Effect of Lipotiechoic acid extracted from *Enterococcus faecalis*on normal and some cancer cell lines

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Abstract

The current study included three parts: the first part is the isolation and identification of 12 isolates of *Enterococcus faecalis* from 45 different samples included 6 samples of urine, 20 samples of feces, 7 samples of skin,10 samples of Crop of the chicken and 2 samples of Liver of the sheep by using the cultural, Biochemical and serological tests from November 2010 to March 2011. Resistance of *E. faecalis* isolates to antibiotics were examined. Some of these isolates were resistant to 9 out of 16 antibiotics were used in this study, (100% to Streptomycin, 92% Tobramycin, 83% to Amikacin, 66% to Erythromycin, 75% Ceftazidime, 84% Augmentin, 33% Cefotaxime, 60% Tetracycline and 91.5% Nalidixic acid). On the other hand, all isolates were sensitive to Genatmicin, Chloramphenicol, Penicillin G, Ampicillin, Vancomycin, Rifampcin and Co-trimexazole. In the second part of the study, lipoteichoic acid (LTA) was extracted from *E. faecalis* that was isolated from feces of infected child and possessed high resistance to antibiotics by using Hot phenol, thereafter partial purification by using Sepharose CL-6B gel filtration chromatography was done. The peak of LTA was extended from fraction 8 to 18. The contents of partial purification of LTA from phosphorous 91.37 mg/ml and 0.022 mg/ml protein were measured. The third parts involved examination of cytotoxic effect of crude and partial purified LTA in normal and several cancer cell lines. Both crude and partial purified extracts at 5000 mg/ml were exhibited high inhibitory activity to Gliobl astoma (AMGM) and Ahmed-Mohammed- Nahi, 2003 (AMN-3) at exposure time 24h. reducing in the percentage of inhibition was noticed when the exposure time was increased. On the contrary, low concentration of both extracts of LTA were induced the proliferation of cancer cells of AMGM and AMN-3 at exposure time 24h. Notably, when the exposure time was increased, the proliferation of AMGM and AMN-3 was decreased. Obviously, the cytotoxicity of the both extracts (crude and partial purified) has slight effect on normal cell line, Rat Embryo fibroblast (Ref).

تأثير حامض التكويك الدهني (LTA) المستخلص من بكتيريا *E. faecalis* في الخلايا الطبيعية وبعض الخطوط الخلوية السرطانية

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الخلاصة

تضمنت الدراسة الحالية ثلاث أجزاء: الجزء الأول عزل وتشخيص اثنا عشر عزله من جرثومة *Enterococcus faecalis* تم الحصول عليها من 45 عينه مختلفة تتضمن (6 عينات من الإدرار، 20 عينة

