Journal of Kerbala University , Vol. 10 No.2 Scientific . 2012

Studying of antibacterial effect for leaves extract of *Callistemon viminalis* in vitro and vivo (urinary system) for rabbits

دراسة تأثير المستخلص المائي والكحولي لأوراق نبات فرشة البطل مختبرياً و حيوياً كمضاد لبعض جراثيم الجهاز البولي في الارانب

Afrah. J. Abd
Biological department /Sciences of collage / Al-Muthanna university

Abstract

This study was designed to evaluate the effects the watery and alcoholic extracts from leaves of *Callistemon viminalis* in vitro to ten of pathogenic bacteria and study the alcoholic extract toward *Streptococcus pneumonia* on urinary system in vivo. The results showed the watery and alcoholic extracts from leaves of *Callistemon viminalis* have antibacterial activity against both Gram positive (*Staphylococcus aureous – Streptococcus pneumonia – Staphylococcus epidermidis*) And Gram negative bacteria (*Klebsilla pneumonia – Klebsilla oxytaci –Proteus vulgaricus - Escherichia coli)* however the watery extract of *Callistemon viminalis* were more potent than alcoholic extract against pathogenic bacteria (80%) . According to results , *Streptococcus pneumonia* pathogen that more sensitive to ward *Callistemon viminalis* alcoholic extract was choosing to injected intraperitonialy as experimental infection in laboratory animals (vivo) which cause morphological and histopathological degenerative lesion of kidney cortex and medella tissue in addittion to change of renal profile test that include blood urea nitrogen, creatinine, creatinine kinase, uric acid, inaddition to calcium. but after alcoholic extract of *Callistemon viminalis* injected in these labratory animals cause significant improvment ($p \leq 0.01$) in the value of blood urea nitrogen, creatinine, creatinine kinase and uric acid. Calcium concentration has small improvement but don't reach significant degree, histopathological studies confirm these results which include regeneration of degenerative lesion for medulla and kidney cortex with convoluted tubules tissue

الخلاصة

صممت هذه الدراسة لتقيم تأثير المستخلصات الكحولية والمائية لاوراق نبات فرشة البطل المزروعة قرب جامعة المثنى في موسم الصيف مختبريا على عشرة جراثيم مرضية ومن ثم دراسة تاثير المستخلص الكحولي على جرثومة *Streptococcus pneumonia* حيويا على الجهاز البولي في الارانب.

اظهرت النتائج الفعالية العالية للمستخلصات المائية و الكحولية لنبات فرشة البطل ضد مجموعة من الجراثيم الموجبة لصبغة كرام متمثلة بـ*Staphylococcus aureous , Streptococcus pneumonia Staphylococcus epidermidis* وبعض الجراثيم السالبة لصبغة كرام ومتمثلة بـ *Klebsilla pneumonia , Klebsilla oxytaci ,Proteus vulgaricus , Escherichia coli* , وكان المستخلص المائي اكثر فعالية على الجراثيم وبنسبة 80% . بالاعتماد على النتائج كانت جرثومة *Streptococcus pneumonia* اكثر حساسية تجاه المستخلص الكحولي لذا استخدمت لاحداث اصابة تجريبية على بعض الارانب المختبرية والتي تسببت بإحداث آفات مظهرية و نسيجية كاحداث تلف في قشرة ولب الكلية اضافة الى احداث تغيرات في الدلائل الحيوية للجهاز البولي والتي شملت تركيز اليوريا في المصل والكرياتنين والكرياتنين كاينيز وحامض اليوريك اضافة الى تركيز الكاليسيوم , بعد حقن الارانب المصابة بالمستخلص الكحولي للنبات ادى الى تحسن معنوي ($p \leq 0.01$) في مستوى تركيز اليوريا في المصل والكرياتنين والكرياتنين كاينيز وحامض اليوريك اما الكاليسيوم وعلى الرغم من وجود تحسن بسيط الا انه لم يصل الى مستوى المعنوية اضافة الى اصلاح الانسجة التالفة في منطقة القشرة واللب والانابيب الكلوية واعادة التنسج من جديد.

