

Antibacterial Effects of Aqueous Extract Green Tea on Multi-Antibiotic Resistant Isolates of burn infection

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Abstract

Burn wound infection is problematic because it delays healing encourages scarring and may result in bacteremia, sepsis or multiple-organ dysfunction syndrome whereby organs from several systems are unable to maintain homeostasis on their own, requiring immediate medical attention. Burn infection may be caused by *Staphylococcus aureus* or *Streptococcus pyogenes* and *Pseudomonas aeruginosa*. Different antibiotics were used for the treatment of burn given topically or systemically. The aim of the present work is to estimate the effectiveness of water soluble green tea extracts on different types of bacteria which were isolated from children suffering from nosocomial burn infection. The study was conducted in Alyarmok Hospital lobby of burns and wounds in Baghdad for the period from July 1, 2011 to 1 June / 2012 . Forty tow samples with positive bacterial cultures were obtained from children with burn infection, they were tested by doing antibiotic sensitivity test with different antibiotics, and the resistant culture of different types bacteria. The bacteria were isolated from infected patients, were tested by aqueous extracts green tea were done and cultured by the brain and heart infusion, blood agar, and Mackonky agar medium. The study revealed that aqueous extract green tea was effective as antibacterial agents, against antibiotic's strains of staph. aureus, Strep(6) isolats, Pyogenes (9)isolats, and *Pseudomonas aeruginosa* (4) isolats ($P>0.05$).

Conclusion

The study conclude that the aqueous extract of green tea was relatively effective as antibacterial agent in vitro and its effect was directly proportional with its concentration (10%), but the concentration of 5%, 15%, and 20% less than effects .

Keyword: burn, *Staph aureus* , *Strep. pyogenes* , *Pseudomonas aeruginosa*, children .

Introduction

Bacteria and fungi are the most common pathogens of burn wounds. These microbes form multi-species biofilms on burn wounds within 48 – 72 hours of injury [1]. Organisms originate from the patient's own skin, gut and respiratory flora, as well as from contact with contaminated health care environments and workers [1, 2]. Gram-positive bacteria are some of the first to colonize burns, followed quickly by gram-negative. Fungal infection tends to occur in the later stages after the majority of bacteria have been eliminated by topical antibiotics [2]. Two bacterial species, methicillin - resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* will be examined, two of the most prevalent infective agents [3]. These two species have proven particularly difficult to treat because they possess a large number of virulence factors and antimicrobial resistance genes [3,4]. Green tea has been found to be superior to black and oolong tea in terms of antioxidant, anti-inflammatory, anti-carcinogenic and antibacterial activity and other health promoting benefits [5,6]. Also green tea components show antiviral and antifungal activities in addition to their inhibitory effects on exotoxins [7].

Various studies have shown significant suppressive effects of green tea against many microorganisms, for example *Salmonella typhimurium* [8], *Salmonella typhi*, *Shigella dysenteriae*, *Yersinia enterocolitica*, *Escherichia coli*, *Staphylococcus aureus*, *Vibrio cholerae*, *Campylobacter jejuni*, *Plesiomonas shigelloides*, *P. aeruginosa* and many other species of bacteria [3,7,8,9,11, 12] .

Patients &Methods

The study was conducted in the city of Alyarmok Hospital lobby of burns and wounds for the period from July 1, 2011 to 1 June / 2012. Forty two samples of cultured bacteria, which were isolated from burn wound of children with wound in Baghdad A total of isolated bacteria tested for these sensitivity to the antibiotics by using Kirby–Bauer disk method, the antibiotics include methicillin, cefotaxime, cefalexin piperacillin, gentamicin, erythromycin and ciprofloxacin [13]. The resistant isolates were selected according to the diameter of inhibition zone, were selected, and their sensitivity to the aqueous extract of green tea of concentration 10 mg /ml stock solution , by using agar –well diffusion [14, 15].

Different concentrations of water soluble green tea (1, 2, 3, 4 and 5 ml)were obtained from the original stock solution of green tea and diluted by using distill water (to obtain a total 10 ml for each concentration)and the bacteria were tested for their susceptibility to the extract using the kirby Bauer method measuring the diameter of inhibition zone . The MIC and MBC of different concentration (1, 2, 3,4 and 5ml) of green tea extract were measured by using Agar –dilution method [2] . MIC (minimum inhibition concentration) which was the lowest concentration of the compound that inhibit the bacterial growth MBC (minimum bactericidal concentration) is minimum concentration of compound which decrease the number of bacterial colonies by 99.9% of culture [2].The statistic analyzed according percentage.

Results

Table 1 shows the antibiotic sensitivity tests of different types of antibiotic on different types of bacteria. The percentage of resistance of staph. aureus to cephalixin, methicillin and Erythromycin was (50, 71.4 and 70.8%) respectively, Streptococcus . Pyogenes resistance was (50, 71.4 and 70.8 %) respectively and Pseudomonas areuginosas for was (75, 78.6 and 62.5 %) respectively, while resistance of Staph. aureus to cefotaxim and gentamicin was (37.5 and 33.3%), Strep. pyopenes resistance was 42.9% for both and Pseudomonas areuginosas resistance was 25% for both antibiotics).