خروج، 7 عينات من الجلد، 10 عينات من حويصلة الدواجن، 2 عينة من كبد أغنام باستعمال الاختبارات الزرعية والكيموحيوية والمصلية للفترة من شهر تشرين الثاني لغاية شهر آذار. فُحصت مقاومة عزلات*E. Faecalis* للمضادات الحيوية، وأظهرت بعض العزلات مقاومتها لـ9 مضادات من أصل 16 مضاداً أُستخدم في هذه الدراسة، (100%) للمضاد Streptomycin، وبلغت مقاومتها لمضاد Tobramycin (92%)، Amikacin (83%)، Erythromtcn (66%)، Ceftazidime (75%)، Augmentin (84%)، Cefotaxime (33%)، Tetracycline (60%)، Nalidixic acid (91.5%). بينما كانت العزلات جميعها حساسة للمضادات: Gentamycin، Chloranphemicol، Penicillin G وAmpicillin وVancomycin وRefampcin وCo-tramoxazole. أما الجزء الثاني من الدراسة فتتضمن استخلاص حامض التكويك الدهني (LTA) من بكتيريا *E. faecalis*، من عزله براز من طفل صغير والتي تميزت بمقاومتها العالية للعديد من المضادات الحيوية المدروسة، بطريقة Hot phenol، ثم نُقي جزئياً بوساطة كروماتوگرافيا الترشيح الهلامي باستخدام هلام Sepharose CL-6B، وامتدت قمة الـLTA من الجزء (8-18)، وبلغ تركيز الفسفور 91.37 مايكرومول/مليليتر، أما تركيز البروتين فكان 0.022 ملغرام/ مليليتر.الجزء الثالث شمل التأثير السمي الخلوي لمستخلص LTA الخام والمُنقى جزئياً في بعض الخطوط الخلوية السرطانية والطبيعية. وأظهر المستخلص الخام والمُنقى جزئياً خصوصاً عند التركيز 5000 مايكروغرام/ مليليتر فعالية تثبيطية عالية في الخلايا السرطانية AMGM وAMN-3 بمدة تعريضية 24 ساعة. ثم بدأت هذه النسب بالانخفاض مع ازدياد مدة التعريض وعلى العكس من ذلك سجلت التراكيز الواطئة من المستخلصين كليهما تحفيزاً للخلايا على التضاعف والانقسام عند 24 ساعة من التعريض، واختفى هذا التحفيز عند زيادة مدة التعريض للخطين لكيهما AMGM وAMN-3. وأبدى المستخلصان كلاهما (الخام والمُنقى جزئياً) تأثيراً تثبيطياً بسيطاً في خط الخلايا الطبيعي Ref.

Introduction

Cancer is one of the most dangerous diseases known in the world; it becomes the most deadly disease after heart attack (1). Cancer is severing complex and very heterogeneous disease. It is not one single disease, but a group of many different diseases, with several common and with many differing characteristics. This is the main reason why searching for better cancer therapies so difficult (2). Surgical therapy is the first line of treatment of cancer at an early stage, surgery may be sufficient to cure the patient by removing all cancerous cells. It becomes ineffective when the tumor metastasizes from the primary site. Radiotherapy side effect is that radiation is not specific to cancerous cells and may damage healthy cells as well. Chemotherapy have serious side effects and its toxicity not limited to cancer cells it extended to normal cells as well, also tumor cells may develop resistance to it (3). So, the researchers went to alternative treatments such as plant extracts, that will destroy the cancer cells without causing damage to normal cells (4). Biological therapy is that form of cancer therapy in which antitumor effects are produced primarily through the action of natural host defense mechanisms augmented by the administration of immunological active substances. Component structures of the bacterial cell wall have an important role in the inhibition of cancer cells. *Enterococcus faecalis* are secondary infection acquired while in the hospitals (5). *Enterococci* have several virulence factors that areresponsible for the disease events, including: resistance to antibiotics, production ofCytolysin, aggregation substance, Gelatinase, Extracellular superoxide, *Enterococcal*surface protein, Hylauronidase, Lipoteichoic Acid (LTA), and Surface

carbohydrate. LTA is highly effective towards the cell membranes, as it is linked to the LTA in various animal cells by the lipid, it is possible to cause inflammation of the arthritis in laboratory animals improve the inflammatory reaction and localized toxicity, Characterized by the ability to link cells, produce Interleukins, Tumor Necrosis factor (1TNF α), Interlenkin-6 (IFN γ), and Nitric Oxide (6) . In the present study, we aimed to Isolation and identification of *Enterococcus Faecalis* from different samples; Extraction of lipotechoic acid from *Enterococcus Faecalis;* Purification the extract of lipoteichoic acid by use chromatography and Study the effect of purified extract lipotechoic acid on cancer and compared them with normal cell in vitro.

