Studying the therapeutic effect of watery &alcoholic extracts of Apium graveolens leaves on urinary tract infections caused by Staphylococcus aureus in rabbits

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Abstract

This study was designed to evaluate the effects of the watery and alcoholic extracts from leaves of Apium graveolens in vitro on pathogenic Staphylococcus aureus bacteria and studying the effect of watery extract on Staphylococcus aureus in urinary system in vivo. The results showed the watery and alcoholic extracts from Apium graveolens leaves have antibacterial activity against Staphylococcus aureus. It was more sensitive toward watery extract of celery concentration (100%) in addition to inhibit the virulence factors for these bacteria which include production of coagulase, protease and hemolysin. According to the results, bacteria was injected intraperitonially as experimental infection in rabbits (vivo) which cause morphological and histopathological degenerative lesion of kidney cortex and medulla tissue in addition to change of renal profile test that include blood urea nitrogen, creatinine, creatinine kinase, uric acid and calcium. However, after injection of watery extract of Apium graveolens intraperitonially in rabbits, significant improvement (p≤ 0.01) had been occurred in the value of blood urea nitrogen, creatinine and creatinine kinase. Calcium and uric acid had a less improvement but don’t reach significant degree (p≥ 0.01). Histopathological studies confirm these results which include regeneration of degenerative lesion for medulla and kidney cortex with convoluted tubules tissue. As general urinary system staphylococcal infection can be diminished by treatment with watery extraction of Apium graveolens leave.

الخلاصة

الهدف من إجراء هذه الدراسة هو تقييم تأثير المستخلصات الكحولية والمائية لأوراق نبات الكرفس مختبرا على جرثومة Staphylococcus aureus ومن ثم دراسة تأثير المستخلص المائي على نفس الجرثومة حييا على الجهاز البولي في الأرانب. اظهرت النتائج الفعلية العالية للمستخلصات المائية والكحولية لنبات الكرفس ضد جرثومة Staphylococcus aureus. ووجد أن هذه البكتريا أكثر حساسية تجاه المستخلص المائي لأوراق نبات الكرفس يركز (100%) إضافةً لتآثر ذلك المستخلص على عوامل المضادة لهذه الجرثومة المعروفة والمتمثلة بنائريات على أنسجات التحليل ونوكليوتيد ومخلوق الدم. وقد احدثت لالقارب المختبریة عددا (20) والتي تسببت في إحداث افادات طهويلة ونسيجية كاحداث تلف في قشرة ونوكليوتيد البداية، واضافة إلى احداث تغيرات في الدائرة الحيوية للجهاز البولي، والتي شملت تركز البكتريا في السائل والكرياتين، والكالسيوم والبوتيفيرین. إضافة الى تركيز<ID>ايفورون الكالسيوم، بعد احكام الحيوانات المصابة بالمستخلص المائي لنبات الكرفس أدى إلى تحسن معنوي (p<0.01) في مستوى تركيز البكتريا في المصل والكرياتين، والكالسيوم، لاما حاسم البكتريا، وتم قياس هذا السبب من وجود تحسن بين السببين إلا أنه لم يصل إلى مستوى معنوي (p≥ 0.01) إضافةً إلى اصلاح الأنسجة التالفة في منطقة القشرة والبلازما الكليه، والاستجابة التلقية من جديد، بصورة عامة يمكن تخفيف إخراج الجهاز البولي بفعل جرثومة Staphylococcus aureus. الكرفس.
Introduction

Medicinal plants have been used in traditional medicine for the treatment of urinary tract disease. At the present time, the interest in the folk medicine is increasing because many patients believed that such products are effective and less harmful (Naema, et al., 2010). This trend stimulates our interest in the scientific investigations of medicinal plants that are used in Iraqi folk medicine for the treatment of urinary tract problems (Yesilada, et al., 1993). Since several plant antimicrobials contain different functional groups in their structure, their antimicrobial activity is attributed to multiple mechanisms (Burt, 2004). Therefore, unlike antibiotics, the potential for bacteria to develop resistance to plant antimicrobials is relatively smaller (Ohno, et al., 2003).

