Evaluation the Cytotoxic Effect of *Lactobacillus Acidophilus* Concentrated Filtrate on Growth of Tumor Cell Lines

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Abstract

This study was designed to evaluate the cytotoxic effect of *Lactobacillus acidophilus* concentrated filtrate on growth of (AMN, REF, RD and $HEPG_2$) tumor cell lines. The isolate obtained from vaginal swabs and identified according to culture characterictics and biochemical tests. *Lb. acidophilus* was grown in MRS broth media and incubated anaerobically for 24 hrs., then culture was centrifuged and sterilized by filtration. The filtrate was concentrated to three fold, then different concentrations (125, 250, 500 and 1000 µg/ml) were prepared and tested against the four types of tumor cell lines. Results showed that concentrated filtrate of *Lb. acidophilus* had a significant cytotoxic effect *($P \leq 0.05$)* against the growth of tumor cells used in this study at the concentrations (125, 250 and 500 µg/ml) when compared with control and the concentration (125 µg/ml) had the highest effect against (AMN, REF, RD and $HEPG_2$) tumor cell lines which showed growth inhibition percentages (90.40, 81.22, 90.37, 84.41)% respectively while the concentration (1000 µg/ml) displayed a significant effect on the HEPG2 cell line with growth inhibition percentage (35.78%).

Introduction

Lactic acid bacteria (LAB) such as *Lactobacillus* are important micro-organism in healthy human microbiotic [1]. They have been associated with several probiotic effects in both nutrition and health for research and commercial development [2]. A probiotic is defined as "live micro-organisms which when administrated in adequate amounts confer the health benefit on the host" [3]. Probiotic (LAB) found mostly in animal intestines, dairy products and human vagina [4]. This gram positive bacteria have an important criteria related to safety like highly tolerance to bile and gastric acidity, lack of potential to develop virulence and have no side effects associated with their use [5]. Recently, many research studies have focused on its protective properties against host diseases [6]. Furthermore potent anticancer agents obtained from food including (LAB) [7]. *Lb. acidophilus* had been studied for possible antitumor properties. Milk that was fermented by *Lb. acidophilus* was able to slow or prevent the growth of breast and colon cancer cells grown in the laboratory [8]. The various (LAB) can inhibit the genotoxcity of dietary carcinogens in vitro and the degree of inhibition was strongly species specific [9]. In other studies, animals that were given *Lb. acidophilus* found to be less prone to DNA damage in the colon after administration of known carcinogens [10]. The orally administration of *Lb. acidophilus* to rats showed a decreased in the incidence of the colon cancer [11]. Also oral supplements with *Lb. acidophilus* reduced the activity of faecal bacterial enzymes such as β-glucuronidase, nitroeductase and azoreductase that are involved in procarcinogen activation in human [12].

Materials and Methods

The growth inhibition was evaluated according to the following steps:

The isolate of *Lb. acidophilus* was supplied by immun. Lab. Biotech. Dept. Al-Nahrain University which previously isolated from vaginal swabs which were cultured on chocolate agar then a loop full was taken and recultured in tubes contain sterile 10 ml of MRS broth and after incubation for 24 hrs. at 37°C (anaerobically). Tubes containing MRS broth medium were inoculated with 1% of fresh culture of *Lb. acidophilus* and incubated an aerobically at 37°C for 24 hrs., then the culture were centrifuged at 6000 rpm for 15 min., supernatant was collected and sterilized by Millipore filter (0.02 µm) [13, 14]. One hundred ml of filtrate was concentrated using oven at 40-45 °C to one fold (50 ml), two fold (25 ml) and three fold

