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RESEARCH ARTICLE

Role of X-ray on theTreatment of UTI Induced By *Escherichia coli*

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Manuscript Info *Abstract*

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This study was conducted to investigate the effect of differentdoses of x-ray on the treatment of experimentally UTI induced by *E.coli* isolate in rabbits which used as an animal model.

E.coli isolate was obtained from patient suffering from UTI in AL-Krama hospital- Baghdad city,the isolate was diagnosis by cultural,morphological, biochemical tests and confirmed by using API 20 E system.

Results of treatment with X-ray showed no growth of *E.coli* from all tested organs kidney,urinary bladder ,spleen,liver,heart and lung in negative control (group 1) which were not infected with *E.coli*while other groups showed various value of bacterial isolation according to the doses of x-ray.

On the other hand group 6 which were treated with high doses of x-ray showed negative results for the isolation of *E.coli* from all the tested organs.

The results of group2(infected without treatment) showed the ability of *E.coli*to infectkidney and bladder with the appearance of acute clinical signs as compared with the negative control.

The results of histopathological changes showed the role of x-ray in decreasing the pathological signs in tissue and giving negative results for *E.coli* isolation in comparison with the positive control(group 2) which showed acute histopathological changes.

Introduction

Urinary Tract Infection (UTI) is an extremely common condition that occurs in both males and females of all age in human and animals,Worldwide, about 150 million people are diagnosed with UTI each year. (Gupta, 2001).

UTI occur when bacteria invade the urinary tract and presence of bacteria in the urine (Anderol*et al*., 2001)

E.coli predominates strongly at most ages (Ann , 2005) , it present between 80-90% of UTI causes and up to 95% of acutepylonephritis (Dezell and Leferve,2000).

The virulence factors of *E.coli* include O antigen(somatic antigen), capsule production especially of type K, haemolysin production as well as the presence of fimbriae (adhesion antigen) and colicin V. (Ann , 2005)

X-ray irradiation is an alternative that has certainadvantages over other current approved ionizing irradiation used in the food industry, such as gamma ray,theX-ray irradiator does not have a radioactive source(Janatpour *et al 2005)* X-ray induced damage of bacterial cells is subject to repair by several enzymatic systems that are specified by bacterial genes, a number of which have been located on the genetic map of *Escerichia coli*(Howard-flander et al.1966).

One interesting example of repair is seen in the case of phage-induced radioresistance of lysogenic bacteria(Trgoreei et al.1977)It differs from other types of repair operating on bacterialDNA in that it appears to depend on products specified bythe bacteriophage.

Damage by ionizing radiation ,leading to cell death is believed to occuerprimairly through random deposition of energy in vital cellular macromolccules which are refered to as targetes .(koteles ,1979)

This study was aimed to investigate the effect of different doses ofx-ray on the treatment of experimently UTI induced by *E.coli*.

Materials and Method

Bacteria: Clinical isolate of pathogenic *E.coli*was obtained from patientsuffering from UTI in the AL-Krama hospital of Baghdad city .

This bacterial isolate was identified by cultural and biochemical tests then the diagnosis was confirmed by using API 20 E system.

Induction of UTI

Abdominal of the rabbitswere disinfected and 1 ml challenged dose of bacterial suspension containing 2.6 x 10^6cfu/ml of *E.*coli was injected directly in to intra- urinary bladder by insulin syringewith needle gauge 30 figure(1).

The rabbit had been watched for symptoms of UTI. (Quinn *et al*., 2006).

Figure (1): Induction of*E.coli* through urinary bladder in rabbit

Experimental Design

EighteenRabbits(1200-1400 gmof different age)were divided equally into 6 groups, 3rabbits for each group .

Group (1): control negative (not infected with *E.coli*)and given only distill water orally for 7 days.

Group (2): control positive inoculatedwith 1 ml of *E.coli*suspension(2.6 x 10^6cfu/ml)in to intra urinary bladder (infected and not treated group).