The urinary tract is the body's filtering system for removing waste liquid, or urine (Ramadan, et al., 2003). A urinary tract infection is caused by bacteria that enter the urinary tract (Parlak, et al., 2007). Urinary tract infection are categorized into either lower tract infection, located in the bladder and/or urethra (cystitis and urethritis) and upper tract infection, located in ureters, collecting system, and parenchyma (pyelonephritis) (Dulczak and Kirk, 2005). Most of UTI are caused by gram-negative bacteria like Escherichia coli, Proteus mirabilis, Proteus vulgaris Klebsiella sp., Pseudomonas aeruginosa, Acinetobacter, Serratia, and Morganella morgante, also UTI are caused by Gram positive bacteria include Enterococcus, Staphylococcus especially coagulase negative staphylococci, and Streptococcus agalactiae (Tangho and Mcaninch, 2004). Treatment of UTI with the appropriate antibiotic can minimize mortality and morbidity but appropriate antimicrobial agents are so difficult due to bacterial resistant's therefor the new diuretic therapy pass mostly toward plant extract like Allium sativum, Apium graveolens, Armoracia lopathifolia, Barbarea vulgaris, Capsella bursapastori, Citrus japonica, Ficus carica, Olea (Ankur, et al., 2010).

Celery seed (Apium graveolens L.) is belong to Apiaceae family. Other common names of celery seed are Smallage, Marsh Water Parsley, Wild Celery, Garden Celery and Marsh Parsley.

Celery seed is medicinal plant has slender and stands about 2-3 feet tall. It has three to five segmented leaves, and flowers with small white petals. Celery seeds are found in the flowers, are very small, tan to dark brown, and have a strong, pleasant smell (AL- Shahat, 1986). It's has been used in traditional medicine for the treatment of urinary tract disease, celery is an excellent diuretic that promotes the flow of urine through the kidneys and increases uric acid excretions, helping to clear toxins from the system (Mills, 1988). This is especially good for gout, where excess uric acid crystals collect in the joints. Its diuretic action may also relieve bladder disorders, cystitis and other kidney problems including stones and gravel. Celery seed is also suggested for treating arthritis and to help reduce muscle spasms, calm the nerves, and reduce inflammation (Spriggs and MeHzer, 2000 and Ehrlich, 2007).

Aim of study: This study was used to investigate the effect for Apium graveolens on Staphylococcus aureus bacteria in addition to investigate the therapeutic effect of this plant on urinary system Staphylococcus aureus infection of rabbits.
Materials & Method

Part I
1-Preparation of plant extracts
A- Watery extract
Fifteen grams of plant sample powders were steeped in 100 ml of cold distilled water and the mixture was then kept in shaker incubator for 24hrs at 35°C then filtered through filter paper (Whatman No.1) and centrifuged for 10 min at 3000 rpm. the filtrate was then left to evaporate in the incubator at 37°C for three days. The dried powder was weighed and transferred to a sterile universal tube in the refrigerator for later usage (Anesini and Perez, 1993).

B- Cold alcoholic extract:
Ethanol 70%, instead of distilled water, was used to obtain a dried powder, for this the same steps mentioned in (1.A) were applied (Anesini and Perez, 1993).

2-Bacterial sample collection
Eighty bacterial isolates were collected randomly from Al-Samawa general hospital. Urine samples were collected from patients suffered from urinary tract infection, 30 samples of them give positive reaction for virulence factors coagulase, hemolysin and protease. Then bacteria were prepared on cultured neutrient agar and incubated at 37°C for 24 hrs. later 1 microliter was taken from bacterial suspension and diffused on nutrient agar by using L- shape spreader, the plates were left for about 5-10 minute to permit the suspension for absorbed by agar, then 5 equal distant holes (10 mm diameter) were made inside the plates for putting different plant extract (watery &alcoholic) concentration 25, 50, 75,100% and distilled water (control). Plates were incubated at 37°C for 24 hrs. the effect of plant extracts on virulence factor were carried out, in addition to its effect on bacteria which calculated by inhibition zones around the different concentrations holes (Crespo, et. al., 1990). Virulent bacteria that give positive the reaction for virulence factor were used in vivo.