Introduction

Callistemon viminalis has a rich history in the use of diverse medicinal flora for traditional healing. These medicinal plants are generally used to treat various medical conditions including skin infections, stomachaches and respiratory conditions [1].

Scientific research on the antimicrobial efficacy of many of the plants used for medicinal purposes in Jamaica is lacking, the presence of active antimicrobial compounds in plants represents a useful area for development of natural products that can be used as substitutes for antibiotics resistant to pathogenic bacteria, further more, they provide the foundation for the development of new antimicrobials[2]. the study will also confirm if there is a biological basis to the claim that the ethno medicinal plant has useful medicinal purposes .[3]

In recently research isolated compounds from the *Callistemon viminalis* plant, such as Sequiterpene Lactons found have effective action against microorganisms:-*Saccharomyces cervisiae* - *Bacillus subtilis Stapylloccus aureus Escherichia coli* . [4], while the flavonoid compo*unds* have the ability to inhibit the platelets aggregation (Anti thrombotic effect) and encourages the dilation of blood vessels [5]. terpenoid compounds also that present in *Callistemon viminalis* characterized by sharp taste, anti-microorganisms, the food conserved, analgesic for pain and tonics .[6].

Callitemon veminalis also contracts the soft tissues of the body, preventing the secretion or excretion of fluids, particularly of the genito-urinary tract. Containing a large concentration of silica, it can help to both reduce hemorrhaging and heal the related wound. These actions also lead to a common use of bottle brush with children to alleviate urinary incontinence and bed wetting[7]. Adult men may also use it to reduce a benign enlargement or inflammation of the prostate. *Callitemon veminalis* has a hemostatic property related to its astringent function in that it can halt the flow of internal bleeding, such as from ulcers, by constricting blood vessels. In continuous with it's diuretic properties make it helpful in relieving water retention and general problems of the urinary tract. It is often used by women as a douche to cleanse the genito-urinary tract from excessive menstruation or a mucosal discharge known as leukorrhea[8] .

This study was used to investigate the effect for Callistemon veminalis on G+ve and G-ve bacteria in addition to investigate therapeutic effect of this plant on urinary system integrity of rabbits.

Materials & Method

Part I

Collection of *Callistemon viminalis* leaves plant

Plant material, leaves of *Callistemon viminalis*, was obtained from the house garden from the period 15 December 2010 to 15 April-2011 after cleaning the leaves from the dust; they put in oven to dry then crushed to produce powdered material .

Preparation of plant extracts

1- watery extract

50 g of powdered plant was taken and added to 500 ml of distilled water then placed in a water bath has $45C^{O}$ for four hours and shake well, the suspension was filtered with a piece of cloth (muslin), and then left to dry on sterile crucible, later the solid layer of the dishes was eliminated using sharp material and convert it to powder for preparation different concentration [9].

2- preperation of cold alcoholic extract

The extraction was applied as in [10] method, about 500 ml of ethanol alcohol in concentration of 80% was added to 50gm of *Callistemon viminalis* plant powder ,the mixture was placed in closed bottle , after 24 hours the bottle content was filtered by a piece of cloth (muslin), the filtered material left to dry and convert it to powder for preparation different concentration 50-75-100 %.[11].

Preparation of bacterial suspension

Special bacterial suspension of dignosed isolets from postgraduate students of science collage-Al muthana university *Pseudomonas aeroginosa , Pseudomonas pseudomelli , Staphyllococcus aureus , Klebsilla pneumonia , Klebsilla oxytaci , Proteus vulgaricus , Streptococcus pneumonia , Staphyllococcus epidermidis , Escherichia coli , Enterobacter* were prepared on Muller Hinton Broth and incubated at 37 C^O for 24 hours, Then 1 micron was taken from each bacterial suspension and diffused on Muller Hinton agar by using L-shape spreader ,the plates were left for about 5-10 minutes to permit the suspension for drying on the agar . Then 3 equal distant wholes were mode inside the plates for putting different plant extract concentration 50,75 and 100 % Plates were incubated at 37C^O for 24 hours, the effect of plant extract on bacteria was calculated by minimum bactericidal concentration (MBC) around the different concentration wholes [12].

