

IN VITRO AND IN VIVO STUDY OF GREEN AND BLACK TEA ANTIMICROBIAL ACTIVITY ON METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS.

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Keywords; green tea , MRSA bacteria , cellulites.

(received 26 May 2011, Accepted 26 June 2011)

ABSTRACT

The *in vitro* study was compared the antibacterial activity of green tea and black tea extracts against methicillin resistant *Staphylococcus aureus* (MRSA). Green tea extract was found to have higher antimicrobial activity on MRSA with an inhibition zone of 20 mm at a concentration of 10 gm/100 ml. For black tea extract, the inhibition zone was 15 mm at same concentration .The minimum inhibitory concentrations for the green tea and black tea were 4mg/ml and 8mg/ml respectively.

The *in vivo* study of the antimicrobial effect of both green and black tea was investigated, by subcutaneous inoculation of four rabbits with MRSA bacteria. After that immediately treated with a series dilution of tested material (green and black tea), the development of swelling and the degree of necrosis were scored. The green tea showed marked reduction in the size of swelling less than 2mm at the concentration of 10mg/ ml; also there is a marked reduction in the severity of necrosis. For black tea a marked reduction occur in the size of swelling (6-10mm) at the concentration of 10mg/100ml; also there is a marked reduction in the severity of necrosis at the same concentration. This study concludes that green and black tea extracts were showed to have an antibacterial activity against MRSA and a therapeutic effect against skin infection caused by MRSA in rabbits.

INTRODUCTION

In the last few decades, many bacteria have become resistant to all available antibiotics that medical research has developed. Once this happens, these new drug-resistant superbugs can become lethal. Methicillin-resistant *Staphylococcus aureus* (MRSA) are superbugs that have become resistant to several types of antibiotics like methicillin. For that MRSA become an increasingly important pathogen in both hospital and community settings⁽¹⁾. It can be transmitted between people and animals during close contact^(2,3). MRSA detected in animals including dogs, cat, rabbit, parrot and pig, some with serious infections^(4,5,6). MRSA involved in a wide variety of skin and soft tissue infections including impetigo, folliculitis, furunculosis, cellulitis, abscesses and wound infections^(7,8,9,10). Therefore, alternative chemotherapeutic agents are needed to be developed and employed to control MRSA.

For the last 10 years, research has shown that green tea has effective antibacterial action against MRSA, one of the superbugs that can spread in hospitals⁽¹¹⁾, particularly in combination with other anti-microbial agents.

The activity of tea against *S. aureus* has enabled two groups to propose tea extracts as a topical antiseptic⁽¹²⁾ and treatment of skin infections caused by *S. aureus* and *Streptococcus pyogenes*⁽¹³⁾.

MATERIALS AND METHODS

Preparation of green and black tea

Green and black tea leaves were ground separately using an electrical grinder. From the resultant powder, 10 grams of each was mixed in 100 ml of distilled water and left for one hour at room temperature. Using the same procedures, concentrations of 5, 2.5 and 1.25 grams of each were prepared⁽¹⁴⁾.

Preparation of bacterial inoculum

A (3-10) colonies of MRSA were transferred to a test tube containing (1) ml of normal saline (0.9%). Spectrophotometer was used to measure the turbidity of bacterial suspension in order to reach bacterial concentration of 10^8 at an optical density of 0.1 at a wave length of 654 nanometers⁽¹⁵⁾.

Testing extracts on MRSA

On a previously prepared Mueller Hinton agar plates, 0.1 ml of the bacterial suspension was streaked on each plate and was left for ten minutes to be absorbed. Four holes were made on the surface of the medium in each plate by using a metal perforator. Separate plates were used for green and black tea. To each hole, 0.1 ml of different concentrations of the same extract was added; the plates were then incubated at 37° C for 24 hours. The inhibition zones were measured in the next day⁽¹⁶⁾. The minimum inhibitory concentrations for the green tea and black tea was then assessed using the method of National Committee for clinical laboratory standard 1997⁽¹⁷⁾. The plates would have the following concentrations of the extracts measured as micrograms/ ml: 64, 32, 16, 8, 4, 2, 1, 0.5, 0.125, 0.06 and 0.03.