Part II
1- Animals
Twenty clinically healthy six month old white Newzealand rabbits were used in the experiment, rabbits were divided into two equal groups. Positive control group administrated food and tap water and injected with 10^3 virulent Staphylococcus aureus diluted suspension intraperitonially, this group suffered from multiple frequent urination time with pus. The second group (treatment group) administrated food and tap water and injected with 10^3 Staphylococcus aureus diluted suspension intraperitonially to induce urinary system disorder, watery plant extract was administrated intraperitonially in the form of two doses 500 mg/ml daily for three weeks after clinical signs of urinary disorders presentation.

2- Sample collection
At 10 weeks whole blood was collected via cardiac puncture from anesthetized (ketamine 50mg/kg-xyllazine 10mg/kg), rabbit were then euthanized with a single cardiac injection fatal plus (concentrated pentobarbital, 360 mg/kg), and kidney tissues was collected for histological studies.

3-Blood chemistries
Blood urea nitrogen, creatinine, creatinine kinase, uric acid and calcium concentration in plasma were determined using commercially available kit (sigma).
4-Statistical analysis

Mean ±SE was used to describe variables. All data are analyzed using Duncan's multiple range tests to determine if the treatment were significantly (P ≤ 0.01) different or not (Duncan, 1955).

Results

The results illustrated that the crude extracts from the leaves of *Apium graveolens* had antibacterial activity against *Staphylococcus aureus* in vitro and in vivo. Virulent bacterial isolated from patient gave positive results for mannitol, coagulase, hemolysin, protease enzymes and acetoin production testes, which insure that these bacteria were pathogenic.

In vitro results indicated that *Staphylococcus aureus* was highly sensitive towards (75,100) % watery *Apium graveolens* leaves extracts when inhibition zones reach to 22 & 25 mm respectively which is agreed with (Shaaban, et al., 2012). While the results related with alcoholic extraction which used at concentrations (50, 75,100) % and watery *Apium graveolens* extract with 50% conc. appear high antibacterial effect and *Staphylococcus aureus* inhibition zones reach to 20 mm. *Staph. aureus* bacteria illustrates more resistance toward 25% conc. of watery and alcoholic extracts of *Apium graveolens*, inhibition zones reach to 14.5, 10.5 mm respectively as explain in table 1.

<table>
<thead>
<tr>
<th>Types of extract</th>
<th>Extract Concentration (%)</th>
<th>Inhibition Zone Diameter (mm) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Watery ~ <em>Apium graveolens</em></td>
<td>25</td>
<td>14.5 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>20 ± 0.57</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>22 ± 1.15</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>25 ± 0.57</td>
</tr>
<tr>
<td>Alcoholic~ <em>Apium graveolens</em></td>
<td>25</td>
<td>10.5 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>20 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>20.5 ± 1.44</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>20.5 ± 0.57</td>
</tr>
</tbody>
</table>

*Staph. aureus* bacteria was selected to inject intraperitonially then treated with 100% watery plant extracts which considered as the more sensitive extracts for this bacteria. Result in vivo reveals presence of significant decrement (P ≤ 0.01) in level of blood urea nitrogen, creatinine and creatinine kinase for treatment group than control group. Uric acid and calcium level for treatment group had some improvement than control group but these improvements do not give significant differences (P≥0.01) as explained in table 2.
Table 2: The effect of *Apium graveolens* renal profile test for infected rabbits with *staph. aureus* intraperitonially.

<table>
<thead>
<tr>
<th>Renal profile test</th>
<th>Control group ± SE</th>
<th>Treatment group ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>blood urea nitrogen</td>
<td>72.65±0.567 a</td>
<td>49.062±0.552 b</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.863±0.015 a</td>
<td>0.59±0.072 b</td>
</tr>
<tr>
<td>creatinine kinase</td>
<td>255.1±24.581 a</td>
<td>106.68±13.049 b</td>
</tr>
<tr>
<td>uric acid</td>
<td>3.2±0.29 a</td>
<td>3.3±0.395 a</td>
</tr>
<tr>
<td>Calcium</td>
<td>5.401±0.389 a</td>
<td>3.574±0.083 a</td>
</tr>
</tbody>
</table>

a: no significant variation  
b: 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 medulla of kidney, for control group animals 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 as present in fig 4 A&B.

