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Study the therapeutic role of Alcoholic Extract of *Plantago lanceolata* aganist infection with *Staphylococcus saprophyticus*

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Summary

The present study was carried out to investigate the antibacterial activity of alcoholic extracts of *Plantago lanceolata* leafes *in vitro* and *in vivo* by inducing urinary tract infection in rats which caused by urethra administration of S.*saprophyticus* isolated from human and animals(cow and sheep)

These extracts showed significant effect ($P<0.05$) on the inhibition of the growth of *S.saprophyticus in vitro* with the superiority of the concentration 200mg / ml of alcoholic extract with the mean of inhibition zone diameter 30 mm against *S.saprophyticus* ,while zone diameter was (26.5 ,21) mm due to the concentration 150, 100mg/ml respectively.

This study included the therapeutic role of doses 150 mg/kg . B.W. of 1.5ml daily orally of alcoholic extract dissolved in DMSO of *plantago lanceolata* leaves in the pathogenesis of *S.saprophyticus* in rats by the urethral infection in compared with the control group (rats injected with *S.saprophyticus* without treatments).The results of histopathological changes showed the role of *Plantago lanceolata* extract on the decreasing of pathological sings in bladder and kidney tissue after 14 and 21 days and gave negative results by decrease congestion in the blood vessels of kidney hemorrhage and few infiltration of inflammatory cells in bladder , in compared with the positive control which showed acute histopathological change.

دراسة الدور العلاجي للمستخلص الكحولي للسان الحمل السناني ضد الاصابه بجرثومة المكورات العنقودية الرمية

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الخلاصة

اجريت هذه الدراسة لتحديد فعالية المستخلص الكحولي لاوراق نبات لسان الحمل السناني في الزجاج وفي الجسم الحي ضد البكتريا العنقوديه الرميه (*S.saprophyticus*) المعزوله من حالات التهاب المجاري البولية UTI في الانسان والحيوان(ابقار واغنام) .وقد اظهرت النتائج ان لهذا المستخلص تاثيرا تثبيطيا معنويا في نمو عدد من عزلات البكتريا العنقوديه الرميه في الزجاج ,اذ اظهرالتركيز 200ملغم /مل لمستخلص أوراق لسان الحمل السناني الاكثر تثبيطا لنمو هذه الجرثومة خارج الجسم الحي وبمعدل قطر تثبيط30ملم , في حين كان معدل قطر التثبيط بتاثيرهذا المستخلص عند التركيز 150,100ملغم /مل هو26.5,21ملم على التوالي.

اختبر التاثير العلاجي بالتجريع الفموي بمقدار.1مل وبجرعة يومية واحدة وبتركيز 150ملغم / كغم من وزن الجسم للمستخلص الكحولي المذاب بالدمسو DMSO لاوراق لسان الحمل السناني في امراضية البكتريا العنقوديه الرميه داخل الجسم الحي بدات المعالجة بعد مرور48 ساعه من حقن جرثومة الاختبار من ظهور الاعراض المرضية واستغرقت مدة العلاج 14 و21 يوم (وبجرعة 240 وحدة تكوين المستعمرة $cfu.ml^{1-}$ عن طريق الاحليل) مع استخدام مجموعة سيطرة موجبة.

أظهرت نتائج التقطيع النسيجي بعد انتهاء مدة العلاج تفوق مستخلص اوراق لسان الحمل السناني الكحولي المذاب بالدمسو لمدة 21 يوما ثم المستخلص الكحولي لمدة 14 يوم في الحد من ظهور العلامات المرضية النسيجية بدأ بقله احتقان الاوعية الدموية للكليه وارتشاح طفيف للخلايا الالتهابية وقلة انسلاخات الخلايا الظهارية في الاحليل وشفيت تماما في اليوم 21من العلاج.

Introduction

Many researchers preferring use of plant extract instead of antibiotics was due to attenmuation of pathogens virulence by plant extract as opposed to the direct killing of pathogenic bacteria with antibiotic as a strategy to combat infections is an interesting concept, the idea that anti-pathogenic molecules that prevents for instance the production of toxins or

abolish the ability of bacteria to adapt to the host environment would give a competitive advantage to the host immune system to allow clearance of the infectious organism (1) .

The uses of *Plantago lanceolata* orally to treat digestive and bronchial disorders and topically to treat skin disorders and eye infections are very widespread ,also it is used for sinus congestion, allergies, lung congestion, colitis, excess of production of mucus, diarrhea and dysentery, cystitis, nephritis and other infections (2) .