Experimental animals

Four adult rabbits weighing from 1200 to 2000 gm. were used. The hair over the both side of chest and abdomen for each rabbit was clipped short with scissors and then completely removed with a solution of hair remover with water. As a rule this depilation was 'carried out several days before the animals were inoculated.

By using 70% ethanol (Disinfectol®, 102 Chem-Lab NV, Zedelgem, Belgium) to disinfect the inoculation area (five regions), waiting five minutes and after evaporation of the ethanol. A 0.1 ml of saline solution containing 10^8 cfu of tested bacteria was injected subcutaneously into each region of rabbits by means of a tuberculin syringe and a 22 gauge needle.

In each animal Post-infection immediate treatment was performed through subcutaneous injection of the both side with 0.1 ml of Series dilutions of a black and green tea suspension, using insulin syringes. ^(18, 19) and one region left without treatment as a positive control.

Rabbits were inspected daily for the development of macroscopic skin lesions depending on vernier caliper measurement.

Description of Skin Lesions

The development, appearance and size of abscesses were scored, a significant marked reaction appear about the site of inoculums after 24 to 48 hours of inoculation

Macroscopic lesions were recorded for five day, and elevated by a diameter of swelling: negative (< 2 mm), small (2-5 mm), moderate (6-10 mm), and sever (>10mm). Necrosis of the area was defined as; negative, slight (<2mm), moderate (2-5mm), and intensive (> 5mm) ⁽²⁰⁾.

RESULTS

The results showed that the inhibitory effect of green tea on MRSA was greater than that of black tea table (1). For green tea, the highest concentration of 10gm/100ml distilled water resulted in maximum inhibition of MRSA growth with an inhibition zone of 20mm, while the lowest concentration of 1.25gm/100ml lead to lowest inhibition zone (5mm) .For black tea, the inhibition zones recorded for the same concentrations were 15mm and 0 mm respectively.

The average values minimum inhibitory concentration (MICs) of the effective extracts are presented in table 1. Macroscopically, the using of green tea sample 10mg/ 100ml showed marked reduction in the size of swelling less than 2mm, in 5gm/ 100ml concentration the size of swelling reduced to 2-5 mm, in 2.5gm/ 100ml concentration the size of swelling equal to 6-10mm and by using 1.25gm/ 100ml concentration of green tea sample the size of swelling was more than 10mm table (2).

In comparison to black tea sample 10gm/ 100ml, marked reduction occur in the size of swelling 6-10mm, and so it's in other concentration 5gm/ 100ml and 2.5gm/ 100ml respectively. And the size of swelling is more than 10mm when the concentration of black tea sample is 1.25gm/ 100ml table (2).

Also the table 3 showed there is a marked reduction in the severity of necrosis (no necrosis) by using the green tea sample in concentration of 10gm/ 100ml, 5gm/ 100ml and 2.5gm/ 100ml. While the degree of necrosis was equal to 2-5mm in concentration of 1.25gm/ 100ml of green tea sample.

In compare to black tea sample in which there is no necrosis occur in concentration of 10gm/ 100ml and 5 gm/ 100ml, while the degree of necrosis was equal to 2-5 mm in both concentration 2.5mg/ 100ml and 1.25mg/ 100ml respectively, as showed in table 3.

The severity of these reactions decreased with increase increment in the concentrations of the tested material and depending on the type of tested materials.

Usually a marked lesion (necrosis) was visible when the dilutions of the tested material are nil.

Table(1): *In vitro* inhibitory and minimum inhibitory effects of green tea and black tea on methicillin- resistant *Saphylococcus aureus* (MRSA)

Green and Black tea concentrations gm/ml	Inhibitory effect of green tea on MRSA	Inhibitory effect of Black tea on MRSA
	Inhibition zone of MRSA	
10gm/100ml D.W 10%	20 mm	15 mm
5gm/100ml D.W 5%	15 mm	5 mm
2.5gm/100ml D.W 2.5%	10 mm	0
1.25gm/100ml D.W 1.25%	5 mm	0
MIC mg/ml	4	8

Table (2): Appearance of swelling in four adult rabbits after subcutaneous inoculation with, MRSA and a serial dilution of black and green tea.