Fig 4 (A)
Photomicrographs of haematoxylin and eosin stained sections of rabbit kidney; (A&B) presence of inflammatory area (spots or patches) in the cortex and medulla 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×).
Discussion

Antibiotic 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 (Lewis, 1995). Natural alternative treatment for bacterial infection may provide pathway for the development of new antimicrobial agents. This study indicated that watery and alcoholic extracts for *Apium graveolens* leaves were more potent against virulence *Staph. aureus* pathogen with inhibition zones to 20-20.5 mm. at conc. 50,75 and 100% of alcoholic extracts respectively this may be due to chemical composition and antibacterial activity for this plant, celery seeds contain higher amount of volatile oils 2-3% many components including limonene (60%) and selenine (10-15%) and various sesquiterpene alcohols (1-3%), e.g. a-eudesmol and (i-eudesmol, santalol) (Fehr, 1979 and Stahl, 1973). Phthalide compounds, 3-n-butyl phthalide and sedanenolide, provide the characteristic odour of the oil (presence of sedanolide and sedanonic anhydride disputed) (Bjeldanes and Kim, 1977 and Bos, 1986). Celery contian limonene and small amount of carvone that contain strong antibacterial effect on *staph aureus*, results in vivo explained significant elevation in BUN and serum creatinine levels were observed ,the idea to use some herbas like celery is to evaluate its effect in improvement of urinary system disorder, celery is an alkalizing food, contains some active ingredients like alkaloids, high level of vitamin C, phthalides (lower cholesterol) ( Al Jawad, *et. al.*, 2011), coumarins (prevent free radicals from damaging cells and enhance the activity of immune defenders that eliminate potentially harmful cells including cancer cells), acetylenics (stop the growth of tumour cells)( Tsi and Tan, 2000) and plant enzymes that aid in maintaining a more alkaline balance(Bingham, *et. al.*, 2001). It produces significant lowering effect in (BUN) blood urea nitrogen, serum creatinine level and an increase in urine.

Figure 5(B)

Figure 5: 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 convoluted tubules, disappearance of congestion in the interstitial spaces for cortex of kidney. (A:H&E, 10×, B:H&E.
levels than normal values, calcium level had small improvement but didn't reach to significant variation and a reduction of its concentration in serum, and this is due to its diuretic effect which may be related to its constituents, particularly allantoins that have diuretic action (Ensminger and Esmingher, 1986). Celery seed also have been shown to possess bacteriostatic and antifungal effects (Choochote, et. al, 2004). Serum creatinine kinase level had significant improvement (p≤0.01), this may be due to reduction in renal tissue damage which correlated with the chemical structure of luteolin, luteolin a flavonoid, had multifunctional effect, is intimately connected with its structure and hydroxyl as its functional group, which participates in electron delocalization and is, therefore, an important determinant for its antioxidative (Horvathova, et. al., 2005).

Our result refer to degenerative change in kidney of control group as supported by histological change in there cortex and medulla, as compared with treatment group, celery play important role in correction the oxidative damage that resulted from Staph. aureus, also protects endothelial cells against oxidative / nitrosative stress. These types of stress decrease the immune system and rob the body important vitamins such as vitamin C, E, D, A and minerals / trace minerals like zinc, selenium, manganese, and molybdenum. Also, antioxidantive nutrients such as L-glutathione, the enzyme super oxid dismutase (SOD) or the amino acid L-cysteine will the body removed faster than normal.( Tayel and El-Taras, 2009).

Virulence factors (protease, hemolysin and coagulase) may be inhibited as explained in results this may be due to its antibacterial effect that include essential oil and oleoresin which are used as inhibitors of Staphylococcus aureus population development, in addition to chemical compositon that contain apiol, neocnidilide, ocimene and proteinase inducing inhibitory factors (Mcfarland and Clarence, 1974, Skariyachan, et. al.,2012 and Hammer, et. al.,1999). In conclusion Apium graveolens have a significant antibacterial and diuretic and attenuating effect that play important role in correction urinary system affection and damage resulted from intraperitonially Staphlococcus aureus infection.

Reference

Choochote,W.; Tuetun, B.; Kanjanapothil, D.; Rattanachanpichai, E.; Chaithong, U.; Chaiwong,P.; Jitpakdi, A.; Tipawangkosol, P.; Riyong, D. and


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