Group (3): infected as in group2 and treated with *X -ray does(k v70 ,MA200, MAS 8.0 ,W 8.6g)* for 7days.

Group (4): infected with *E.coli*as in group2 and treatedwith *X -ray does (KV 90,MA 300,MAS 7.5 ,W8.4g)* for 7 days

Group(5): infected with *E.coli* as in group2 and treatedwith *X -ray does (KV 100,MA 300,MAS 7.5 ,W 8.9g)* \ for 7days

Group(6): infected with *E.coli* as in group2 and treated with *X -ray does (KV 106,MA 300,MAS 10.5 w,8.6g)* \ for 7 days.

KV: Kilo voltage , MA :MiliAmper, MAS:MiliAmper per second, W:wight

Results and Discussion

*E.coli*was diagnosed according to the cultural ,biochemical tests and confirmed by using API 20 E systemkitas listed in the following table (1):

Table (1):Cultural and biochemical tests of *E.coli* isolate.

Bacteria	**Cultural examination**		**Biochemical tests**	
E. coli	Gram stain	-	Indol	+
	Blood agar	Hemolysis	SimmonCitreate	-
	MacConkey agar culture	Rosy colonies lactose fermentation	Catalase	+
	EosinMethylene Blue agar	metalic sheen colonies	Vogesproskauer	+

-

Results of API 20 E system kit were confirmed the diagnosis of *E.coli*isolate

The results of the present study are agree with Anandkumar*etal*., (2003)who found that *E.coli* was the first causes of UTI,andalso with Mushtaq*et al*. (2005) who showed that the inoculated rats with 2.6×10^6 cfu/ml were led to efficient colonization of *E. coli* within 24 hrs, after inducing experimental infection by this bacteria .*Escherichia coli* that cause UTI and other uropathogens are distinguished from related ofspecific virulence determinants , microbial adaptations promoting success in the urinary tract (Johnson, 2003 ; Ann , 2005).

Clinical Signs

Before UTIinduction healthy animals showed normal feces, normal urine with yellow color , no fever, while after inducing infection with *E.coli* animals were suffered from anorexia, dehydration, fever (39.5-39.8 c) , frequency of uriaion , dysuria,cloudy of urine increased gradually from the first day after infection **,**results of thetreated with x-ray (group 3and4)showed severe uriaion, shine in eay, emaciation, rough body coat fever (39-39.5 c), increase in the up take of water and dehydration while (group 5and6) showed normal urine with yellow color without cloudy or fever.

Macroscopical signs

Macroscopicalexamination of urinary bladder and kidney after 48 hr post infection with *E. coli* showed the symptom of acute UTI as follow, rabbits viscera showed emaciation and atrophy, wasting skeletal muscle, while intestine lumen showedodema, congestion, with thickening its wall and hemorrhagiculceration of intestinal lumen and enlargement of bladder as in figure (2) while figure(3)showed normal animals.

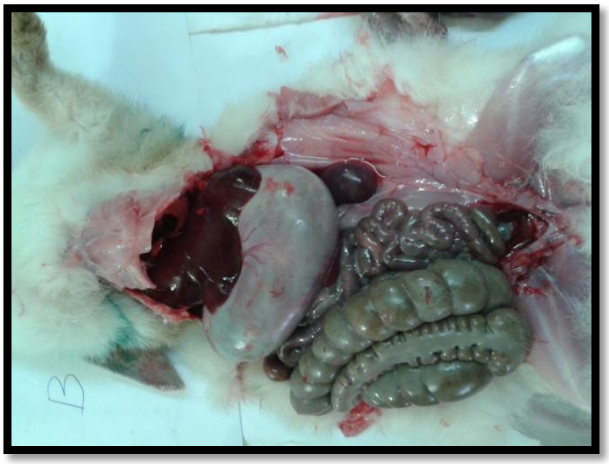


Figure (2):
Pathological gross examination innormal visceraPathological gross examination in infected viscer