The score of swelling size, diameter: nil, < 2 mm; small, 2-5 mm; moderate, 6-10 mm; sever, >10mm respectively.

Materials	Concentrations Of materials	Size of swelling			
		Nil	small	Moderate	sever
Black tea	1.25 gm	0	1	2	1
	2.5 gm	2	1	1	0
	5 gm	1	2	1	0
	10 gm	1	2	1	0
Green tea	1.25 gm	0	1	2	1
	2.5 gm	2	1	1	0
	5 gm	3	1	0	0
	10 gm	4	0	0	0

Table (3): Appearance of necrosis in four adult rabbits after subcutaneous inoculation with, MRSA and a serial of dilution of black and green tea.

Materials	Concentrations Of materials	Degree of necrosis			
		Nil	Slight	Moderate	Intensive
Black tae	1.25 gm	2	1	1	0
	2.5 gm	2	1	1	0
	5 gm	4	0	0	0
	10 gm	4	0	0	0
Green tae	1.25 gm	2	1	1	0
	2.5 gm	3	1	0	0
	5 gm	4	0	0	0
	10 gm	4	0	0	0

The score of necrosis size, diameter: negative, slight, < 2 mm; moderate, 2 -5 mm and intensive, > 5 mm respectively.

DISCUSSIONS

Many *in vitro* and *in vivo* studies had proved the antibacterial activity of green tea catechins on some bacteria. Green tea is generally considered to be a safe nontoxic beverage and its consumption is usually without side effects.

Green tea is not fermented during processing preserving the enzymes and olive green color while black tea is fermented before drying. Fermentation can destroy some of the active components of black tea ^(21, 22).

In this study the *in vitro* effects of different concentrations of green and black tea on MRSA were tested. Various Concentrations of green tea were found to clearly inhibit growth MRSA *in vitro*. The inhibitory effect was found to be proportional to the amount of green tea present in the aqueous concentration. The minimum inhibitory concentration (MIC) for MRSA was found to be 4 micrograms/ml.

The mechanism by which green tea inhibits the growth of MRSA was explained by many authors. The polyphenols which are constituents of tea extracts have previously been shown to have antibacterial activities against human and animal disease- related bacteria and phytopathogenic bacteria and food born bacteria ⁽²³⁾. A Study published in 2008 have revealed that tea polyphenols can inhibit the growth of MRSA in the laboratory. These authors suggest that drinking two to three cups of green tea per day is generally safe and may provide the necessary benefits ⁽²⁴⁾. Toda *et al* (1992) showed that a mixture of tea polyphenols could protect rabbits from experimental infection with *Vibrio cholera* and they suggest that patients suffering from Cholera could benefit if tea extract were added to oral rehydration solutions ⁽²⁵⁾.

MRSA strains have been detected in animals (including dogs, a cat, a rabbit, a parrot and a pig), some with serious infections ^(4, 5, 6). Some strains have been linked to skin and soft tissue infections and severe necrotizing pneumonia ^(26, 27, 28). This organism can be involved in a wide variety of skin and soft tissue infections including impetigo, folliculitis, furunculosis, cellulitis, abscesses and wound infections ^(29, 30, 31, 32). Many animal models have the ability to describe the pharmacokinetics (PK) and pharmacodynamics (PD) of antimicrobial therapy. *In-vivo* studies have a distinct advantage over *in-vitro* models in the ability to determine which PK/PD dosing index is most closely associated with efficacy ⁽³³⁾. Animal models are useful in determining the impact of drug concentration on the rate and extent of antimicrobial killing. They can also determine post-antibiotic effects on bacterial growth, which is often of longer duration than when measured with *in-vitro* techniques.

Other study found that intradermal inoculations was able to reproduce *S. aureus* skin infections in rabbits similar to those seen in the field This infection model is thus suitable for further rabbit *S. aureus* research. It has also a potential for other *S. aureus* research, for instance in the field of MRSA or *S. aureus* isogenic mutant strains, other models bypass the epidermal barrier by injecting the bacterial inoculums directly intradermally or subcutaneously ⁽³⁴⁾.