The Gram-positive bacterium *Staphylococcus saprophyticus* can cause up to 5 to 15% of uncomplicated UTI (3).

S. saprophyticus has also been isolated from 7% of rectal swabs taken from carcasses of cattle and pigs. The microorganism is a common contaminant of various food samples, especially of raw beef and pork. (4)

The virulence factors of *S. saprophyticus* include adherence to urothelial cells by means of a surface-associated protein, lipoteichoic acid; a hemagglutinin that binds to fibronectin, a hemolysin; and production of extracellular slime (5).

This study was aimed to study the therapeutic role of *Plantago lanceolata* (alcoholic extrat) against infection with *S.saprophyticus*

Materials and Methods

Clinical isolates of pathogenic *S.*saprophyticus were obtained from UTI patients and animals (cows and sheep) in Baghdad city. Diagnosis of all these isolates (from human and animals) were depended on the cultural, macroscopical examination and biochemical tests, then the diagnosis was confirmed by using API Staph system.

Organic solvent extraction of the *Plantago lanceolata* was carried out by using ethanol (95% ethyl alcohol). This was done by using Soxhlet apparatu. (6)

Agar-well diffusion method was used to check the activity of plant extract *in vitro* (7). To achieve this purpose, for *S.saprophyticus* pure colonies were selected.

Different concentrations of plant extract (100,150,200 mg /ml) were poured in the wells; other two well were filled with 0.1 ml of DMSO and with D.W as a control.

Stock solutions were prepared by mixing 1.5g from extract with 10 ml, of DMSO it was filtered through whatman (No.1).to prepare the concentrations of 150 mg/ml. This concentration was used for daily dosing of treated groups.

Twenty Four rats (190-200 B.W and 2-3mouth) were divided equally into four groups, six rats in each group (treatment begin after 48 hrs. after inducing infection).

Group (1): control negative (not infected group which given only DMSO orally for 14 and 21 days)

Group (2): control postive (infected and not treated group).

Group (3): infected and treated orally with 150mg/kg B.W of alcoholic extract of *Plantago lanceolata* for 14days.

Group (4): infected and treated orally with 150mg/kg B.W of alcoholic extract of *Plantago lanceolata* for 21days.

Two parts of each rat (kidney and Bladder) put in 10% neutral formalin solution till further study histopath.

Results

Staphylococcus saprophyticus was detected by morphological and biochemical tests listed in the following table (1):

Table (1): Morphological and biochemical tests of *S.saprophyticus*

Bacteria	Morphological examination		Biochemical tests	
S. saprophyticus	Gram stain	+	Urease	+
	Blood agar	Non hemolysis	Motility test	-
	MacConkey agar culture	No groth	Catalase test	+
	Mantol Salt agar culture	clear colonies (ferment manitol)		

Results of the phytochemical screening of *Plantago lanceolata* leaves extract showed positive result for phenol, coumarins, steroids, terpenoids, resins, saponins, flavonoids, tannins and glycosides, and absence of alkaloids.

The results of inhibition zone diameter for alcoholic extract against *S.saprophyticus* were (20,26.5,30) mm, due to the three concentration of extract 100 ,150 ,200 mg/ml respectively as in Figure (1) and Table (2).

Table (2): In-vitro antibacterial activity of *P.lanceolata* extract in different concentrations on *S.saprophyticus* growths (diameter of inhibition zone in mm.)

Concentration mg/ml of *P. lanceolata* L.	***S.saprophyticus* (inhibition zone-mm) (Mean ±SE)**
100	20.0±0.58 c
150	26 .5 ±0.34 **b**
200	30 ±0.29 **a**
90% DMSO	0.00±0.00

Values represent mean ±S.E

Different small letters mean significant (P<0.05) results between different concentrations.