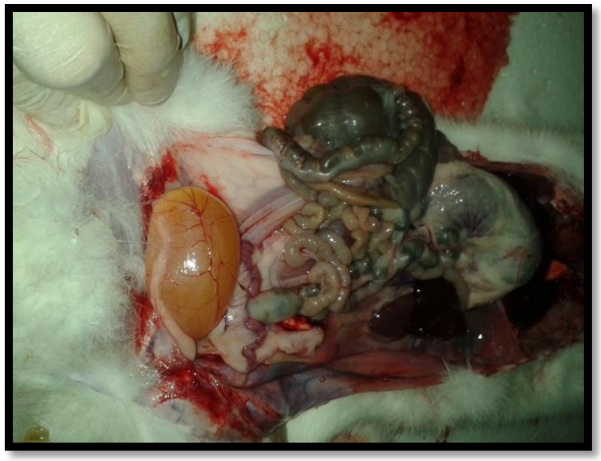


Figure (3):

Results of treatment with X-ray radiation showed negative growth of *E. coli* from all tested organs kidney,urinary bladder ,spleen ,liver,heart and lung in group 1 which were not infected with *E.coli* while other groups showed various value of bacterial isolation according to the x-ray dose .
The results showed sever infection in groups 2and 3 while the increase in x-ray dose result in reducing the rate of infection .
On the other hand group 6 which were treated with high dose of x-ray showed negative results for isolation of *E.coli* from all tested organs.Bacterial isolation gave positive results in group 2and negative result in group 1while the treatment with x-ray gave positive for bacterial isolation in group 3 and 4 after 7 days but gave negative results in groups 5and 6after 7 days,as in table (2)
These results wereagreed withwhat was mentioned by. (Gomes *etal*. , 2005). The adherence of *E.coli* to urothelial cells , is the first step in the pathogenesis of urinary tract infection (Svanborg and Godaly , 1997).

Bacteria that cause urinary tract infections typically transmitted to the urethra from the bowel, *E. coli* are able to attach to the bladder wall and form a biofilm that resists the body's immune response. (*Salvatore et al,.2011*).

Table (2): Bacterial isolation (on nutrent agar) from the internal organs of rabbit infected with virulent *E.coli*at7 days post-challenge.

Group	No.	Kidney	Urinary bladder	Spleen	Liver	Heart	Lung
Control	1	-	-	-	-	-	-
	2	+++	+++	+++	+++	+++	++
	3	+++	+++	+++	+++	++	++
	4	+++	++	++	+++	++	+
	5	+	+	+	-	-	-
	6	-	-	-	-	-	-

\- = negative (0) colony
\+ = mild (1-5) colonies
++ = moderate (6-10) colony
+++ = severe (over than 11 colonies)

Result of Histopathological Examination

The histopathological changes in kidney tissues after 7 days of injection with *E.coli* (group II) showed mononuclear lining cells of renal tubule in the tissue &vacuolar degenerationand enlargement epithelial cell .figure (4).
Bladder tissue showed lesion represented by congestion blood vessels in the lumen leaperia epithelial layer &polymononuclear cells (PMNS) , mononuclear cells (MNS) infiltration with odema in subepithelial layer and

neutrophils attachment to the endothelial cell Figure (5),as compared with the negative control group1 which showed normal structure of kidney and bladder Figure (6),(7).