The resistance of Staph .aures to ciprofloxacin and pipracillin was 33.3 and 41.7%), Strep .Pyogenes resistance was 35.7% for both and Pseudomonas areuginosas was sensitive to both antibiotics. The study shows that aqueous extract green tea was effective against different types of resistant bacteria (Staph aureus, strep. pyogenes and Pseudomonas areuginosas) as in table 2. The diameter of inhibition zone was (8.25, 10.14, 11.20, 13, 40, and 15.00) mm for different concentrations of water soluble green tea extracts, to strepto .

pyogenes was 00.0, 2.75, 4.42, 6.75, 8.85) mm and for Pseudomonas areuginosa was (1.00, 5.32, 7.12, 9.25, 12.25) mm^[13]. Table -3 showed the effect of different MIC concentration on the bacteria .

Table 1: Percentage of resistant bacteria to different antibiotics

Antibiotics	Staph aureus	Strep. pyogenes	Pseudomonas areuginosas
	No: 24 Resentence%	No: 14 Resentence%	No: 4 Resentence%
Cephalexin	12 (50%)	10 (71.4%)	2 (50%)
Cefotaxime	9 (37.5%)	6 (42.9%)	1 (25%)
Methicillin	17 (70.8%)	10 (71.4%)	2 (50%)
Erythromycin	15 (62.5%)	11 (78.6)	3 (75%)
Gentamicin	8 (33.3%)	6 (42.9)	1 (25%)
Piperacillin	10 (41.7%)	5 (35.7%)	0 (0%)
Ciprofloxacin	8 (33.3%)	5 (35.7%)	0 (0%)

Table 2: Inhibition zone (mm) of aqueous extract green tea on bacteria

Bacteria	Inhibition zone in different concentration (mm)				
	1mg/ml	2mg/ml	3mg/ml	4mg/ml	5mg/ml
Staph .aureus	8.25	10.14	11.20	13.40	15.00
Strep.pyogenes	0.00	2.75	4.42	6.75	8.85
P. areuginosa	1.00	5.32	7.12	9.25	12.25

Table 3: MIC Concentration Inhibition of aqueous extract green tea on the bacteria

Bacteria	MIC concentration of aqueous extract green tea			
	5% conc.	10%	15%	20%
Staph .aureus	0	6	2	2
Strep. pyogenes	0	9	3	4
Pseudomonas areuginosas	0	4	1	1

Table 4: values of MIC and MBC of aqueous extract green tea

Bacteria	Water Soluble green tea		
	MIC	MBC	Total
Staph .aureus	2	4	6
Strep. pyogenes	4	5	9
Pseudomonas areuginosas	3	1	4

Discussion

The results of this study revealed that the aqueous extract green tea was relatively effective as antibacterial agent against Pseudomonas areuginosas,

which was efficient than on staph. aureus and strep. Pyogenes .The antibacterial activity of green tea was due to the presence of catechin compound, which induce rapid leakage of small molecules entrapped in

the intraliposomal space and aggregation of the liposome which caused lyses, and kill the bacteria^[12]. The study also showed that the antibacterial activity of green tea was parallel to that of different types of antibiotics and black tea^[13].

We conclude that the aqueous extract of green tea was relatively effective as antibacterial agent in vitro and its effect was directly proportional to its concentration. Its antibacterial effect was directly proportional with its concentration according to the values of MIC & MBC as in table 4; the results revealed that the aqueous extract green tea were more effective as antibacterial agent against staph. aureus, *Pseudomonas aeruginosa* than *Strep. Pyogenes*, which was due to the bactericidal effects of catechins of green tea, which is related to the Gallic acid moiety and the hydroxyl group member, which cause

