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Antibacterial Activity of the Extracts of Chicken Gizzard Membrane Against Some Pathogenic Bacteria Isolated from Urinary Tract Infections

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A r t i c l e i n f o

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ABSTRACT

This study aims to develop a natural antibacterial material with broad spectrum activity against bacteria implicated in stones formation and urinary tract infection. Instead of the use of excessive and non-safe antibiotics and to block the prevalence of multi-drug resistant (MDR) bacterial isolates, which have increased in the last few years.

The aqueous extract of the chicken gizzard membrane was prepared with three different methods and used ***invitro*** against some pathogenic bacteria isolated from patient suffering from UTI and urinary stones. The activity of chicken gizzard membrane was determent according to the presence or absence of bacterial growth in cultured plates treated with the aqueous extract.

The present study found that using a cold aqueous extract of the chicken gizzard membrane prepared freshly and used directly without any treatment had remarkable effectiveness compared with other extract, which heat treated and used after one hour or 24 hours of extraction.

We have tested a natural material, which may represent a good candidate for development as an antimicrobial for the most common UTI causative agents in the presence of urinary stones, such as: ***E.coli, Proteus mirabilis*** and ***Staphylococcus aureus***.

UT infection associated with the urinary stones remains a major problem, because of the need for treatment with medical drugs (antibiotics) for a long time as well as the possibility of back infection again. The natural antimicrobial material such as the chicken gizzard membrane is an excellent candidate solution to this problem

الخلاصة

تهدف هذه الدراسة الى تطوير مادة طبيعية ذات فعالية مضادة واسعة الطيف ضد البكتريا المسببة لالتهابات المجاري البولية، بدلا من الاستخدام الواسع وغير الامن لمضادات الحياة ، فضلا عن منع ظهور السلالات البكتيرية المتعددة المقاومة للعقاقير والتي بدأت بالتزايد في السنوات القليلة الماضية.

حضر المستخلص المائي للغشاء الداخلي لقانصة الدجاج بثلاثة طرق مختلفة واستخدم ضد بعض الاجناس البكتيرية المعزولة من مرضى يعانون من اصابات المجاري البولية. قدرت مدى فاعلية هذا المستخلص بالاعتماد على قياس اقطار مناطق التبيط في اطباق النمو البكتيري.

وجدت الدراسة الحالية ان استخدام المستخلص المائي البارد لغشاء القانصة المحضر انيا والمستخدم مباشرة دون اية معاملة قد اظهر فاعلية ملحوظة مقارنة بالمحاليل الاخرى.

اختبرنا هنا مادة طبيعية قد تمثل منطلقا لتطوير مضادا بكتيريا ناجح لاغلب مسببات التهابات المجاري البولية مثل اشيريشيا القولون ، الزائفات الزنجارية والمكورات العنقودية ، اذ ان اصابات المجاري البولية تتطلب العلاج بمضادات الحياة لفترة طويلة مع امكانية تكرار الاصابة مجددا ،الامر الذي يدفع الى ظرورة وجود مادة طبيعية بديلة ذات فاعلية عالية لحل هذه المشكلة

INTRODUCTION

Urinary tract infections (UTI) are one of the common chronic and recurrent bacterial infections. Uropathogens, which are able to form biofilm, constitute a major etiological factor in UTI, especially among elder patients who are subject to long-term catheterization. It is caused by the capacity of the microorganisms for efficient and permanent colonization of tissues and also adhesion to diverse polymers used for urological catheter production such as propylene, polystyrene, silicone, polyvinyl chloride or silicone coated latex [1].

Osteopontin production, with associated mucosal damage due to UTI, may allow easier crystal retention and nucleation resulting in stone formation [2].

Attempts to treatment of an infected urinary tract in the presence of stones almost fail. Relapse or reinfection is the rule under these circumstances and this suggests that stones may act as a source of infection in which bacteria are protected from the effect of antimicrobial drugs The Relationship between infection of the urinary tract and urinary stones is well known and has been reported for many years. Stone enhance the susceptibility of the

urinary tract to infection, and infection can predispose to stone formation. [3].

Despite the fact that accurate knowledge of stone composition is of great value when setting up therapeutic advice to prevent stone recurrence, it was not demonstrated until the beginning of 1950 that the structure and internal arrangement of calculi, which is impossible to elucidate using chemical methods, is crucial in determining the mechanism of formation of different kind of stones [4]. Urinary stones may contain a mixed bacterial flora. Two types of ***Proteus (P. mirabilis*** and ***P . rettgeri)*** were isolated from different parts of a stone isolated from a patient. Also, ***Escherichia coli*** and ***Proteus mirabilis*** were isolated from one of the stones.

the Bacteria inside urinary bladder stones were shown to survive after exposure of the stones to a 3% solution of iodine in alcohol, or a concentration of an antibiotic excess of that required to inhibit the growth of the isolated organism. Stones were shown in the fluid culture media in which they were incubated, even if they had first been exposed to antimicrobial agents. Moreover, after exposing of stones to bacterial cultures, micro-organisms that eventually penetrated into the stone were protected against high concentrations of antibiotics to which they were otherwise sensitive. The findings may help to explain the reasons of persistence of infection and the ineffectiveness of drugs of urinary tract infection in the presence of urinary tract stones [3].

