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Antimicrobial Effect of Green Tea Extract on Uropathogenic *Escherichia coli* Isolates

Hana Mohammed Ahmed*, Babiker Saad Almugadam, Shima Mubarak Mohammed Baher and Khansa Elneel Suliman Elbasher

Department of Microbiology, Faculty of Medical Laboratory Sciences, University of El Imam El Mahdi, Kosti city, Sudan

Corresponding author:
Hana Mohammed Ahmed

aumahd2010@gmail.com

Assistant Professor, Department of Microbiology, Faculty of Medical Laboratory Sciences, University of El Imam El Mahdi, Kosti City, Sudan.

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Abstract

Background: The development of antibiotic resistance in bacteria is a growing problem worldwide especially in uropathogenic Escherichia coli (*E.coli*) isolates.

Objective: This work was designed to assist the in vitro antimicrobial effect of green tea extract against uropathogenic *E.coli* isolates and to determine the MIC and MBC of green tea extract.

Materials and Methods: This is experimental laboratory based study. A total of 10 uropathogenic *E.coli* isolates were collected from urinary tract infected patients attended Kosti Teaching Hospital during period from September to October 2017. All isolates were identified base on colonial morphology, Gram stain, and standard biochemical tests. Each isolate was subjected to evaluation of their susceptibility to green tea extract using well diffusion and broth dilution method. Data was analyzed using statistical package for social sciences (SPSS) software version 17.

Results: The means of inhibition zone of bacteria to 25%, 50%, and 100% concentrations of green tea extract were 10 mm, 18 mm, and 30 mm respectively. The MIC and MBC of green tea extract for most isolates were 200mg/ml and 400mg/ml respectively.

Conclusions: Green tea extract has in vitro antimicrobial effect on uropathogenic *E.coli.* Implementation of in vitro and in vivo studies to evaluate the antimicrobial effect of green tea and the efficacy of its catechins in the treatment of UTIs can lead to discover of a cheapest therapy.

Keywords: Green tea extract, MBC, MIC, Uropathogenic *E.coli*, UTI

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Introduction

Urinary tract infections (UTIs) are the most common type of infection worldwide, and have resulted in billions of dollars in medical care costs [1,2]. *E.coli* is the most important cause of 80-90% of all UTIs [3]. Uropathogenic *E. coli* infects the urinary tract by producing special surface proteins (adhesions), which give them the ability to attack the epithelial cells that line the urinary bladder [4,5]. If pathogenic *E. coli* is in the bladder, and is not eliminated, it may travel up the ureters to the kidneys and cause pyelonephritis [3,6]. The development of antibiotic resistance in bacteria is a growing problem worldwide. A numbers of *E.coli* isolates have been collected from urine specimens of patients with UTI that are resistant to antimicrobial agents commonly uses to treat UTIs [7,8]. Therefore, the treatment options were replaced with a second or third choice of antibiotics, which are much more expensive [8]. This challenges have been receiving growing interest to find alternative antimicrobial agents from plant extracts that need to be developed and use to control multidrug resistant bacteria [9,10]. Camellia sinensis (green tea) is one of the most popular beverages in the world and has been reported to have antimicrobial effects against various pathogenic bacteria [11]. Tea can be cultivated in many regions from sea level to high mountains. It is generally safe nontoxic, cheap, and available. These properties make it a very good alternative antimicrobial agent. Many studies on the antibacterial activity have shown that green tea inhibits the growth of *E. coli* by it is polyphenolic components (also known as catechins). The bactericidal action of catechins is due to its hydrogen peroxide generation [12]. Also Polyphenols are anti-inflammatory agents that inhibit clinical symptoms of UTIs [13]. Catechins induce production of cytokines such as IL-12 and IL-10,

blocking the connection of conjugated R plasmid in *E. coli* that have bactericidal and antitoxin effects, and decrease tumor necrosis factor alpha gene expression which is important in pathogenesis of *E. coli* infection [7]. Catechin-copper II complexes damage the cytoplasmic membrane of *E.coli* [14]. This work was designed to assist the in-vitro antimicrobial effect of green tea extract against uropathogenic *E.coli* isolates and to determine the minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) of green tea extract against it.

Materials and Methods

This is experimental laboratory based study, carried out in the Department of Microbiology, Faculty of Medical Laboratory Sciences, University of El Imam El Mahdi in Kosti city, White Nile state, Sudan. A total of 10 uropathogenic *E.coli* isolates were collected from urinary tract infected patients attended Kosti Teaching Hospital during period from September to October 2017. All isolates were identified based on colonial morphology, Gram stain, and standard biochemical test [15]. Each isolate was subjected to evaluation of their susceptibility to green tea extract using well diffusion and broth dilution method. This study was approved by Ethic Review Committee, University of El Imam El Mahdi. All data was analyzed using statistical package for social sciences (SPSS) software version 17.