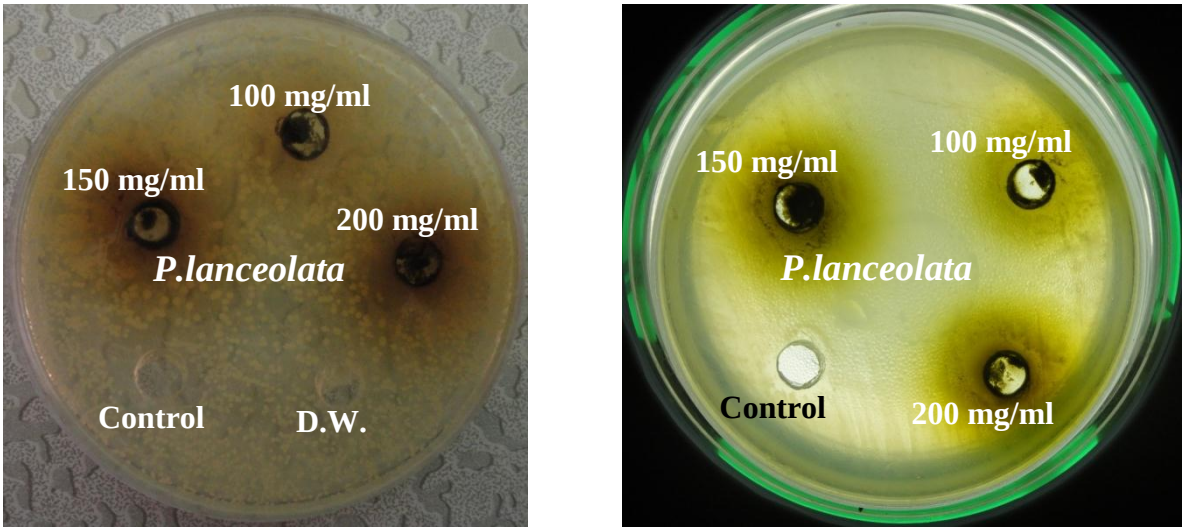


Figure (1): Sensitivity of *S.saprophyticus* to alcoholic extract of *P.lanceolata* (mg/ml).

The histopathological changes in bladder tissue after2 days from challenged by virulent *S.saprophyticus* isolate in positive control group without treatment were showed lesion represented by inflammatory cells infiltration in subepithelial layer, odema and congestion blood vessels with inflammatory cells by particularly neutrophils attachment to the endothelial cell Figure (2),

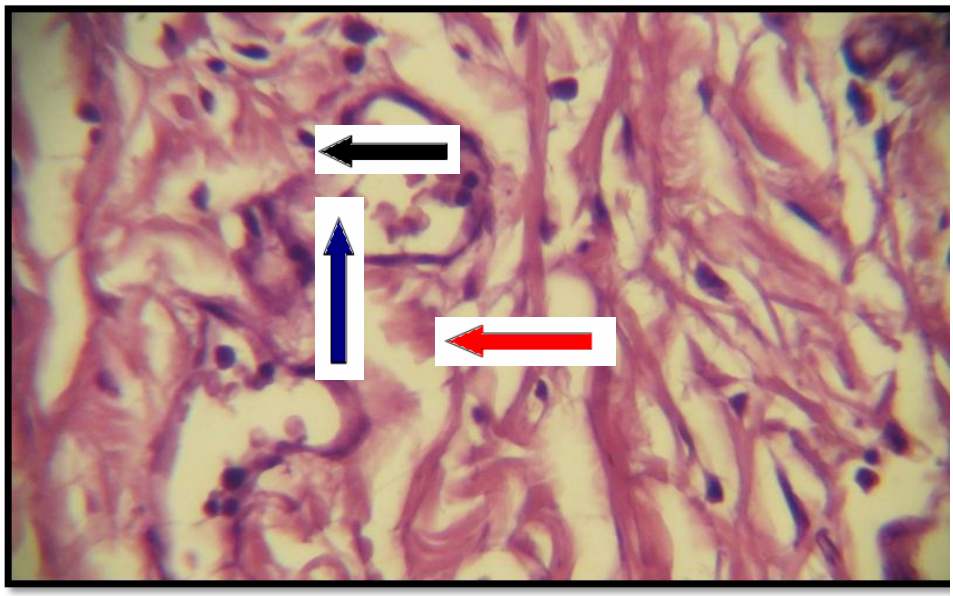


Figure (2). Histological section in urinary bladder of one animals at 2 days post infected with *S.saprophyticus* shows congestion blood vessels → &inflammatory cell in their lumen (neutrophils) → ,& odema with mononuclear cell in subepithelial layer → *(H&E stain 40X).

in kidney acute cellular degeneration of epithelial lumen cells characterized by enlargement and vacuolar generation of epithelial cell which lead to close renal tubules figure (3),as compared with the negative control group which showed normal structure of kidney and bladder Figure (4), (5).