Material and Methods

Samples were collected from suspected cases in Al children Educating Hospital and Kadimiya Hospital. The total are 45 samples included 6 samples from the urinary tract infections, 20 feces samples, and 7 samples of the skin wounds, burns and infections also collected 10 samples from crop of the chicken, and 2 samples from livers of sheep, from November 2010 to March 2011. The samples were cultured on the Brain Heart Infusion Agar with 5% of sheep blood, Streptococcal Faecal Broth, Enterococcus Confirmatory Agar, Kenner Faecal Broth (KF), Esculinblood Agar (7). For primary identification, cultures reading were performed as well as Microscopic examination using Gram stain. The second step of identification was performed by using API 20 strep system (8). Sensitivity to antibiotics was performed according to (9) and Extracted, purification of the liopteichoic acid (LTA) by hot phenol according to (10). Cytotoxic effect of purified extractedliopteichoic acid on cancer cell lines and normal cell in vitrowas studied according to (11).

Results

The results of the present study showed obtaining of 12 isolates belong to *Enterococcus faecalis* out of total 45 samples included 5 isolates obtained from faeces, 2 from Urinary tract infections, 4 from crop of the chicken and 1 from skin lesion. The *Enterococcus faecalis* isolates were identified depending upon Culture characteristics Microscopic examination and Biochemical test as shown in table (1), which shows its ability to grow on 10, 45 ^{0}C, and with High concentration of salt up to 6.5% of sodium chloride and pH =9.6, also recorded the ability of isolates to grow at high concentrations of salt on the esculin media which contain high concentrations of bile salts up to 40%, this indicates the ability of *E faecalis* to reaction with iron through conversion of asculinase to aesculetinto yield black color at 45 ^{0}C (12). The sensitivity test of 12 isolates of *E. faecalis* against 16 of antibiotic showed that all isolates were sensitive for Gentamicin, Chloramphincol, Penicillin G, Ampicillin, Vancomycin, Rifampcin, Co-trimoxazole in Fig. (1), and resistance to 9 antibiotics (100% to Streptomycin, 92% to Tobramycin, 83% to Amikacin, 66% to Erythromycin, 75% toCeftazidime, 84% to Augmentin, 33% to Cefotaxime, 60% to Tetracycline and 91.5% to Nalidixic acid) as in Table (2). The extracted of Lipotechoic acid (LTA) from feces isolation because it is have higher resistance for many antibioticsso high virulence, by used hot phenol method (65^{0}C) to extracted large quantities of LTA with a small amount of protein and nucleotides. The Gel Filtration Chromatography by a gel Spharose CL-6B was used for purified partially of LTA. The effects of crude and purified LTA were tested on two cancer cell lines (Glioblastoma cell line, AMN-3) and normal cell line (Ref), by using Two-fold different concentrations ranging from (625-5.000) mg/ ml For three exposure time (24,48,72) hours.

The results showed that high growth inhibition was significantly observed after 72 hours exposure time. Except partially purified LTA extract showed high growth inhibition effect on AMGM after 24 hours as in Fig. (2).

Table (1) The results of Biochemical test for *Enterococcus faecalis* isolates

Biochemical test	Result
Catalase	-
Motility	-
Growth at 45 ^{0}C	+
Growth at 50 ^{0}C	+
Survives 60 ^{0}C for 30 min	+
Growth at pH 9.6	+
Growth in 6.5 % NaCl	+
Growth on 40% bile	+
Growth in 0.04% $K_2Te\ O_3$	+
Carbohydrates fermentation	
Arabinose	-
Lactose	+
Mannitol	+
Raffinose	-
Sorbitol	+
Glycerol	+
Aesculin hydrolysis	+

Table (2) The results of antibiotics resistance test of *Enterococcus faecalis* isolation

Antibiotics	Resistance	Resistance of (2) isolation from urine	Resistance of(5)isolation from feces	Resistance of (1)isolation from skin	Resistance of (4) isolation from vesicle
Streptomycin	**% 100**	**R**	**R**	**R**	**R**
Tobramtcin	**% 92**	**1/2 R**	**R**	**R**	**R**
Amikacin	**% 83**	**1/2**	**R**	**0**	**R**
Erythromycn	**% 66**	**0**	**R**	**0**	**3/4 R**
Ceftazidime	**% 75**	**1/2 R**	**R**	**0**	**3/4 R**
Augmentin	**% 84**	**R**	**R**	**R**	**2/4**
Cefotaxime	**% 33**	**0**	**3/5 R**	**0**	**1/4 R**
Tetracycline	**% 60**	**1/2 R**	**4/5**	**0**	**2/4 R**
Nalidixic acid	**% 91.5**	**1/2 R**	**R**	**R**	**R**