Antibiotic therapy is the most common treatment for UTI. Fluoroquinolones, nitrofurans, beta-lactams, aminoglycosides, trimethoprim and sulfonamides are used predominantly. However, the biofilm, due to its complex structure, constitutes an effective barrier to the antibiotics used in the treatment of urinary tract infections. In addition, the growing number of multidrug resistant strains limits the usage of many of the currently available chemotherapeutic agents [1]. The etiology of UTI (the pathogens traditionally associated with UTI) are changing many of their features, particularly because of antimicrobial resistance and are also affected by underlying host factors that complicate UTI, such as age, diabetes, spinal cord injury, or catheterization. Consequently, complicated UTI has a more diverse etiology than uncomplicated UTI, and organisms that rarely cause disease in healthy patients can cause significant disease in hosts with anatomic, metabolic, or immunologic underlying disease [5]. Therefore, it seems important to search for new methods of treatment.

The inner lining (membrane) of the chicken gizzard, which has a yellowish-gold color is called ***Jineijin*** (*ji* = chicken; *nei* = inner; *jin* = gold). This substance has been in use for about 2,000 years, At that time, ***jineijin*** was described as a treatment for diarrhea [6]. According to Chinese studies, a large dose of ***jineijin*** could even affect people with normal digestion: 45-60 minutes following ingestion of the roasted ***jineijin*** powder (5 g) in healthy individuals, the gastric secretion was increased by 30-37% compared with the control group [7]. An additional property of ***jineijin*** was : breaking down masses, being used for any kind of stagnation in the internal organs, for lower abdominal masses in women, for gallstones and kidney stones, and for tumors [8].

[9] notes that ***jineijin*** is effective for treating dyspepsia, food stasis, and infantile malnutrition.

Jineijin has trace amounts of digestive enzymes and these enzymes cannot be a major source of the action of this substance because of in our digestive system, there is a release of digestive juices with enzymes in quantities far higher than one would be obtained from ***jineijin.*** The active component that has been isolated from the ***jineijin*** is called ventriculin. It had been primarily used to treatment of pernicious anemia, which often resulted from poor absorption of vitamin B12, and for chronic gastritis, one of the main causes of pernicious anemia in adults. Ventriculin was later replaced by other drugs [9].

Because of many of attempts to treatment an infected urinary tract in the presence of stones almost fail, The purpose of the present study is to use the extract of chicken gizzard membrane (***jineijin***) as a new method or drug to the urinary stones and UTI, which is caused by pathogenic bacteria.

MATERIAL AND METHODS

Preparing of chicken gizzard membrane powder

After killing a chicken, about 2Kg of fresh and raw chicken gizzard was taken out and cut with a knife to remove the thick inner membrane immediately; then the membranes were washed to clean and dried in the sun then milling to get fine powder, which stored in a cool and dry well-closed container, keep away from moisture and strong light/heat. The general characteristics of the powder are listed in Table 1.

Table 1: General Characteristics for Chicken Gizzard Membrane Powder

Source	Color	Nature/ in flavor	pH after dissolved in water
Chicken	yellow /green	sweet	6.0

The preparation of the aqueous extract of the chicken gizzard membrane

The aqueous extract of the membranes prepared and used in three different ways:

1- Hot water extract in two ways:

A: Dissolve 25 g of powder in 250 ml of hot distilled water and left for 24 hours, then filtrated using filter papers and the filtrate was distributed in the empty dishes and left to dry then we scraped it as powder.

B: Dissolve 25 g of powder in 250 ml of distilled water and left for 1 hour in water bath at 60°C, then filtrated using filter papers and the filtrate was distributed in the empty dishes and left to dry then we scraped it as powder.

2- Cold water extract (C): Dissolve 20 g of powder in 40 ml of distilled water at room temperature then filtrated using filter papers and the filtrate was used immediately without any treatment.

Bacterial isolates and growth conditions

Bacterial isolates and their sources are listed in Table 2. Bacteria were grown overnight in Tryptone Soya Broth

(TSB; Oxoid, Basingstoke, UK) and then washed three times in phosphate buffered saline (PBS; NaCl 8 g/ l, KCl 0.2 g/ l, Na2HPO4 1.15 g/ l, KH2PO4 0.2 g/l). Bacteria were then resuspended in TSB to an OD660 nm of 0.5 for all isolates.

Preparing of nutrient agar plates inoculations

Nutrient agar plates was inoculated with (1 ml) of bacteria prepared as described above. By using the cork borer wells were made in some of these plates. These plates were used in the next step to determinate the antimicrobial activity of chicken gizzard membrane powder against all bacterial isolates which used in this study.