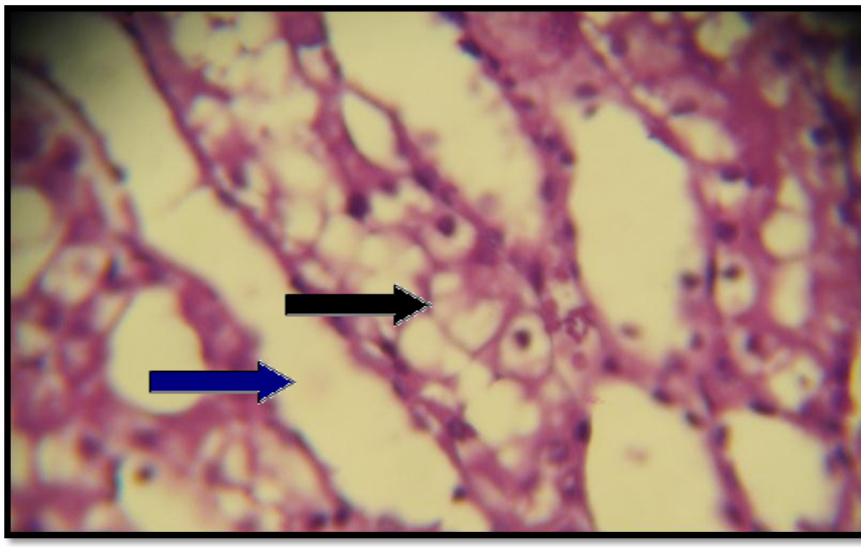


Figure (3): Histological section in kidney at 2 days post infected with S.saprophyticus shows dilatation of renal tubules with vacuolation → & enlargement of the cell lying renal tubules → (H&E stain 40X).

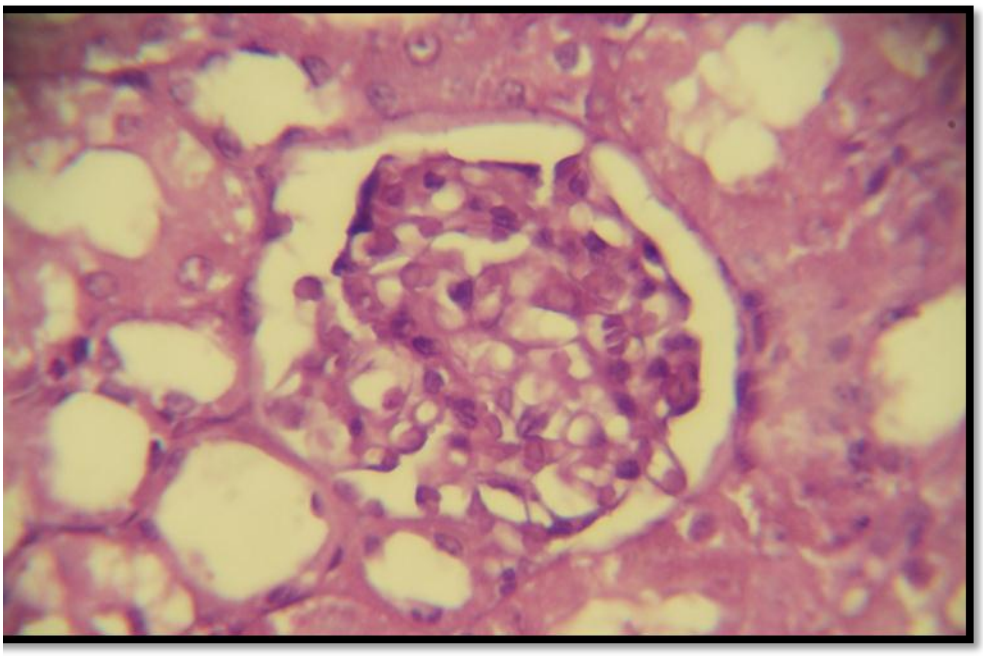


Figure (4): Histological section in normal animal showed normal structure of kidney (E&H Stain 40X)