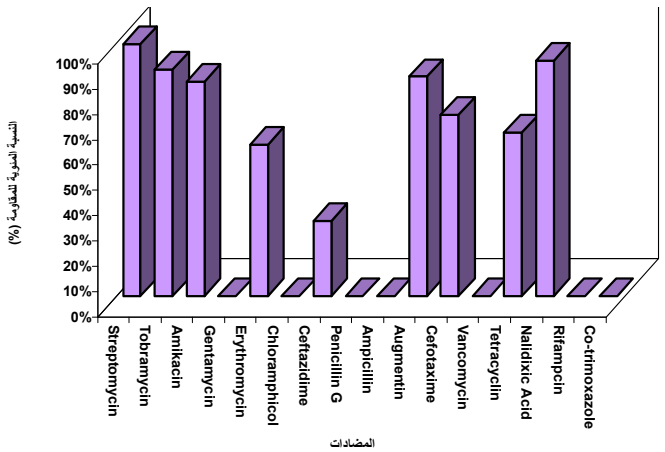

Fig. (1)Antibiotics sensitivity test of *E. faecalis*

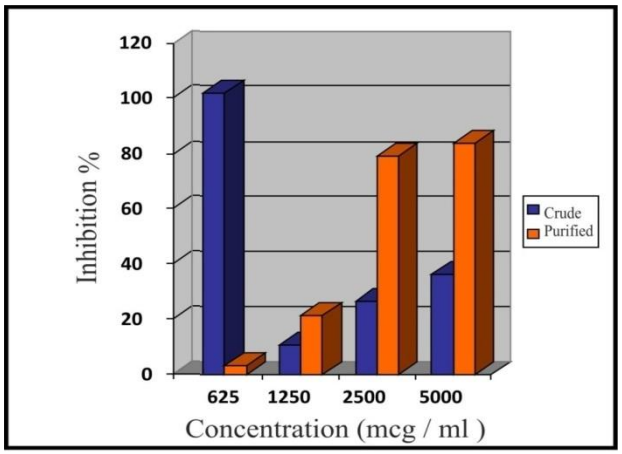

Fig (2) The Inhibitiory effects of AMGM cell line growth after exposoure to different Concentration of LTA extracted (Crude, Purified) after 24 hrs.

Discussion

*Enterococcus faecalis*are rich sources of isolation from human and animal the isolation of *Enterococcus faecalis* were tested for their sensitivity to different types of antibiotics and results showed that most of the isolates were highly resistance to the antibiotics used in this work the results was agree with (13). *E facalis* has opened anew window on cancer treatment by used LTA extracted by hot phenol was the method choice it give the best result of other method were compared with this technique. The amount of crud and partial purification of LTA resulted from using Sepharose CL-6B gel filtration chromatography was consistent to that resulted in the study of (14). Cancer treatment is encountered by various significant problems, LTA could unlock secrets that aid cancer treatment. In the present study showed that LTA as a causative agents of benign infection in cancer cell lines. cytotoxic effect of crude and partial purified of LTA in normal and several cancer cell lines were examined. In general the tow types of extracts are more effective on AMGM than on AMN-3 cell line, this means that AMGM cell line was more sensitive than AMN-3 cell line. Whereas Ref normal cell line was non sensitive to all concentrations of the tow types of extracts of LTA so it was used for a comparison with tumor cell lines. LTA extract can enhance reduction in tumor cells but not in normal cells, similar results were reported by (15). Crude and partial purified LTA extracts both showed cytoxic effect of cancer cell lines AMGM and AMN-3 with concentration and exposure time dependent.

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