(12.5 ml) [15]. Cell lines were supplied by tissue culture unit Cancer Research Center / Baghdad, Iraq., [(Rhabdomyosarcoma (RD), Mice mammary gland carcinoma (AMN), Rat Fibroplast (REF), Liver Carcinoma ($HEPG_2$)] cell lines. Cell suspension was prepared for each types of cell lines and seeded in a well (96 well tissue culture plates) as (200 µl) in each well and incubated at 37 ^oC for 24 hr. After the incubation time, the wells examined for the formation of cell monolayer, then 200 µl / well from each concentration (125, 250, 500 and 1000 µl/ml) of concentrated filtrate of *Lb. acidophilus* were added to the wells (three replicate for each concentration). Also 12 replicates were made for the control which contained only the cells with (200 µl/ml) of serum free medium, then the plates were wrapped with Parafilm and incubated at 37 ^oC for 48 hr. in an incubator supplemented with 5% CO_2. After the incubation period, the media was decanted off and 50 µl/well of neutral red dye were added, then after 2 hr. the plates were washing with (PBS) to removed the excess dye and the results were read using the ELISA reader at wave length 492 nm [16]. The percentage of growth inhibition was calculated according to the following equation [17].

Growth inhibition =

$$\frac{Absorbanseof\ control - Absorbanseof\ treated\ cells}{Absorbanseof\ control} \times 100$$

Statistical Analysis

The values of the investigation parameters were given in terms of mean ± standard error. Differences between means were assessed by analysis of variance (ANOVA) and Dunkin test using SPSS computer program at the probability of *($P \leq 0.05$).*

Results

The concentrated filtrate of *Lb. acidophilus* had a significant cytotoxic effect on growth of AMN cell line (*$P \leq 0.05$*) when compared with the control at the concentration of 125 µg/ml with growth inhibition rate (90.40%), followed by (44.41, 23.80 and 10.36)% at the concentration of (250, 500, 1000 µg/ml) respectively Table (1). The significant cytotoxic effect *($P \leq 0.05$)* of concentrated filtrate of *Lb. acidophilus* on REF cell line started at concentration 500 µg/ml with growth inhibition percentage 34.12% when compared with control and the significant effect was increased at the lower concentration (250, 125 µg/ml) which had (45.39, 81.22)% respectively, but their was no significant effect at the concentration 1000 µg/ml as shown in Table (2). Maximum growth inhibition percentage on RD cell line was (90.34%) at concentration 125 µg/mlwith a lesser effect at the other concentration (250, 500, 1000 µg/ml) and the percentage were (49.66, 28.04, 20.43)% respectively Table (3). Results in Table (4) showed that a high effect of this extract on the $HEPG_2$ cell line at the concentration 125 µg/ml (84.41) and was significantly lower at the rest concentrations (250, 500, 1000 µg/ml).

Table (1)
Cytotoxic effect and growth inhibition percentage (G1%) of Lb. acidophilus concentrated filtrate on AMN cell line.

Extract concentrate µg/ml	*O.D. mean ± S.E.*	*G1%*
Control	0.521 ± 0.18	
125	0.050 ± 0.006 [a]	90.40
250	0.291 ± 0.003 [b]	44.14
500	0.397 ± 0.011 [b]	23.80
1000	0.467 ± 0.009 [c]	10.36

Different letters = significant differences (*$P \leq 0.05$*) between means.

Table (2)
Cytotoxic effect and growth inhibition percentage (G1%) of Lb. acidophilus concentrated filtrate on REF cell line.

Extract concentrate µg/ml	*O.D. mean ± S.E.*	*G1%*
Control	0.293 ± 0.024	
125	0.055 ± 0.002 [a]	81.22
250	0.176 ± 0.006 [b]	45.39
500	0.193 ± 0.021 [b]	34.12
1000	0.322 ± 0.001 [c]	9.89

Different letters = significant differences (*$P \leq 0.05$*) between means.

Table (3)
Cytotoxic effect and growth inhibition percentage (G1%) of Lb. acidophilus concentrated filtrate on RD cell line.

Extract concentrate µg/ml	*O.D. mean±S.E.*	*G1%*
Control	0.592 ± 0.016	
125	0.057 ± 0.004 [a]	90.37
250	0.298 ± 0.011 [b]	49.66
500	0.426 ± 0.009 [b]	28.04
1000	0.471 ± 0.031 [b]	20.43

Different letters = significant differences *(P≤ 0.05)* between means.

Table (4)
Cytotoxic effect and growth inhibition percentage (G1%) of Lb. acidophilus concentrated filtrate on $HEPG_2$ cell line.

