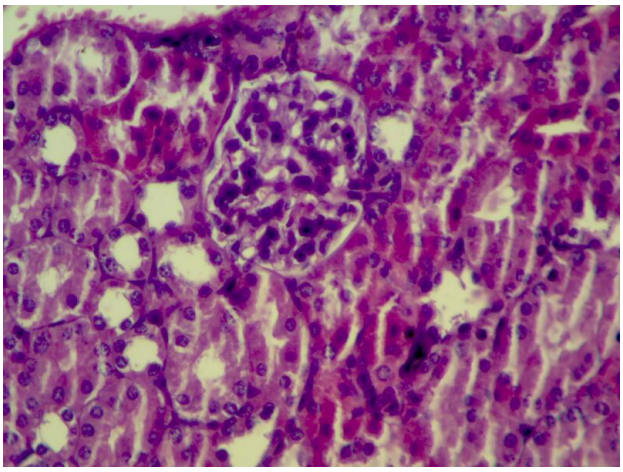
Figure, (11): section in lung shows severs inflammatory cell infiltration, destruction of alveoli oedema and congestion of blood vessels (H&E 400 X).



Figure, (12): Section in urinary bladder (positive control) shows Simple inflammation of the lining of the urinary bladder and inflammatory cell infiltration (H&E 400 X).



Figure, (13): Section in kidney (treated group) shows look like appearance but with necrosis and mild inflammatory cell infiltration (H&E 400 X).



Figure, (14): Section in kidney (treated group) shows look -like normal appearance (H&E 400 X).

Urinary tract infection is an infection which could affect the kidneys, ureters, bladder or urethra and it's subjected as one of the most common infections in humans (Geetha et al., 2011). Histological of kidney appeared damage, necrosis of renal tubule and inflammatory cell infiltration after 1st week of urinary tract infection (Figure 9, 10) (Woods etal.,1986) showed high production of elastase and protease in strains isolated from urinary tract infections, these factors have been shown to play an important role in pathogenesis of *P. aeruginosa* induced infections. Lung revealed sever inflammatory cell infiltration, destruction of alveoli oedema and congestion of blood vessels after 1st week of urinary tract infection with *Pseudomonas aeruginosa* .(Figure, 11). *P .aeruginosa* often produced a chronic pulmonary infection, leading to excessive inflammation and lung injury.(Gregory etal.,2007)

In urinary bladder section showed simple inflammation of the lining of the urinary bladder and inflammatory cell infiltration after 4th weeks of urinary tract infection (Figure, 12). The third group show look like appearance but with necrosis and mild inflammatory cell infiltration in kidney after 2nd weeks of treatment with ethanolic extract

of chamomile recutita flowers (Figure, 13) while showed look -like normal appearance in kidney after 4th weeks of ethanolic extract of chamomile recutita flowers (Figure, 14).Chamomile flowers extract had been exhibited antioxidant and antimicrobial activities (Roby etal.,2013). It could be concluded that Chamomile recutica flower extract has potent protective role against UTI and appears by correction between biochemical parameters and histological studies.

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