Preparation of the plant extract

Obtained a green tea from the local markets in Kosti of dried leaves stored in the bags packed (this type of green tea is Chinese origin). Aqueous extract was prepared by mixing 50gm of *Camellia sinensis* non fermented leaves with 500ml distilled water in volumetric flask capacity of 1000 ml and left stuck with stirring in water bath for 24 hours at a temperature of 40°C, later the extracts filtered through several layers of sterilized gauze first then through filter paper. Then the filtered extracts poured in clean and sterile petri dishes and left to dry in the oven at 50°C for 3 days. 25%, 50%, and 100% concentrations were prepared from the stuck of green tea extract using distal water [16].

Agar well diffusion method

It was done to evaluate the inhibitory effect of different concentrations of the green tea extract. Bacterial suspensions equivalent to 0.5 McFarland standards was prepared from each isolate. 0.1ml of the suspension was spread on MH agar using sterile cotton swab. Then pores with 5mm diameter were made by the piercing cork in the rate of 3 pores for each plate. 0.1ml of each concentration of the extracts laid down in pores. Each plate was incubated aerobically at 37°C for 24hours. The inhibition zones of each isolate to different concentrations of green tea extract were measured in mm [16,17].

Broth dilution method

It was used to determine minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of green tea extract. The inoculums was prepared by making a direct broth suspension of isolated colonies using sterile peptone water medium (from non-selective medium, incubation not more than 18-24 hour), then the suspension was adjusted to achieve turbidity equivalent to 0.5 McFarland turbidity standards. Different concentrations (25, 50, 100, 200, and 400mg/ml) of green extract were prepared by dissolve the powder of each concentration in 1ml of peptone water then 100µl of adjusted inoculum was added to each concentration, and to a positive control tube containing only broth. The procedure was done for each isolate. All tubes were mixed and incubated aerobically at 37°C for 24 hour. MIC and MBC were measured. MIC is a first concentration in the tube showed non visible growth. MBC was detected by subculture of the tubes next to MIC tube and reported as first concentration has negative growth in subculture [18].

Results

The means of inhibition zone of bacteria to different concentrations of green tea extract were displayed in **Table 1**. The MIC and MBC of green tea extract for most isolates were 200mg/ ml and 400mg/ml respectively, except for isolate number 6 and 10. The MIC and MBC for number 6 and 10 was 100mg/ml and 200mg/ml respectively as shown in **Table 2**.

Discussion

In the last several years, the frequent occurrence of infections with beta-lactamases producers has been observed globally. The advent of beta-lactamases producers has established a great hazard to the use of many classes of antibiotics particularly cephalosporin. The high costs of treatments make attention to importance of alternative cheaper therapy. In this regard, plant extracts are being comprehensively researched by scientists. Green tea is consumed worldwide now and its beneficial physiological and pharmacological effects are very well known. As we reported in this study the means of inhibition zone of bacteria to 25%, 50%, and 100% concentrations of green tea extract were 10 mm, 18 mm, and 30 mm respectively. And the MIC and MBC of green tea extract for most

Table 1 The means of inhibition zone diameter (mm) of bacteria for different concentrations of green tea extract.

Extract concentration	100%	50%	25%
Diameter of zone (mm)	30	18	10

Table 2 The minimum inhibitory concentration and minimum bactericidal concentration of green tea extract.

Isolate number	MIC (mg/ml)	MBC (mg/ml)
1	200	400
2	200	400
3	200	400
4	200	400
5	200	400
6	100	200
7	200	400
8	200	400
9	200	400
10	100	200

isolates were 200mg/ml and 400mg/ ml, respectively. Our data suggests that the green tea extract has highly in vitro antimicrobial activity against uropathogenic *E.coli* isolates that strongly consistent with the results of Passat et al. study that reported, green tea water extract has antimicrobial effect on *E.coli* strains with MIC 275 mg/ml, which is slightly low when compare with our study [18]. Also our study agrees with Tiwari et al. study that reported green tea extract has antimicrobial effect against *E.coli,* while we disagree with Tiwari et al. study in the value of MIC as they reported it is 88.30mg /ml. The main cause of difference in the value of MIC between Tiwari et al. study and our study may be the use of green tea extract combined with ciprofloxacin in Tiwari et al. study [19].

Conclusions

Green tea extract has in vitro antimicrobial effect on uropathogenic *E.coli.* Implementation of in vitro and in vivo studies to evaluate the antimicrobial effect of green tea and the efficacy of its catechins in the treatment of UTIs in future can lead to discover of a new cheaper therapy region.

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