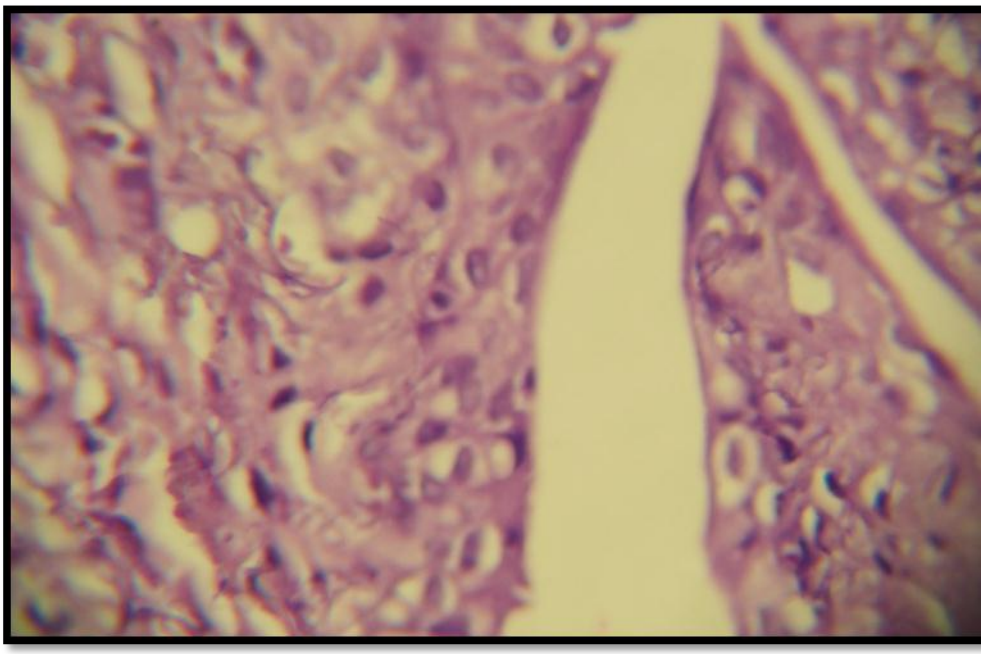


Figure (5): Hisological section in urinary bladder of normal animal showed normal structure of urin bladder (H&E stain 40X).

after 14 days from treatment showed a few infiltration of mononuclear cells around and intra-acini lumen & moderated hyperplasia of smooth muscle in kidney (figure 6),

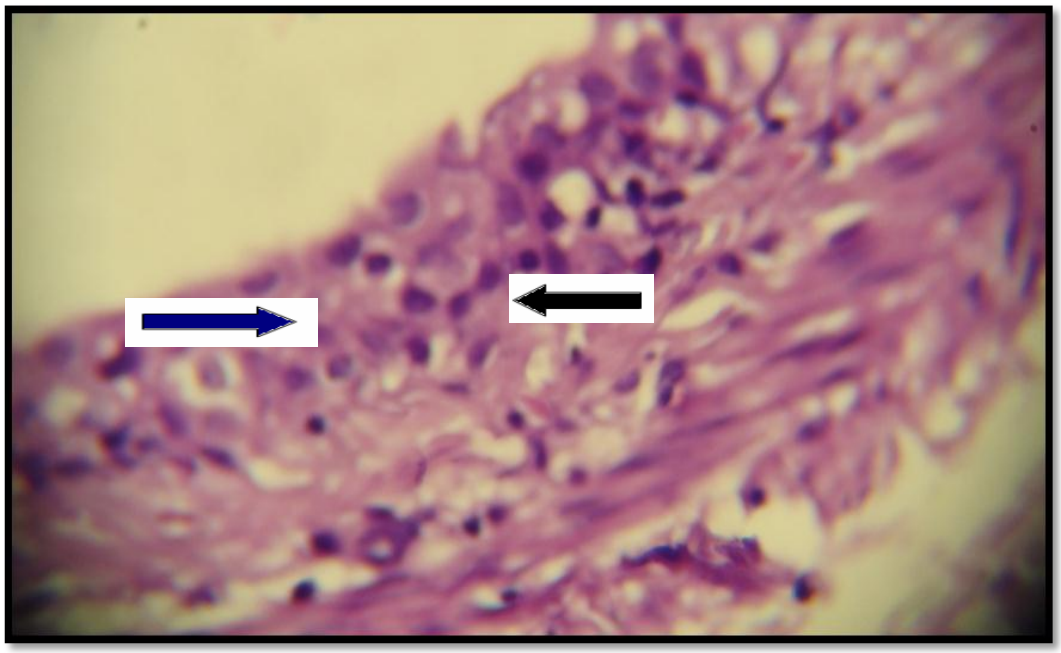


Figure (6) : Histological section in kideny in animal infected with S.*saprophyticus* at 14 days and treatment with alcoholic extract *Plantago lanceolata* showed hyperplasia of mucosal bapal layer (→)&mononucler cell inflteration in subepitheial layer(➡) (H&E40X)

, but in other cases no clear pathological lesion where reported in bladder (figure 7).

