The effectiveness of inhibitory of Cinnamomum zeylanicum extract against Proteus mirabilis (invitro and invivo)

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Abstract
The study designed to show the antimicrobial activity of cinnamomum zeylanicum extract against *P. mirabilis*. The present study was divided to two parts, the first part (invitro) including sensitivity test of *P. mirabilis* against extract and amoxicillin. where, the results of inhibition efficacy of extract toward *P. mirabilis* showed very high inhibition efficacy of extract, which reached to 30 mm, while amoxicillin showed non inhibition effect against bacterial growth. In the second part (in vivo) used 20 albino mice *Mus musculus* that divide randomly to four groups (each group consist 5 mice), first group; control group administrated only normal diet and water, second group; injected intraperitoneally with ($2x10^8$ CFU/ml), third group; injected intraperitoneally with (2×10^8 CFU/ ml) and treated with amoxicillin, forth group; injected intraperitoneally with (2×10^8 CFU/ ml) and treated with extract. The urea and creatinine levels were increased and showed high significant changes ($P < 0.01$) in groups (second, third) compared with control group but its back to normal when treated with extract. The microscopic examination showed many changes in kidneys of groups (second, third) including damage glomerulus, convolute tubules, fibrosis, and infiltration of lymphocytes, congestion and thickening wall of blood vessels. The kidneys tissue in forth group showed recovery in the glomerulus and convolute tubules. It was concluded from this study that the cinnamomum zeylanicum has been antimicrobial activity against *P. mirabilis*.

Introduction
Cinnamomum zeylanicum is the oldest herbal medicine, has mentioned in Chinese texts since 4 000 years ago [1]. Cinnamomum is an evergreen tree, belonging to the Lauraceae family. The leaves and barks of Cinnamon are used as flavoring agent in foods and for various applications in medicine [2]. cinnamon consist of the most important components include; cinnamaldehyde and trans-cinnamaldehyde (Cin), thus contributing to the fragrance and to the various biological activities observed with cinnamon [3]. The bark of cinnamon include; procyanidins and catechins [4]. Procyanidins components include both procyanidin - A and B-linkages [5-7]. These procyanidins extracted from cinnamon and berries also have antioxidant activities [6-8].

Proteus is a genus of Gram-negative bacteria, its cause infections in different human systems, with *Proteus mirabilis* causing 90% of these infections. *Proteus mirabilis* commonly inhabits cows, dogs and birds, and can cause nosocomial infections when colonizing human feces in hospital settings [9]. The recognized species of proteus include: *P. mirabilis*, *P. penneri*, *P. vulgaris*, and *P. myxofaciens* [10]. All Proteus species (except *P. myxofaciens*) are also associated with opportunistic human infections. *P. mirabilis* is most often isolated from infections; it is hypothesized that the reason may be a higher carriage rate of *P. mirabilis* in human intestines. *P. mirabilis* has isolated from the throat, respiratory tract, skin, and burns and is speculated to be a possible cause of gastroenteritis and *P. mirabilis* is also a major cause of complicated urinary tract infection (UTI) and diseases [11,12]. The aim of current study is to reveal the potential activity of cinnamomum zeylanicum extract against *P. mirabilis*.

Materials and Methods
Plant extract
Powder was collect from Kirkuk market. Five gram of powder was suspended in 200 ml distilled water for 24 hours. Then, the rotary evaporator used to concentrate the mixture. Finally, it was stored at a temperature of 4°c until use [15].

Bacteria
A. collection
Different clinical samples such as urine, purulent material from wounds, ear swabs, sputum collected from patients suspected of bacterial infection at microbiology lab were cultured to isolate the bacteria.

B. Cultivation and Identification
The cultured of collecting samples done by used Blood agar and MacConkey agar. Then, dishes incubated at 37 ^oC for 24 h. The colonies morphology characteristics including size, shape and haemolytic nature were recorded. Isolated and identified of suspected Proteus colonies done according to Barrow and Felthan, they method to isolation include; positive for nitrate reduction; H2S gas production; methyl-red and urease reactions; carbohydrates fermentation and negative for lactose fermentation. P.mirabilis was identified by maltose fermentation and ornithine decarboxylase production [13].