Determination of antibacterial activity of chicken gizzard membrane

5g of the membranes powder A and B was added separately to 10 ml of distilled water (final concentration =0.5g/ml), then filtered and sterilized using millipore filter unit. While the filtrate of powder C was shacking and used directly. 50µl from each filtrate (A,B & C) was transported by micropipette to nutrient agar plates and spread on all the surface of agar, then 0.1ml of bacterial broth for each isolate was spreaded on the surface of the agar and incubated at 37°C for 24 hrs.

In other way 50µl of filtrate was transported to each well in cultured agar plate and incubated at 37°C for 24 hrs. After the end of the incubation period, the presence or absence of bacterial growth in the dishes was investigated. Also the diameters of inhibition zones have been identified.

RESULTS AND DISCUSSION

Bacterial isolates

Eight samples of urine were collected from patients have turned stones in their urinary tract and the urine culture for these samples was positive. The genus and numbers of the isolated bacteria are listed in table 2. From these readings we see that ***Proteus*** was prevalent in the events of urinary tract infections, followed by ***E. coli*** with a slight lead and then finally come genus ***Staphylococcus***.

Antibacterial activity of chicken gizzard membrane powder

After the end of the incubation period, the growth of bacteria and the diameter of inhibition zones were examined and the results were explained in Table 3. The extract which prepared with method A has not any activity against the tested bacteria, also we can see that the extract B have a little activity. While the cold water extract has a good activity represented by the diameters of inhibition zones.

This study show that infected urinary tract could has one or more of calculi (stones). We conclude from this that the bacterial infection may be one of the reasons for the emergence of calculi. Other study recorded that infection of urinary tract by ***Escherichia coli*** causes higher than normal expression of promoter protein osteopontin and mucosal damage at renal tubular cells. These suggest that urinary infection may promote stone formation by mucosal damage and elevate promoter protein osteopontin at tubulus cell, allowing easier crystal retention and nucleation [2].

Table 2: Number, Percentage of Bacterial Isolates and Source of Isolation

Patient has urinary stones	Urine culture	Type of isolate	Serial number	Percentage (%)
1	+	*Escherichia coli*	1	37.5
2	+	*Escherichia coli*	2	
3	+	*Escherichia coli*	3	
4	+	*Proteus mirabilis*	4	50
5	+	*Proteus mirabilis*	5	
6	+	*Proteus mirabilis*	6	
7	+	*Proteus mirabilis*	7	
8	+	*Proteus mirabilis*	8	12.5

Table 3:Antibacterial Activity of Extract of Chicken Gizzard Membrane Powder

Type of isolate	Type of extract	Diameters of inhibition zones (Mean±SD)	Growth (Streaking method)
Escherichia coli	A	0±0	+
	B	2±0.1	+
	C	4±0.1	_
Proteus mirabilis	A	0±0	+
	B	2±0.1	+
	C	5±01	_
Proteus mirabilis	A	0±0	+
	B	1±0.1	+
	C	4±0.1	_

A; chicken gizzard membrane extract treated with hot water for 24hr. _: presence of growth
B: chicken gizzard membrane extract treated with hot water for 1hr. + : No growth
C: chicken gizzard membrane extract without any treatment.

In many studies the ***Escherichia coli*** was the predominant uropathogen (80%) isolated in acute community-acquired uncomplicated infections, followed by ***Staphylococcus saprophyticus*** (10% to 15%), While ***Enterococci, Klebsiella, Enterobacter*** and ***Proteus species***, infrequently cause uncomplicated cystitis and pyelonephritis [5].

The current study recorded some different result when it found that the ***Proteus species*** was dominant causative of UTI, followed by ***Escherichia coli*** then ***Staphylococcus***. [10] observed ***Proteus mirabilis*** is a pathogenic gram-negative bacterium that frequently causes kidney infection, typically established by ascending colonization of the urinary tract.

Many researches study the urinary tract infections and the effectiveness of the use of antibiotics as a first option in the treatment [11-15].

Microbiological confirmation of a urinary tract infection (UTI) takes 24-48 hrs. In the meantime, patients are usually given empirical antibiotics, sometimes inappropriately [16].

The use of antibiotics for a long time may lead to the emergence of new isolates of antibiotics resistant bacteria, which have been sensitive in the past. Sometimes the use of antibiotics leads to the emergence of side effects, which vary in severity from simple cross to high-risk to human health. Thus there is a need to use an alternative treatment of antibiotic must be effective against bacteria cause UTI

and urinary stones and have few or no side effects at the same time.

Chicken gizzard is the muscular stomach of chicken [17]. In Nigeria, chicken gizzard is a major source of protein to the population and is widely consumed [18]. ***Jineiji*** has trace amounts of pepsin and amylase, Due to the presence of these enzymes of protein digestion explains why there is remarkable effectiveness of the gizzard membrane against tested bacteria.

Because of these materials affected by heat, this explains the fact that the non-heat treated extract was more effective compared with another which extracted using hot water.

This study advises focusing on the use of fresh cold-aqueous extract of chicken gizzard membrane to treat UTI and stones instead of the extravagant use of antibiotics.

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