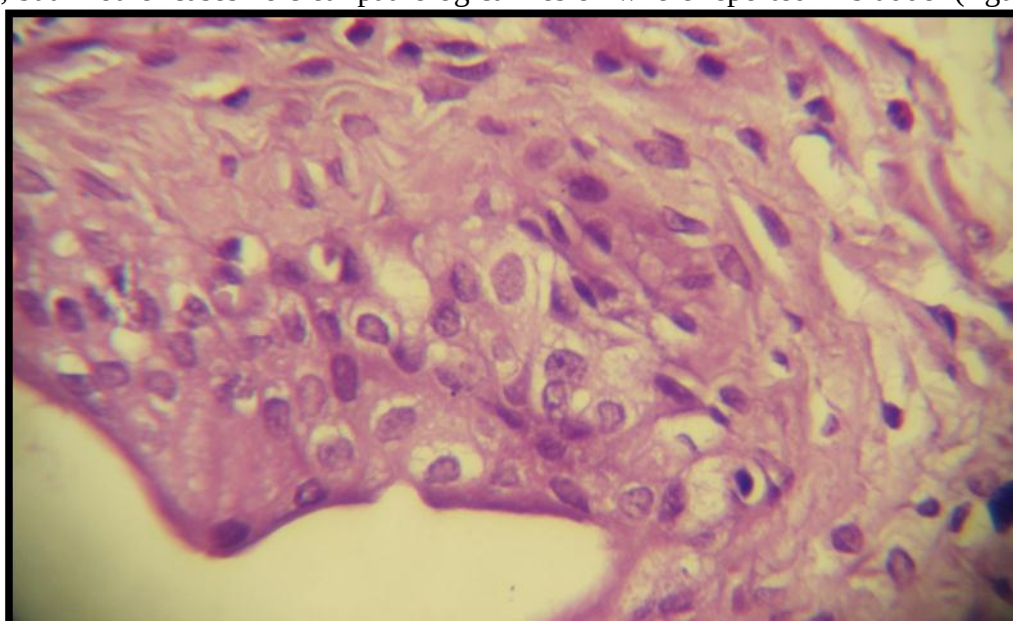
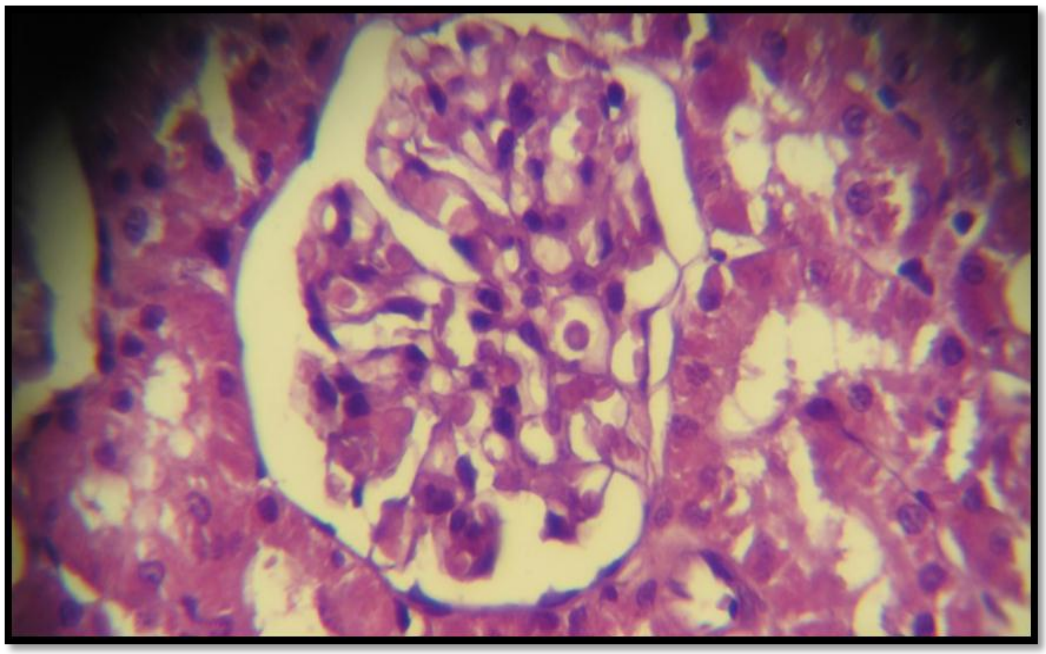