Part ɪɪ

1- Animals

Clinically healthy six month old about1.5 kilo female white newzealand rabbits were used in the experiment (rabbits are divided to tow groups control group administrated food and tap water and injected with 10^{-3} *streptococcus pneumonia* suspension (as this bacteria consider the two more susceptible to this plant extract) intraperitonially and treatment group T2 administrated food and tap water and injected with 10^{-3} *Streptococcus pneumonia* suspension intraperitonially to induce respiratory pneumonia then injected with alcoholic extract of *Callistemon viminalis* after 36 hrs of respiratory signs presentation, plant extract was administrated intraperitonially in the form of two dose 500 mg daily for three weeks.

2- Sample collection

At 10 weeks whole blood was collected via cardiac puncture from anasthesized (ketamine 50mg/kg-xylazine 10mg/kg) in EDTA tubes. Rabbits, then euthanized with a single cardiac injection fatal plus (concentrated pentobarbital, 360 mg/kg), blood was collected in EDTA tube, kidney tissues was collected for histological studies.

Blood chemistries

Blood urea nitrogen, creatinine, creatinine kinase, uric acid and calcium concentration in plasma were determined using commercially available kit (sigma)

Statistical analysis

Mean±SE was used to describe variables. All data are analyzed using ANOVA table and Duncan's multiple range test was used to determine if the means were significantly ($P \leq 0.01$) different or not [13] .

Results

The results illustrated indicated that the two crude extracts from the leaves of *Callistemon viminalis* showed antibacterial activity against both Gram positive (*staphylococcus aureas – streptococcus pneumonia – staphylococcus epidermidis)and* Gram negative bacteria (kl*ebsilla pneumonia – klebsilla oxytaci –proteus vulgaricus - Escherichia coli)* however the crude extract of *Callistemon viminalis* were more potent antibacterial (agent against Gram positive bacteria than Gram negative results related with *pseudomonas pseudomelli* appear more resistant for all watery extract and all alcoholic extract for *Callistemon viminalis* leaves , these results did not occupied with [9], while results related with *Klebsilla pneumonia* appear high sensitivity for 50-75% concentration of watery extract about2.2cm , 2cm respectively , while watery extract 100% concentration and alcoholic extract 50% concentration appear intermediate result MBC reach about 0.8cm for each concentration alcoholic concentration 100% for *Callistemon viminalis* leaves , extract appear more resistant MBC reach about 0.6cm .results related with *Klebsilla oxytaci* bacteria illustrate more resistant for 50 %concentration Of watery extract about 0.6cm but appear more sensitive for 100 % concentration of watery extract and 50%-75%- 100% concentrations of alcoholic extract for *Callistemon viminalis* leaves , MBC reach about (1.6-1.2,1-2.2) cm