C. the effectiveness inhibitory
The inhibition potential of antimicrobial agents (amoxicillin (25 µg) and extract) against bacteria was done by using Modified Kirby-Bauer disk diffusion method [14]. The inoculate was prepared by growing Proteus species on agar plates and colonies were transferred with inoculating loop into 3 ml of normal saline in a test tube. Muller-Hinton agar plate surface was evenly inoculated with the organisms using a sterile swab. The wet swab was then used to inoculate the Muller-Hinton agar by evenly streaking across the surface. The antibiotic and extract discs were applied

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to the surface of the inoculated agar and the plates were incubated overnight at 37 ^{o}C. The zone diameter growth-inhibition observed was measured [14].

Animal model

Twenty adult albino mice, (wt 25-28 g) obtained from requirements medicals company – Samara, Iraq, and kept ventilation cages with standard diet.

Experimental design

In current study 20 albino mice divided to five groups (each group consist five mice) as follow:

1. First group: Control group feeding standard diet, then killed.
2. Second group: Mice injected intravenously with 2.0 x 10^8 bacteria, and then killed after infection.
3. Third group: Mice injected intravenously with 2.0 x 10^8 bacteria. After that, treated with amoxicillin (25mg/kg) for three week, and then killed.
4. Forth group: Mice injected intravenously with 2.0 x 10^8 bacteria. After that, treated with 1ml/50mg extract for three week, and then killed

Prepare of blood solution

Blood collections occur, under anesthesia, from mice heart blood and put in EDTA tubs. The tubes were centerfigation 5000 cycle/min for 10 min. then, the serum was taken and stored until used.

Histological study

Kidneys from each mice were cut out rapidly, and fixed by 10% formalin. Dehydration was done by ascending grades of ethanol and followed by clearing then tissue samples by xylene before being impregnated with melted paraffin wax, embedded and blocked out. Tissue sections thickness were stained with haematoxylin-eosin [16].

Statistical analysis

The analysis of data was done by using a statistical Minitab program. The results were analyzed statistically using Analysis of Variance (ANOVA) test, in order to evaluate the significance of variability between treated and control groups [17].

Results

Inhibition efficacy of extract and antibiotic toward P. mirabilis

The results of inhibition efficacy of extract toward *P. mirabilis* showed very high inhibition efficacy of plant extract, which reached to 30 mm, while antibiotic showed non inhibition effect against bacterial growth (Fig. 1).

Figure (1): show the sensitivity test.

Biochemical test

Urea tests

The level of urea showed significant changes ($P<0.05$) between groups. As shown in figure (1). The level of urea in bacterial infected mice group (52.4 mg/dl) and bacterial infected mice and treated with amoxicillin group (49.6 mg/dl) showed significant change compared with control group(26.9 mg/dl), but in bacterial infected mice and treated with extract group showed non-significant changes compared with normal mice.

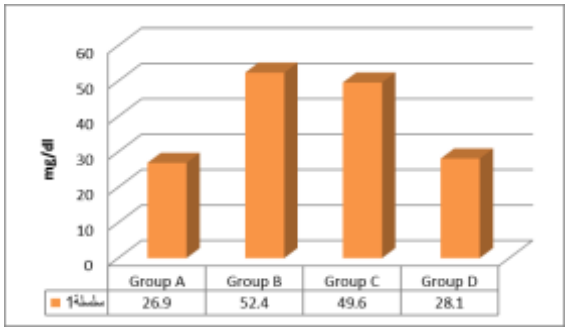

Figure (3):Urea levels

Creatinine tests

The level of creatinine showed significant changes ($P<0.05$) between groups. As shown in figure (2). The level of creatinine in bacterial infected mice group (2.8 mg/dl) and bacterial infected mice and treated with amoxicillin group (2.3 mg/dl) showed increased change compared with control group(0.6 mg/dl), but in bacterial infected mice and treated with extract group showed non-significant changes compared with normal mice.