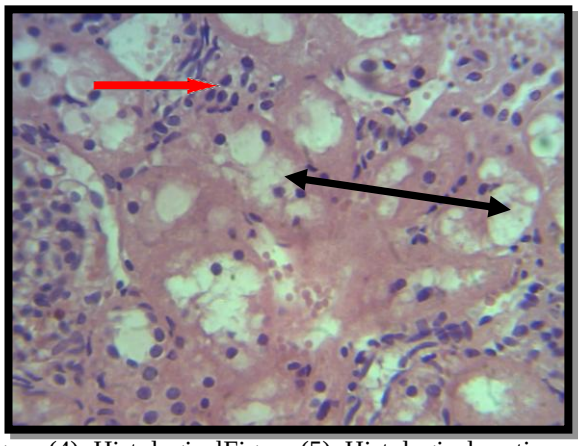


Figure (4): HistologicalFigure (5). Histological section in kidney of one animals at 7 days post infected with E.coli (G1) at 7 days post infected with *E.coli*(G1) mononuclear lining cells of renal tubule shows polymononuclear cells (PMNS) in the interstitial tissue are the main lesion and mononuclear cells (MNS) infiltration in the kidney in addition &odema in subepithelial layer with to vaculation ,degeneration and enlargemetcongestion blood vessels and neutrophils epilithelial cell

(E&H Stain 40 x) (E&H Stain 40X)

Figure (6): Histological section in normal animal (G2) showed normal structure of kidney (E&H Stain 40X)

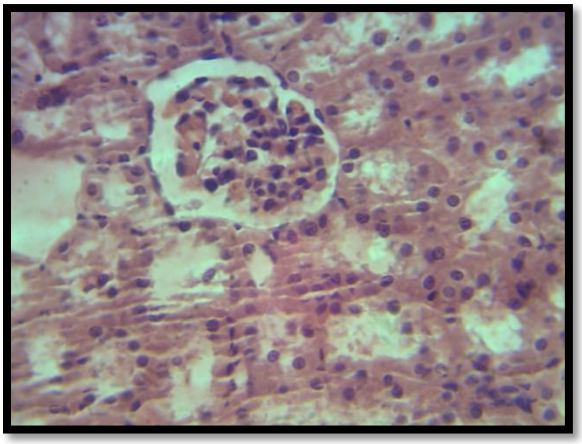
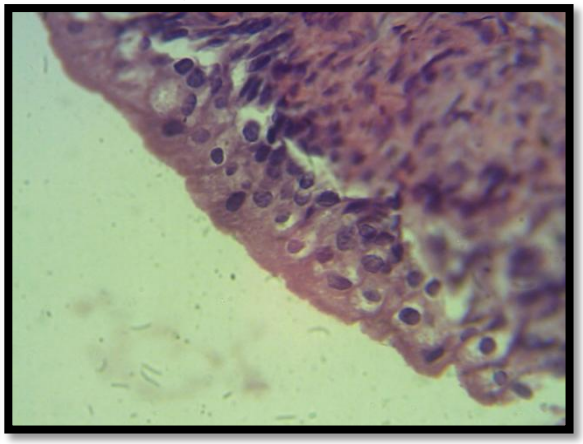


Figure (7): Histological section in urinary bladder of normal animal (G2) showed normal structure of urin bladder (H&E stain 40X).



This pathological finding, which confirmed the bacterial isolation, characterized by marked suppurative reaction, necrosis and congestion of blood vessels. These results are in acceptance with those previously reported by several investigators. Radostits et al., (2007) reported that typical pathological finding when induce chronic infection.