respectively . While these bacteria appear intermediate result for 75% concentration of watery extract concentration MBC reach about 0.8cm. result related with *proteus vulgaricus* bacteria appear intermediate for 75% concentration of watery extract and 100% concentration of alcoholic extract for MBC reach about 0.8cm for each concentration .while this bacteria appear more resistant for 50% concentration of watery extract MBC reach about 0.4 cm , also the same resultS appear with 50-75% concentrations of alcoholic extract for MBC reach about 0.2cm for each concentration but this bacteria illustrate more sensitive for100% concentration of watery extract for MBC reach about1cm. results related with *staphylococcus aureus* appear intermediate for50-75% concentration of watery extract MBC reach about 0.8cm for each concentration , but this bacteria appear more sensitive for 100% concentration of watery extract and all alcoholic extract concentration 50%-75%-100% which MBC reach about (1.8-2.1) cm respectively .results related *staphylococcus epidermidis* appear intermediate for 75% concentration of watery extract and reach MBC about 0.8cm but this bacteria appear more sensitive for50%-100% concentration of watery extract and MBC reach about 1.6cm for each concentration , the same result appear with all alcoholic extract 50%-75%-100% concentration and reach MBC about (2.1-2.3) cm respectively ,Results related with *Escherichia coli* bacteria appear high sensitivity for all watery extract 50%-75%-100% concentration and MBC reach about(1.4-2.1.4) cm respectively and also results indicate more sensitivity for all coholic extract concentration 50,75,100% concentration and MBC reach about (1.1-2.1.6) cm respectively . results related with *Enterobacter* appear high sensitivity for all watery and alcoholic concentration and MBC reach about (0.6-1-1.2) cm for 50,75,100 % concentration of watery extract respectively and (1.6-1,4-2)cm for 50,75,100% concentration of alcoholic extract of *Callistemon viminalis* leaves .finally results related with *streptococcus pneumonia* appear intermediate for 50% concentration of watery extract and MBC reach about0.8 cm but this bacteria appear high sensitive for 50%-75 % of watery extract and MBC reach about 1-2.2 concentration respectively , the same results appear with all alcoholic extract concentration 50%-75-100% and MBC reach about 2-2-4.4cm respectively. The result above illustrate that *streptococcus pneumonia* appear the most sensitive for100% concentration of alcoholic extract for *Callistemon viminalis* leaves. occupied with ,[14]

Fig 1: is appear sensitivity of *streptococcus pneumonia* toward alcoholic leaf extract. This bacteria was selected to inject intraperitonially then treated with alcoholic extract for *Callistemon viminalis* leaves after 36 hrs from respiration signs presentation.

Results in vivo indicate presence of significant variation ($p \leq 0.01$) in level of blood urea nitrogen, creatinine, creatinine kinase and uric acid for G2 animals, calcium concentration had small improvement but these improvement don't reach to significant variation, as explained in table 1:

Table (1) indicate the effect of *Callistemon viminalis* leaves on renal profile test for infected rabbits with *streptococcus pneumonia*.

Renal profile test	G1 animals	G2 animals
blood urea nitrogen mg/dl	41±0.816 a	27.5±1.5 b
Creatinine mg/dl	0.533±0.007 a	0.346±0.01 b
creatinine kinase u/l	839.7±26.408 a	275±6.191 b
uric acid mg/dl	2.56±0.162 a	2.02±0.038 b
Calcium mg/dl	6.86±0.352 a	7.91±0.41 a

Results are expressed as mean ± standard error.
a: no significant variation
Different letters between groups refer to significant variation under ($p<0.01$).
Degree of freedom :1, 9

These results are supported by histopathological examination

Results indicates presence of inflammatory area (spots or patches) in the cortex and medella of kidney, increase the numbers of cells in the wall of proximal and distal convoluted tubules, with enlargement of the cells in the wall of collecting duct and distal convoluted tubules, hypertrophy of bowman capsule, mononuclear cells present in the interstitial space between renal tubules, damage in cilia for G1 animals as present in fig 1 and fig 2