References

- Salimi, H. Yakhchali, B., Owlia, P., Lari, A. R. Best Approach for Burn Wound Infection. In Medical Microbiology .24th Ed 2010. McGraw Hill.2010; 78: 4311-4319.
- Geo F, Karen C, Janet S, Stephen A. Ntimicrobial Chemotherapy, Measurement of Antimicrobial activity. Jawetz Medical Microbiology, 24th ed. 2007: 168.
- Stapleton, P.D., S. Shah, J.M. Hamilton-Miller, Y. Hara, Y. Nagaoka, A. Kumagai and P.W. Taylor. Anti-Staphylococcus aureus activity and oxacillin resistance modulating capacity of 3-O-acyl-catechins. Int. J. Antimicrob. Agents.2004; 24: 374-380.
- Jazani, N.H., S. Shahabi and A. Abdi Ali. Antibacterial effects of water soluble green tea extracts on multi-antibiotic resistant isolates of *Pseudomonas aeruginosa*. Pak. J. Biol. Sci 2007; 10: 1544-1546.
- Siddiqui, I.A., V.M. Adhami, M. Saleem and H. Mukhtar. Beneficial effects of tea and its polyphenols against prostate cancer. Mol. Nutr. Food Res. 2006;50: 130-143.
- Tiwari, T.P., S.K. Bharti, H.D. Kaur, R.P. Dikshit and G.S. Hoondal. Synergistic antimicrobial activity of tea and antibiotics. Ind. J. Med. Res. 2005;122: 80-84.
- Kiessoun Kon. atél. Antibacterial activity of aqueous extracts green tea against bacterial infection .2011; 11: 4-7.
- Lee, Y.L., T. Cesario, Y. Wang, E. Shanbrom and L. Thrupp. Antibacterial activity of vegetables and juices. Nutrition.2003; 19: 994-996.
- Kim, S., C. Ruengwilysup and D.Y. Fung. Antibacterial effect of water-soluble tea extracts on food borne pathogens in laboratory medium and in a food model. J. Food Prot.2004 67: 2608-2612.
- Sharquie KH, AL-Turffy EH Antibacterial effect of black tea in vitro and vivo. Journal of Dermatology, 2000; 27: 706 -10.
- Yoko Y, Yoshimasa y, Yukihiro H, Tadakatsu S. A combination effect of green tea catechins, with antibiotics on *Helicobacter pylori* growth in vitro. Current Microbiology, an International journal, Vol .47, 2003: 244 -49.
- Masatomo H, Kazuko T. Multiple effects of green tea catechin on the antifungal activity of antimycotics against *Candida albicans* Jurnal of antimicrobial Chemotherapy 2003; 19: 1-4.
- Baron EI, Fingold SM, Peterson LR. Laboratory Methods for diagnosis of bacterial infections. Bailey and Scotts Diagnostic Microbiology, 9th ed 1994; Mosby company Missoni USA : 148.
- National Committee for Clinical Laboratory standards. Performance standards for antimicrobial disk susceptibility test. Twelfth information supplement .2002; Document M100-S12, Vol 22, No.1.
- Boyanova L, Derejian S, Koumanova R, Katsnova N et al. Inhibition of *Helicobacter Pylori* growth in vitro by Bulgarian propolis : preliminary report. J. Med Microbial 2003 ; 52 (5) : 417-9 .15 – Sawaye AC, Palmo AM, Caetans FM.
- Al-Rejaie, S.S. Effect of green and black teas on immobilization induced stress in male wistar albino rats. Int. J. Pharmacol.2009; 5: 137-145.
- A.Y. Obaid, O.A. Abu-Zinadah and H.K. Hussein. The Beneficial Effects of Green Tea Extract and its Main Derivatives in Repairing Skin Burns of Rabbit. International Journal of Biological Chemistry. 2011; 5: 103-115.

فعالية خلاصة الشاي الأخضر المائي على أنواع مختلفة من البكتيريا التي تم عزلها من الأطفال الذين يعانون من الحروق

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الملخص

تعد أخماج الحروق مشكلة بسبب تأخر شفاء الجرح والذي قد يؤدي إلى تجرثم الدم ، أو متلازمة الاختلال حيث أن الأجهزة غير قادرة على الحفاظ على التوازن ، الأمر الذي يتطلب عناية طبية فورية وقد يكون سبب الأخماج هو انتهازية بعض أنواع الجراثيم كالمكورات العنقودية والمسبحية وكذلك الأورجينيوزا الزائفة وهناك عدة مضادات حيوية تعطى موضعياً أو عن طريق الفم أو الحقن كعلاج لتلك الأخماجات. الهدف من هذه الدراسة هو تقدير فعالية خلاصة الشاي الأخضر المائي على أنواع مختلفة من البكتيريا التي تم عزلها من الأطفال الذين يعانون من الحروق في المستشفيات. أجريت هذه الدراسة في مستشفى اليرموك ردهة الحروق والجروح للفترة من 1 تموز 2011 إلى 1 حزيران / 2012 . وقد تم عزل 42 عينة والمأخوذة من الأطفال الذين يعانون أخماجات الحروق وقد تم اختبارها المضادات الحيوية عليها حيث أنها كانت مقاومة لمختلف المضادات الحيوية وكذلك تم اختبار حساسيتها لمستخلص الشاي الأخضر المائي وتركيز 10% . كشفت الدراسة أن مستخلص الشاي الأخضر المائي وتركيز 10% كان فعالاً ضد الجراثيم ، لسلاسل المكورات العنقودية : (6) عزلات ، المكورات المسبحية القححية (9) عزلات، والأورجينيوزا الزائفة (4) عزلات ($p > 0.05$) .

الاستنتاجات : أستنتجنا أن مستخلص الشاي الأخضر الذائب في الماء كان فعالاً نسبياً كعامل مضاد للجراثيم في المختبر ، وتأثيره كان يتناسب طردياً مع تركيزه (10 %) ، لكن التراكيز 5% و 15% و 20% هي أقل تأثيراً .

الكلمات الرئيسية : الحروق ، المكورات العنقودية الذهبية ، المكورات المسبحية القححية ،الأورجينيوزا الزائفة ، الأطفال .