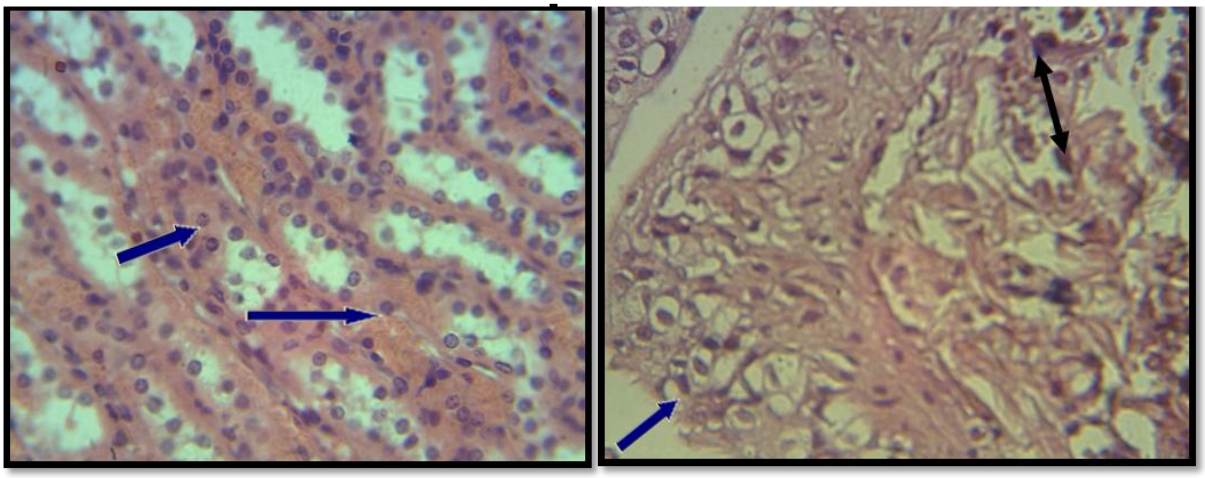
The results showed the development of the bacterial pathogensis which attributed to severity and ability of *E.coli* to grow and multiply and induce inflammation of kidney &bladder, these results came in agreement with Alwan, 1996 who explained that aggregated mononuclear cell indicated immune response the infection characterized by a rapid and massive multiplication of bacteria and a general necrosis of the infected quarter.The ability of *E.coli*to cause disease has been attributed to its ability to produce virulence factorswhich allow them to

colonize and subsequently produce disease these results came in agreement with what was reported by Quinn et al ., 2006 .

Results of group 3 which were treated with X-rayshowedacute cellular degeneration of epithelial lumen cells characterized by vacuolar generation cell which the main lesion in the kidney figure(8),as well as bladder showed congestion blood vessels in submucoa with slight cellular infiltration & vaculation of epithelial laning cell of mucosa figure(9).

Figure (8): Histological section in kidney of one animals at 7 days post infected with E.coli (G3) shows acute cellular degeneration of dillates renal tubular → *(H&Estain40X)

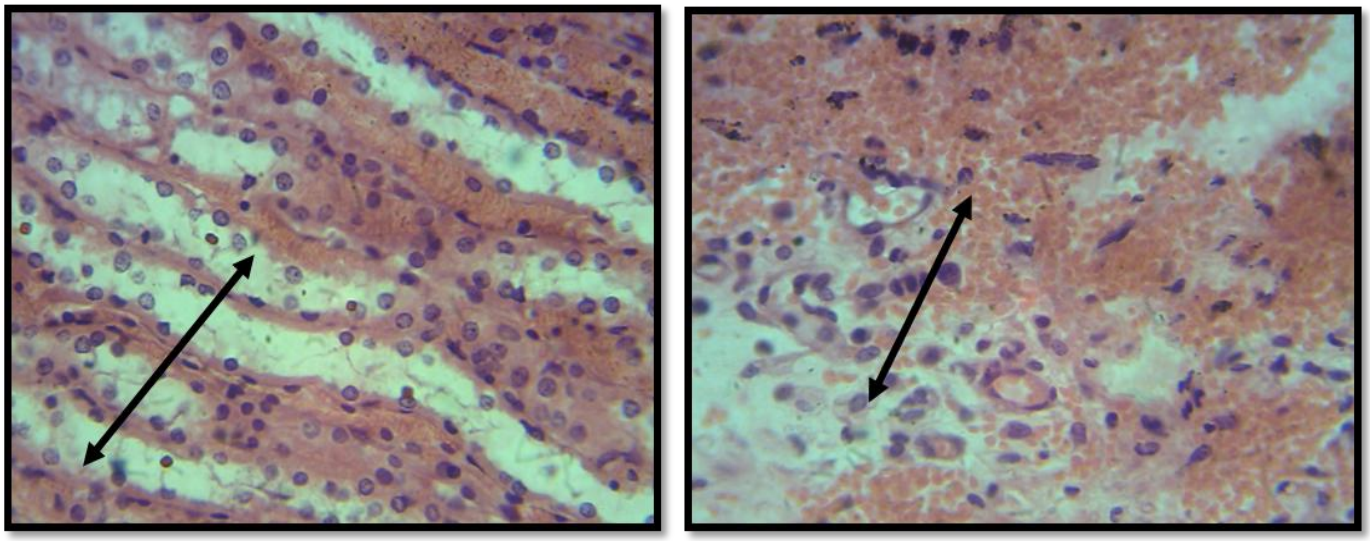
Figure (9) :Histological section in urinary bladder of one animals at 7 days post infected with E. coli(G3) showscongestion blood vessels in submucosawitht slight cellular infiltaration ↔ &vaculation of epithelial laning cell of mucosa → (H&E stain 40 X)