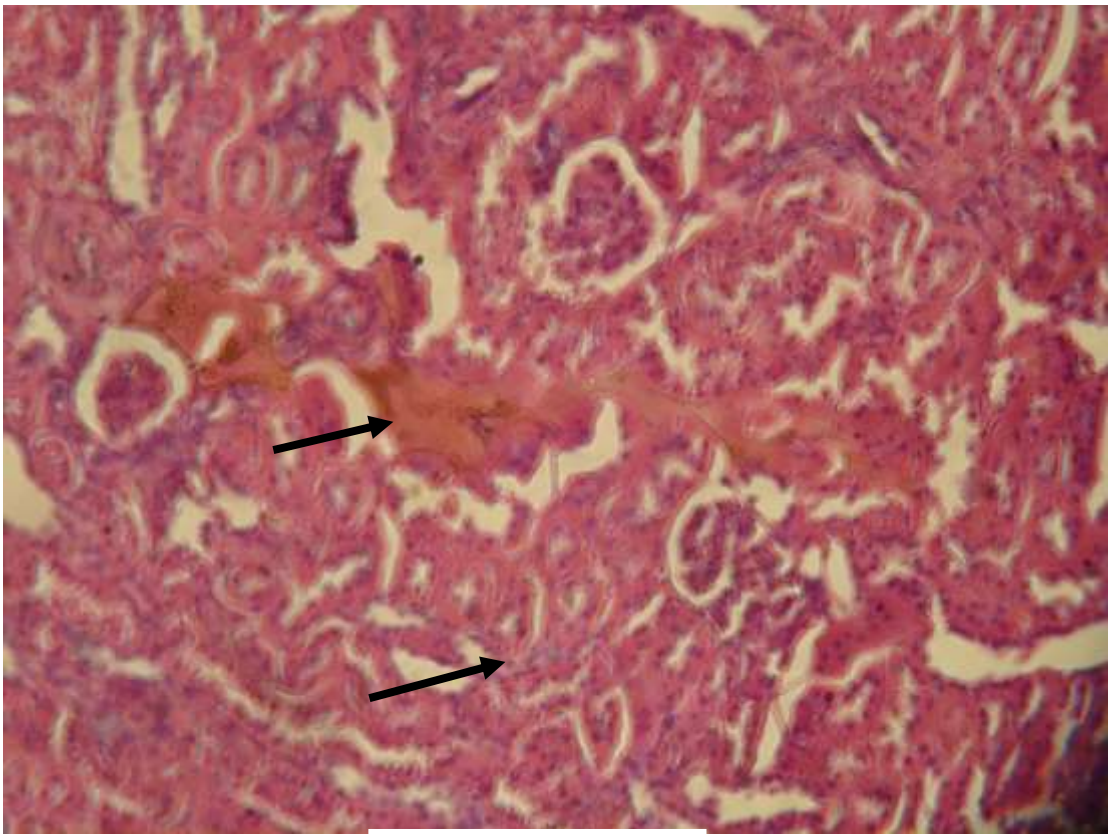

Fig 2 (A)