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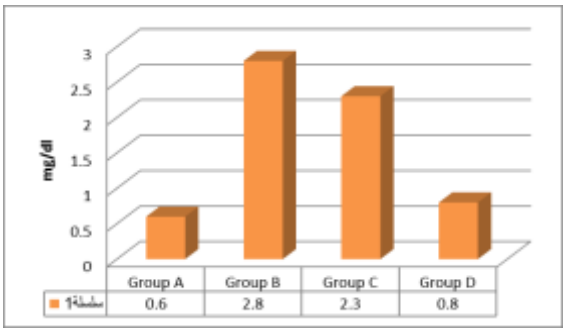

Figure (3): Creatinine levels

Histological examination

A. Control group

The microscope examination of normal kidneys showed normal structure of kidneys and demonstrated normal glomerulus sizes and shapes, normal shapes and structure of convolute tubules (Fig.4).

B. group injected with bacteria

The cross sections that prepared from this group showed damage glomerulus in most regions with destruction the convolute tubules and severing infiltration of lymphocytes (Fig. 5).

C. group injected with bacteria and treated with amoxicillin

The histological examination showed thickening wall of blood vessels with congestion of blood vessels and damge of glomerulus and infiltration of lymphocytes with present the fibrocytes (Fig.6).

D. group injected with bacteria and treated with extract

The microscope examination showed recovery of kidneys tissue demonstrated normal glomerulus, normal shapes and structure of convolute tubules (Fig. 7).

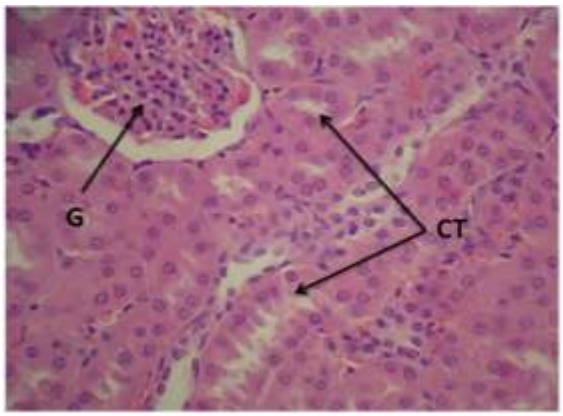

Figure (4): kidney of control group showed normal glomerulus (G) and normal convolute tubules (CT) 400X H&E.

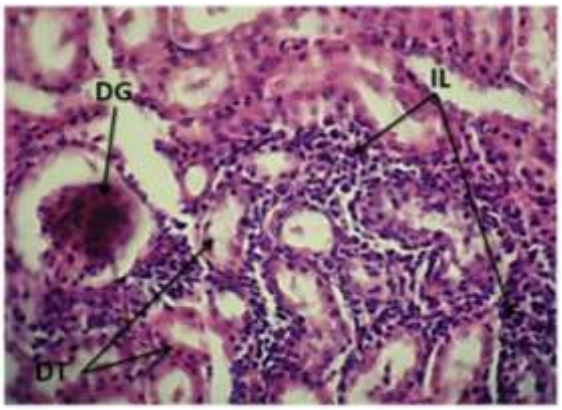

Figure (5): kidney of injected bacteria group showed damage glomerulus (DT), damage tubules (DT) and infiltration of lymphocytes (IL) 400X H&E.