Result of group 4 showed acute cellular degeneration and cystic dilation of renal tubules in kidney figure (10)while bladder showed hemorehagic&nutrophilsinfiltaration in subepithelal layer of mucosa figure (11).

Figure (10): Histological section in kidney infected with E.coli (G4) shows acute cellular degeneration and cystic dilation of renal tubules (H&E stain 40 X)

Figure (11) Histological section in urinary bladder of animal at 7days post infected(G4) ,shows hemorehagic&nutrophilsinfiltaration in subepithelal layer of mucosa (H&E stain 40 X)



The animals of group 3and 4 (infected & treated) for 7 days did not showed complete recovery at the dose (70 and 90 KV) with treatment of x ray and clinical signs were relatively mild.

The result of group5, kidney showed lessnecrosis of epithelial lumen cell of renal tubular &acute cellular degeneration of renal tubular were the main lesion in the kidney figure(12)where similar to their reported in the group 3 but lesspathological lesion ,as well as in bladder shows no clear pathological changes except moderated congestion in the blood vessels figure(13) ,while in group 6 kidney &bladder showed no clear pathological lesion as in figure (14 &15).

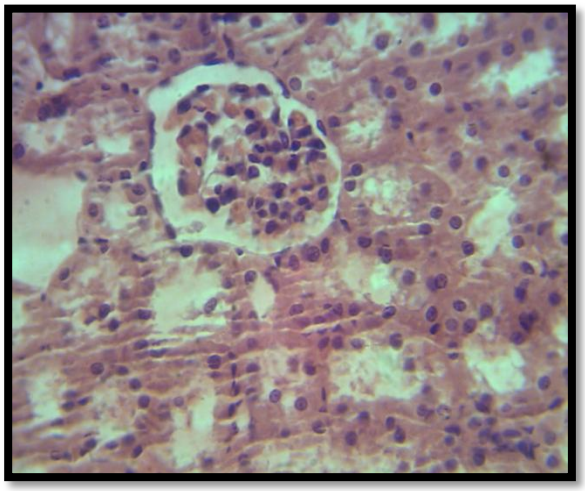
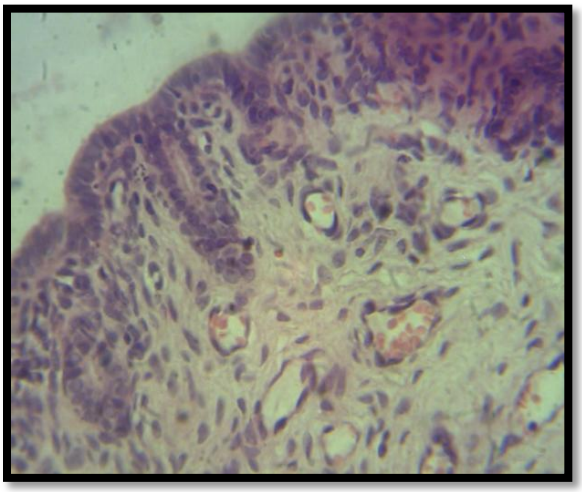


Figure (12)Histological section in kidney (G5) less necrosis of epithelial lumen cell &acute cellular degeneration of of renal tubular are the main lesion in the kidney . (H&E stain 40X).