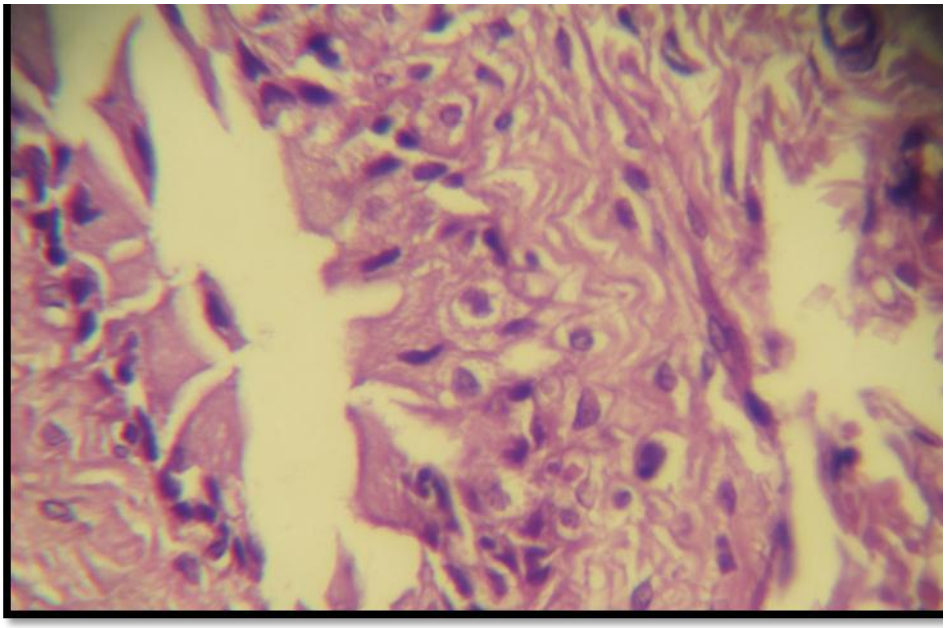
Figure (7)Histological section in urinary bladder at 14 days of treatment of alcoholic extract of Plantago lanceolata , shows no clear pathological lesions (H&E40X).

Group four which treated with alcoholic extract of Plantago lanceolata leaves (after 21 days from treatment) showed no clear pathological lesion in kidney (figure 8).



Figuer (8): Histological section in kidney at 21 days from treatment with acolholic extract of Plantago lanceolata showes no clear pathogenic lesion (E&H40X)

As well as in bladder no clear pathological lesion except a few monocytes cell infiltration in subepithelial layer (Figure 9) .



Figuer(9)Histological section in urinary bladder at 21 days from treatment with alcoholic extract of Plantago lanceolata shows no clear pathological changes except slight infiltration of mononuclear cell in subepithial layer (H&E40X)

Discussion

The results showed the superiority of the concentration 200mg /ml in all plant extracts and this may be due to the solubility of high amount of active ingredient which inhibited the bacterial growth, these results come in agreement with that mentioned by (8)

(9) who found *Plantago lanceolata* have good cure because of its contain acidic phosphatase and naphthol phosphatydrolase and speeds up tissue healing besides the effects of mucilage, vitamin C and zinc.

In addition to the active molecules ,acidity of alcoholic extract play as antibacterial substance for the growth of bacteria due to the free hydrogen ions which band to the molecules and change the microbial environment (10)

The current study agrees with (11) who found *S.saprophyticus* causes odema and degeneration in thier epithelial lumens cell and hyperplasia of epithelial lumen cell in bladder.The results of histopathological changes were not showed any clear pathological changes in kidney and bladder in the group which treated with alcohol extract of *Plantago lanceolata* leaves dissolved in DIMSO (treated for 21 days), this refers to the role of this extract in killing of bacterial cells and repaired of tissue because this extract contain active ingredient which may act as antibacterial agent such as tannins and these results came in agreement with that mentioned by (12), the tannins play a role in healing tissue and form protective layer over the exposed tissue (13).

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