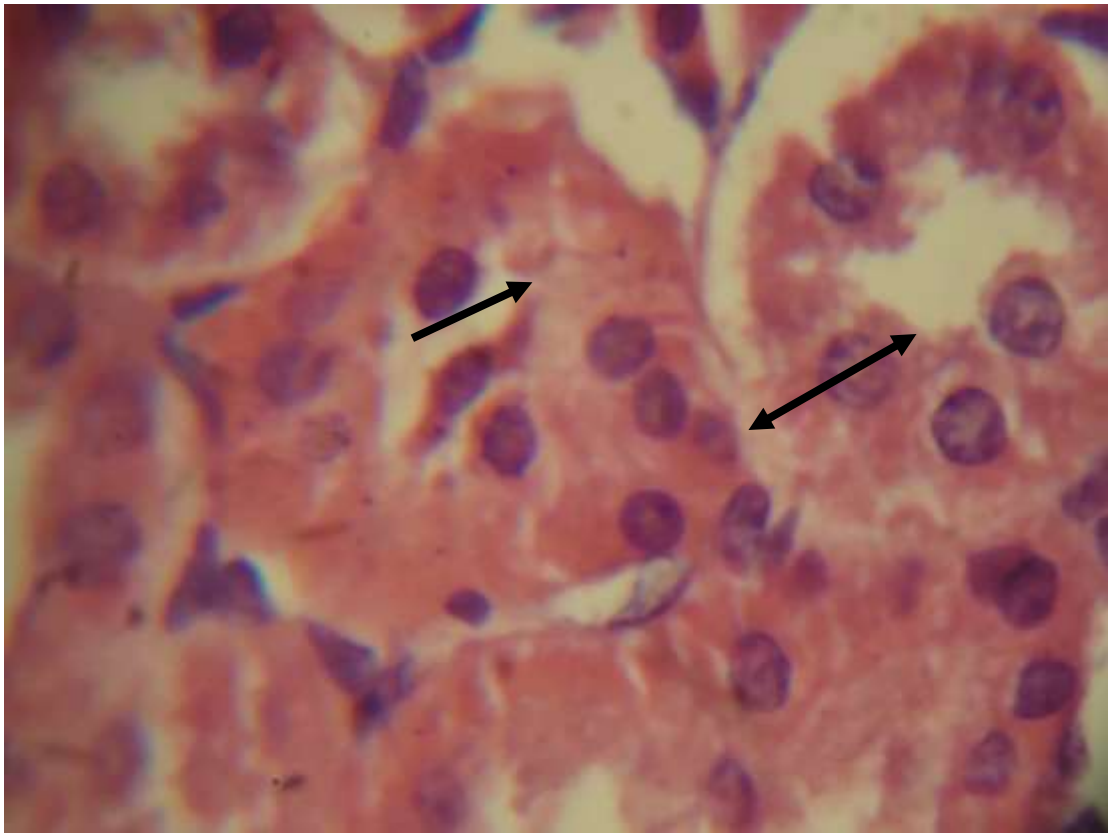

Fig 2 (B)

Photomicrographs of haematoxylin and eosin stained sections of rabbit kidney; (A&B) presence of inflammatory area (spots or patches) in the cortex and medella of kidney, increase in the wall of proximal and distal convoluted tubules, with enlargement of the cells in the wall of collecting duct and distal convoluted tubules, hypertrophy of bowman capsule, presence of mononuclear cells in the interstitial spaces between renal tubules damage in cilia. (A:H&E, 10×, B:H&E, 100×).

Histopathological examination for G2 animals reveal moderate regeneration for cells of collecting ducts, proximal and distal convolted tubules, disappearance of congestion in the cortex of kidney, as present in fig2 A and B