Figuer(13)Histological section in urinary bladder (G5) shows no clear pathological changes except moderatedcongestion in the blood vessels (H&E40).

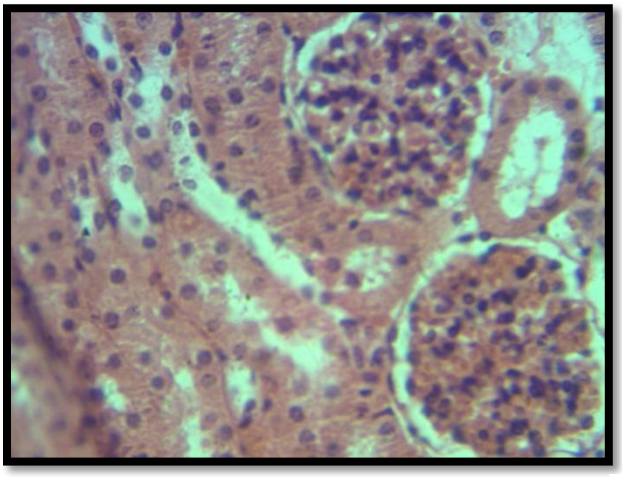


Figure (14)Histological section in kidney (G6), showes no clear pathogenic lesion (H&E40X).

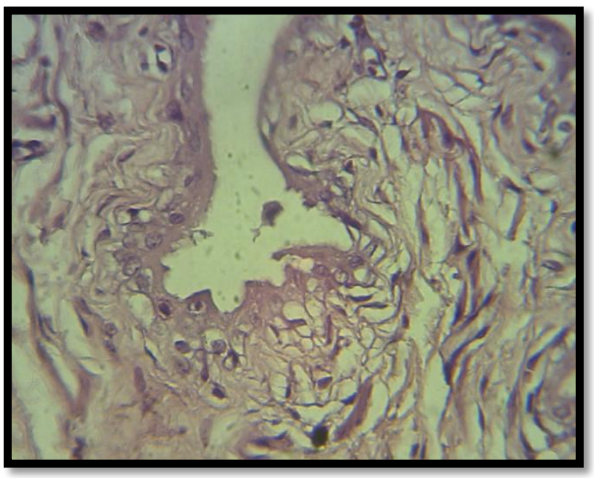


Figure (15)Histological section in urinary bladder (G6) , showes no clear pathogenic lesion (H&E40X).

The results of histopathological changes were not showed any clear pathological changes in kidney and bladder in the group 6 which treated with high dose of x-ray, this refer to the role of this x-ray in killing of bacterial cells and repaired of tissue becauseX-ray irradiation is an effectivetreatment and has successfully been used to sterilize several food products .(Mahmoud 2009a,b; Mahmoud and Burrage 2009),and these results came in agreement with that mentioned by(Mahmoud and Burrage 2009).Treatment with X-ray significantly reduced the population of the tested pathogens on kidney &bladder, compared with the control negative.

Absorption of UV photon energy causes photochemical damage in microorganisms by formation of lesions, in particular through dimerization of adjacent pyrimidine nucleotides. Accumulated lesions may overwhelm the cellular capacity for repair,induce mutations, inhibit replication and thus finally kill the organism (Friedberg et al. 1995**).**

X-ray radiation, which could break the DNA backbone, causing physical breaks and mutations.
In addition to the generation of mutations through direct breakage of the DNA backbone, x- ray irradiation of biological material is known to result in the formation of reactive radicals, which in turn damage DNA and other cellular macromolecules, resulting in cell death(Jay Krishnan et al ,.2010**).**

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