Many study demonstrated green tea's antimicrobial activity against a variety of gram-positive and gram-negative pathogenic bacteria that cause cystitis, pyelonephritis, diarrhea, dental caries, pneumonia, and skin infections⁽³⁵⁾.

Green tea polyphenols are potent free radical scavengers due to the hydroxyl groups in their chemical structure. The hydroxyl groups can form complexes with free radicals and neutralize them, preventing the progression of the disease process and the inflammation reactions⁽³⁶⁾. Also the green tea polyphenols have demonstrated significant anti-oxidant, anti-carcinogenic, anti-inflammatory, thermogenic, probiotic and anti-microbial properties in numerous human, animals and in vitro studies (37, 38, 39, 40).

Other workers have proposed epigallocatechin gallate as the main anti-bacterial component of green tea⁽⁴¹⁾, particularly in combination with other anti-microbial agents. The activity of tea against *S. aureus* has enabled two groups to propose tea extracts as a topical antiseptic⁽⁴²⁾ and treatment of skin infections caused by *S. aureus* and *Streptococcus pyogenes*⁽⁴³⁾.

Other study has shown that green tea extracts can inhibit the growth of methicillin resistant *Staphylococcus aureus* (MRSA)⁽⁴⁴⁾. Using electron microscopy, the same workers have also shown that the method of action for this effect is by inhibition of cell division⁽⁴⁵⁾.

In conclusion the study showed that green and black tea extracts have an antibacterial activity against MRSA and a therapeutic effect against skin infection caused by MRSA in rabbits.

دراسة الفعالية ضد جرثومية للشاي الأخضر و الأسود على جرثومة methicillin resistant *Staphylococcus aureus* (MRSA) داخل وخارج الجسم الحي.

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الخلاصة

أجريت الدراسة المختبرية لمقارنة التأثير التثبيطي لكل من الشاي الأخضر و الأسود ضد البكتيريا المرضية العنقودية المقاومة للمثيسلين ، أظهرت الدراسة إن خلاصة الشاي الأخضر لها تأثير تثبيطي على الجرثومة methicillin resistant *Staphylococcus aureus* (MRSA) في الوسط الزراعي حيث كانت منطقة التثبيط (٢٠) ملم عند التركيز (١٠ غم / ١٠٠ مل) بينما كانت منطقة التثبيط للشاي الأسود (١٥) ملم عند التركيز ذاته. وجد ان اقل تركيز مثبط للشاي الأخضر و الأسود (٤) مايكرو غرام / مل و (٨) مايكرو غرام / مل على التوالي .

ولقد أثبتت الدراسة داخل الجسم التأثير المضاد للجراثيم لكل من الشاي الاخضر و الشاي الاسود، وذلك من خلال حقن اربعة أرناب تحت الجلد بالبكتيريا المرضية MRSA، وبعد ذلك تمت عملية حقن المنطقة مباشرة

بتخافيف متعدده من عينات الشاي الاخضر و الشاي الاسود، لقد تمت عملية قياس درجة الالتهاب و التورم الحاصله في منطقه الحقن. اظهرت الدراسه ان للشاي الأخضر تأثير واضح في انخفاض حجم التورم الحاصل في المنطقه الى اقل من ٢ملم في التركيز المساوي ل ١٠ ملغرام/١٠٠مل، وتأثير واضح في انخفاض درجة التقرح الحاصله في المنطقه. كذلك اظهرت الدراسه ان للشاي الاسود تأثير واضح في انخفاض حجم التورم الحاصل في المنطقه بين ٦-١٠ ملم في التركيز المساوي ل ١٠ ملغرام/١٠٠مل وكذلك تأثير واضح في انخفاض درجة التقرح الحاصل في المنطقه و في نفس التركيز. لقد اثبتت الدراسه ان للشاي الاخضر و الشاي الاسود تأثير علاجي للامراض الجلدية في الارانب الحاصله بسبب الاصابة MRSA .

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