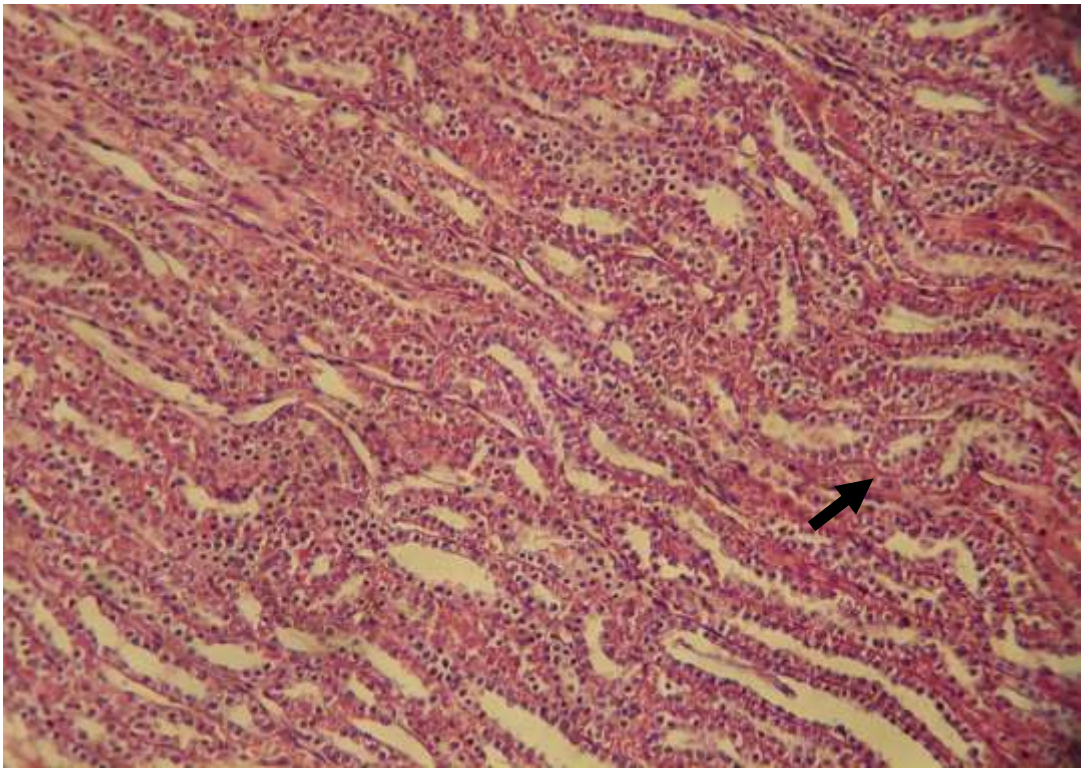

Fig 3(A)