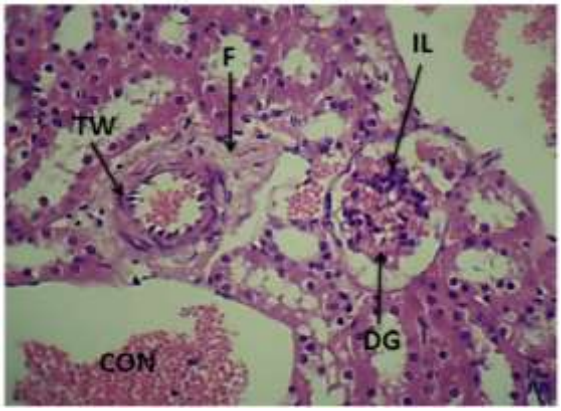

Figure (6): kidney of injected bacteria and treated with amoxicillin group showed damage glomerulus (DT), congestion (CON), infiltration of lymphocytes (IL), thickening wall (TW) of blood vessels and fibrocytes (F) 400X H&E.

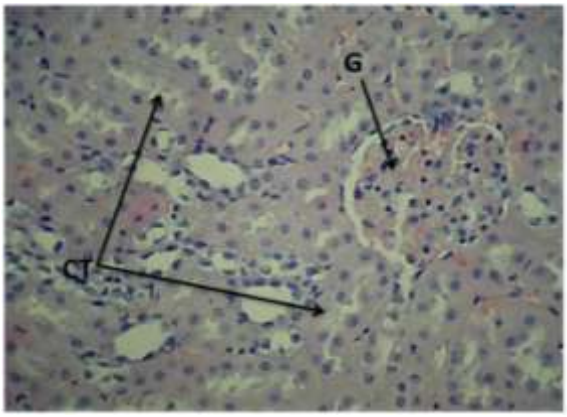

Figure (7): kidney of injected bacteria and treated with extract group showed normal glomerulus (G) and normal convolute tubules (CT) 400X H&E.

Discussion

The results of current study show a high antimicrobial activity of Cinnamomum zeylanicum extract against *Proteus mirabilis* as appeared in an inhibitory test. Where the inhibition zone diameter reached to 30 mm. This results agreement with Goni et al. (2009) who referred that the antibacterial activity of a combination of cinnamon and clove oils against Gram-positive bacteria *(Enterococcus faecalis, L. monocytogenes, S. aureus,* and *B. cereus*), as well as against Gram-negative bacteria (*Pseudomonas aeruginosa, Salmonella choleraesuis, Escherichia coli* and *Yersinia enterocolitica)* [18]. A study carried by Hili et al. (1997) stated that cinnamon oils have potential action against various types of bacteria (*P.aeruginosa, Staphylococcus aureus, and Escherichia coli*) and yeast (*Schizosaccharomyces pombe, Torulopsis utilis, Candida albicans,* and *Saccharomyces cerevisiae*) [19].

Wong et al. (2014) referred that the oil from cinnamomum zeylanicum possesses anti-bacteria activity against *Bacillus subtilis* and *Escherichia coli* [20]. Priyanga et al. (2013) referred that the cinnamomum zeylanicum has potential anti-bacteria action against a wide variety of bacteria (*Acinetobacter spp, Bacillus spp, Brucella melitensis, Clostridium spp, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Helicobacter pylori, Klebsiella pneumonia, Listeria ivanovii, Listeria monocytogenes, Mycobacterium smegmatis, Mycobacterium tuberculosis, Proteus mirabilis, Pseudomonas aeruginosa, Saccharomyces cerevisiae, Salmonella spp, Staphylococcus spp and Streptococcus spp*) [21]. In study carried by Al-Ani et al. (2011) to show the toxicity affect of *Proteus mirabilis* in the rats kidneys. They found after treated rats with *Proteus mirabilis* different changes occur in kidneys tissue including damage in glomerulus, damage convolute tubules with infiltration of mononuclear cells and congestion of blood vessels with interstitial fibrosis [22].