Extract concentrate µg/ml	*O.D. mean±S.E.*	*G1%*
Control	0.584 ± 0.020	
125	0.019 ± 0.004 [a]	84.41
250	0.334 ± 0.004 [b]	42.80
500	0.346 ± 0.007 [b]	40.75
1000	0.375 ± 0.012 [b]	35.78

Different letters = significant differences (*P≤ 0.05*) between means.

Discussion

Results showed that concentrated filtrate of *Lb. acidophilus* had cytotoxic effect on tumor cell lines used in this study. This might be due to its' ability to produce Lactic acid, bacteriocins and hydrogen peroxide [18. 19]. Most anticancer drugs currently in use exert their effect via the induction of apoptosis (programmed cell death) which is important for many human tumors and chemotherapy-induced tumor cell death [20]. Hydrogen peroxide exhibited the ability to induce apoptosis in various cell lines by fragmentation of DNA, decrease the level of CD_{95} and activate the differential expression of some specific gene (P_{53}) which is required for apoptosis [21]. Another study showed that soluble polysaccharide derived from *Lb. acidophilus* inhibited cancer cell proliferation and these polysaccharide proved to be less cytotoxic to normal cells than the whole cells of this bacteria [22], and this explain why many cancer therapy agents are limited in their use [23]. A similar observation was confirmed that polysaccharide fraction of *Lb. acidophilus* causing a death of HT-29 cancer cell lines by inducing apoptosis, also polysaccharide isolated from *Lb. acidophilus* were significantly regulated the expression of BC1-2 interacting protein and cell division cycle protein [24]. Statistical analysis showed that a high significant cytotoxic effect of concentrated filtrate on growth of (AMN, REF, RD and $HEPG_2$) cell lines occurred at lowest concentration (125 µg/ml) with growth inhibition percentages (90.44%, 81.32%, 90.37% and 84.41%) respectively, but the effect was lower at the higher concentration. An explanation of this behavior might be due to the variation in cell lines properties, selectivity of their receptors and their interference with cell response pathways [25].

Conclusions

The concentrated filtrates of *Lb. acidophilus* exert significant antitumor activity on a variety of cancer cell lines. On going study is evaluating this result in an attempt to use this extract as adjuncts in cancer therapy by using the animal models.

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الخلاصة

أجريت هذه الدراسة لتقييم الفعالية السمية للراشح المركز لبكتريا حامض اللاكتيك *Lb. acidophilus* ضد أربعة أنواع من خلايا خطوط الزرع السرطانية ($HEPG_2$, RD, REF, AMN). عزلت البكتريا من المسحات المهبلية وتم تشخيصها باستخدام الاختبارات الشكلية والبايوكيمياوية، وقد استخدم وسط MRS السائل المغذي لتنمية البكتريا حيث زرعت عليه وحضنت لمدة ٢٤ ساعة. تم فلترة المزروع البكتيري باستخدام الفلاتر (0.02µm/milipore filter). تم تركيز المزروع المفلتر لثلاث مرات (three fold) وتم تحضير عدة تراكيز (١٢٥، ٢٥٠، ٥٠٠ و ١٠٠٠ µg/ml) واختبرت ضد الأنواع الأربعة من خطوط الخلايا السرطانية. أظهرت النتائج امتلاك الراشح المركز وبتراكيز µg/ml (١٢٥، ٢٥٠، ٥٠٠) فعالية سمية ذات قيمة معنوية عالية على نمو الخلايا السرطانية ($HEPG_2$, RD, REF, AMN). وكانت النسبة المئوية للتأثير السمي %(٩٠.٤٠، ٨١.٢٢، ٩٠.٣٧، ٨٤.٤١) بالترتيب عند مقارنة النتائج بخلايا السيطرة، وامتلك التركيز 125 µg/ml أعلى فعالية سمية على جميع أنواع الخلايا، بينما كان التركيز 1000 µg/ml ذو أقل فعالية سمية وكان تأثيره معنوياً على خلايا $HEPG_2$ فقط بنسبة مئوية مقدارها (٣٥.٧٨%).