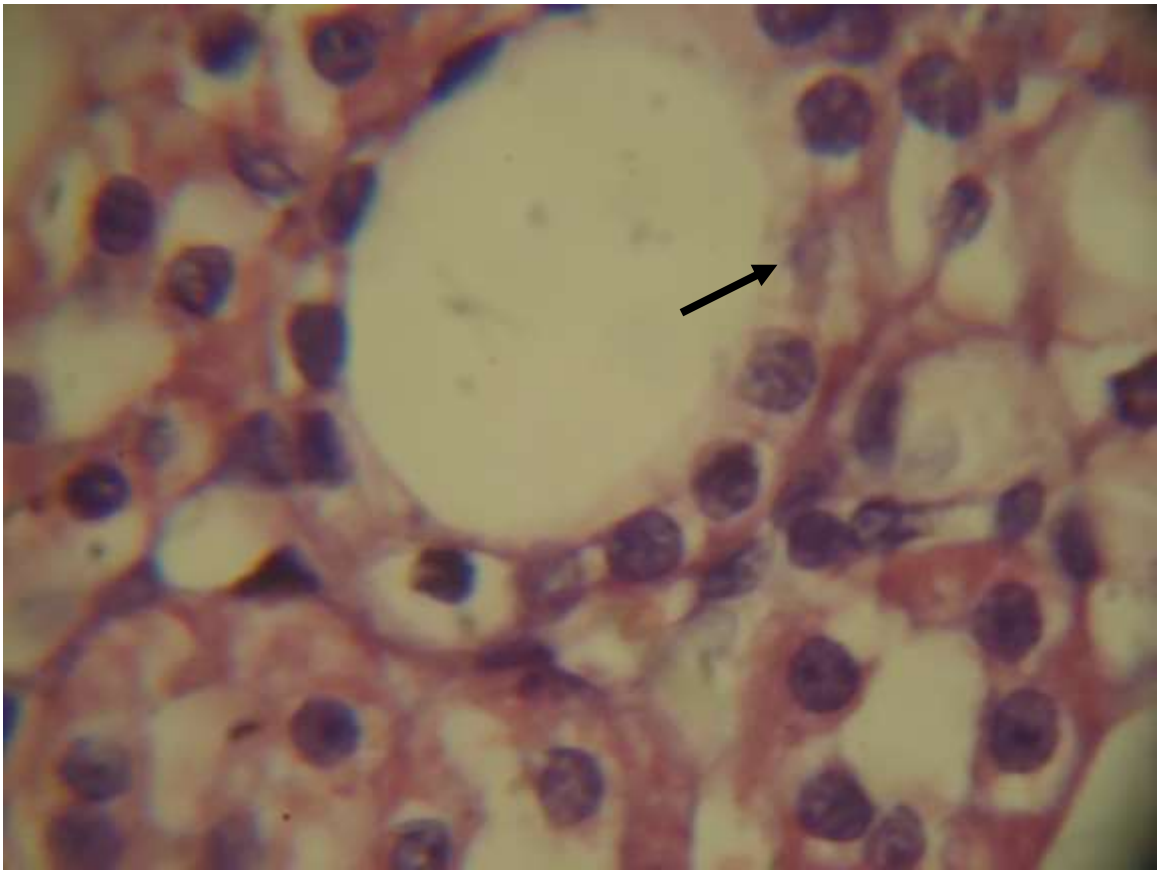

Fig 3 (B)

Photomicrographs of haematoxylin and eosin stained sections of rabbit kidney; (A&B) presence of reveal moderate regeneration for cells of collecting ducts, proximal and distal convolted tubules, disappearance of congestion in the interstitial spaces for cortex of kidney. (A:H&E, 10×, B:H&E, 100×).

Discussion

Anti biotic was medical miracles during the second world but are now becoming impotent bacterial weaponry. This has caused an urgent need for the search of new and innovative ways to control bacterial invasions especially by multi-resistant pathogens ,[15].

Natural alternative treatment for bacterial infection may provide path way for the development new antimicrobial agents. This study indicated that watery and alcoholic extracts for Callistemon viminalis leaves were more potent against Gram positive MBC of o.8- 4.4 mg / ml than Gram negative bacteria. this may be due to chemical composition and anti bacterial activity for basic oil gain by watery extraction for *Callistemon viminalis* leaves which analyzed by gas chromatography these oils represent about 98%from that total agents of free fatty acids that present in this plant, *Callistemon viminalis* plant also contain another antimicrobial composition in about 1.8 senol which represent 8% concentration in additional to 6.4% alpha-benin complex which represent potent anti microbial agent .[16],[17].

Our result refer to degenerative change in kidey of G2 group as supported by histological change in there cortex and medulla, kidney is one of multiple organ affected by spesis. Sepsis is the leading cause of acute renal failure which mostly develops as part of a spectrum of organ dysfunction. Acute renal failure occurs in approximately 45% of critically ill septic patients [18]. The association of sepsis with acute renal failure results in approximately 70% mortality compared with 45% mortality in patients with acute renal failure without sepsis. Our results refer to significant increament in the level of blood urea nitrogen, creatinine, creatinine kinase and uric acid for T1 group as compared with T2 animals S. pneumoniae pneumonia did not predictably affect plasma urea levels, these data show that in this model S. pneumonia induced renal dysfunction, specifically glomerular filtration is impaired, as shown by a significant increase in the level of urea and uric acid [19]. Our data also reveal that serum creatinine and creatinine kinase levels were significantly higher than normal level Creatinine is a small and freely filtered solute by the glomeruli of the kidney. Crn is produced from the break down of creatine in muscle while creatinin kinase mostly reveal presence of damage for heart tissue which an indicator for presence of multiple organ dysfunction. A reduced glomerular filteration rate (GFR) leads to retention of Crn in the blood. If we assume that Crn is produced at a constant rate in an individual, then a 50 percent reduction in GFR results in proximate doubling of the plasma Crn concentration [20] These data indicate that animals suffered from infections and kidney damage, while data for G2 animals represent significant improvement this may be due to therapeutic effect of bottle brush leave alcoholic extract on this bacteria.

In summary, we have shown that intratraperitonial injection of S. pneumoniae induces pneumonia and kidney damage. We show a clear establishment of pneumonitis in this model. Multiple organ dysfunction may occurred and time-dependent fashion after S. pneumoniae-induced pneumonia and was manifested as renal, and cardiac dysfunction. This model may serve to be a very useful rabbit model to study gram-positive sepsis and multiple organ dysfunction syndrome (MODS).

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