Cinnamomum zeylanicum extracts inhibit both gram positive and negative bacteria. Antibacterial activity back to cinnamon [23]. Ali et al. (2009) referred that the cinnamomum zeylanicum extracts has been important protective role against microorganisms of the Dental Plaque. Where, the In-Vitro study and scanning electron microscopic assessment showed high antibacterial activity of cinnamomum zeylanicum against microorganisms of the Dental Plaque (*Streptococcus aureus, Streptococcus mutans, Enterococcus coli* and *Lactobacillus*) [15]. Also, mohammed et al. (2005) referred that the cinnamomum zeylanicum extract has been antibacterial activity against gram positive and gram negative bacteria. They showed inhibitor effect of cinnamomum zeylanicum extract against P. aeruginosa, Escherichia coli, P. mirabilis, Bacillus subtilis, S. aureus and Salmonella typhi. They showed a good inhibitor effect of cinnamomum zeylanicum extract against P. mirabilis [24]. In study carried by Sakr & Ashraf (2014) to show the protective effect Cinnamomum zeylanicum extract against the toxicity of cypermethrin in the rats. They found that the cypermethrin lead to damage glomerulus, blood haemorrhage, and damage convolute tubules with infiltration of mononuclear cells but after treated the rats with cinnamon, the kidney tissues were recovery [25].

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تحديد الفعالية التثبيطية لمستخلص نبات الدارسين ضد بكتريا ***Proteus mirabilis*** (خارج وداخل الجسم)

انتصار عبد الجبار شمخي

قسم علوم الحياة ، كلية العلوم ، جامعة تكريت ، تكريت ، العراق

الملخص

صممت الدراسة الحالية لاظهار الفعالية التثبيطية لمستخلص نبات الدارسين ضد *P. mirabilis* . الدراسة قسمت الى جزئين, الجزء الاول (خارج الجسم) والذي تضمن اختبار الحساسية ضد *P. mirabilis* المستخلص ومضاد الاموكسلين. حيث كانت نتائج الفعالية التثبيطية للمستخلص ضد البكتريا عالية جدا وصلت الى تكوين منطقة تثبيط يصل قطرها 30 mm, بينما الاموكسلين لم يظهر اي فعالية تثبيطية ضد نمو *P. mirabilis*. ام الجزء الثاني من الدراسة (داخل الجسم) تم استخدام 20 من الفئران البيض والتي قسمت عشوائيا الى اربع مجاميع (كل مجموعة تحتوي 5 فئران), المجموعة الاولى هي مجموعة السيطرة والتي جرعت بحمية طبيعية وماء فقط, المجموعة الثانية والتي حقنت داخل الصفاق بجرعة ($2x10^8$ CFU/1ml) من البكتريا, المجموعة الثالثة والتي حقنت داخل الصفاق بجرعة ($2x10^8$ CFU/1ml) من البكتريا وتم معالجتها بمضاد الاموكسلين, المجموعة الرابعة والتي حقنت داخل الصفاق بجرعة ($2x10^8$ CFU/1ml) من البكتريا وتم معالجتها بالمستخلص. اظهرت معايير الدم تباين في مستويات اليوريا والكرياتينين حيث ازدادت واظهرت فروقات عالية المعنوية ($P < 0.01$) في المجاميع المحقونة بالبكتريا والمحقونة بالبكتريا ومعالجة بالاموكسلين مقارنة مع مجموعة السيطرة, بينما في المجموعة المحقونة بالبكتريا ومعالجة بالمستخلص كانت مستويات اليوريا والكرياتينين طبيعية. الفحص المجهري اظهر تغيرات في الكلى في المجاميع المحقونة بالبكتريا ومعالجة بالاموكسلين تضمنت تحطم الكبيبات والنبيبات الملتوية البولية, تليف, ارتشاح الخلايا اللمفية واحتقان الاوعية الدموية. نسيج الكلى في المجموعة المحقونة بالبكتريا ومعالجة بالمستخلص اظهرت الكبيبات والنبيبات البولية مقاربة لما هو موجود في مجموعة السيطرة. استنتج من هذه الدراسة بان مستخلص نبات الدارسين فعالية تثبيطية ضد بكتريا *P. mirabilis*.