Cytotoxic Effect of the Locally Isolate *Lactococcus lactis* Cell Wall Proteins on Tumor Cell Lines

Moyassar B. H. Al-Shaibani[*1], Ali Abbdul-Rahman T. Al-Shekhly[**] and Mohammad R. Abdul-Majeed[***]

[*] Department of Biotechnology, College of Science, University of Al-Nahrain, Baghdad-Iraq.

[**] Nanotechnology and Advanced Materials Research Centre, University of Technology, Baghdad-Iraq.

[***] Deceased.

[1]E-mail: moyassarbasil@yahoo.com.

Abstract

This project was conducted to study the activity of cell wall components present in one of a human microflora which is *Lactococcus lactis* on *AMN-3* and *Hep-2* tumor cell lines *in vitro*. Twenty samples of dairy products (bucolic sour yoghurts, pasteurized milk and raw milk) were collected; nine isolates of *Lactococccus* were isolated by propagating in *MRS* broth medium followed by subjecting the isolates to microscopic, cultural, physiological and biochemical tests. The isolates were grown in *M17* broth medium, at 37ºC for 6 hrs followed by extraction cell wall proteins using sonication method followed by estimation the concentrations of extracted proteins depending on standard curve of bovine serum albumin. The cytotoxic activity of different concentrations was studied on *AMN-3* and *Hep-2* tumor cell lines for three incubation periods (24, 48 and 72 hrs) in addition to normal rabbit embryo fibroblast (*REF*) cell line for 72 hrs only. The results showed the presence of four isolates belong to the genus *Lactococcus lactis*. The result showed a clear cytotoxic activity of these crude extracts with high significances on these tumor cell lines during the three incubation periods, suggesting that the cytotoxic effect of *CWP* is a dose and time dependent, but on *REF* cell line, there is no significant effect reported. It is concluded that *CWP* of *L. lactis* may possess some specificity in cytotoxicity on cancer cells but not on normal cells.

Keywords**:** AMN-3, Hep-2, *L. lactis*, cell line, Cytotoxicity and REF.

Introduction

The intestinal flora is a complex ecosystem consisting of over 400 bacterial species that greatly outnumber the total number of cells making up the entire human body. These metabolically active bacteria reside close to the absorptive mucosal surface and are capable of a remarkable repertoire of transforming chemical reactions. The esophagus has a flora similar to that of the pharynx [1]. The empty stomach is sterile due to gastric acid. The normal flora of the duodenum, jejunum and upper ileum is scanty but the large intestine is very heavily colonized with bacteria among which are; Bacteriodes (mainly members of the fragilis group which outnumber *Bacteroides fragilis* itself), Bifidobacteria, Anaerobic cocci, *E. coli*, *Streptococcus faecalis*, *Clostridia*, *Lactobacilli* and less common inhabitants; *Klebsiella*spp., *Proteus* spp., *Enterobacter* spp. and *Pseudomonas aeruginosa*. The intestinal flora can be thought of as a chemical factory with massive levels of active enzymes. All rapidly growing bacterial species in the small intestines produce metabolic by-products that can be absorbed. Some of the absorbed products are utilized for energy immediately in the epithelial cells of the gut; others may be acted upon by the detoxification systems in the liver; while others are passed [2].

The enteric flora comprises approximately 95% of the total number of cells in the human body and can elicit immune responses while protecting against microbial pathogens. The beneficial role of the normal flora is the prevention of other more pathogenic bacteria from gaining a foothold in the body. The gut bacteria seem to be responsible for the normal structure and function of the intestine: they degrade mucin, epithelial cells and carbohydrate fiber and their metabolism

produces vitamins, especially vitamin K [3]. However, the resident bacterial flora of the gastrointestinal tract may also be implicated in the pathogenesis of diseases such as inflammatory bowel disease (ulcerative colitis and Crohn disease). Any compound taken orally, entering the intestine through the biliary tract or by secretion directly into the lumen is a potential substrate for bacterial transformation. So the colonic microflora is important to health [4]. Since the 1980s, many efforts have been made to better understand the molecular basis of LAB technological properties and to obtain better control of industrial processes involving LAB. This knowledge has led researchers to investigate their potential use for new applications, such as the production of heterologous proteins in bioreactors, in fermented food products or directly in the digestive tract of humans and other animals. Some LAB used as probiotic strains, naturally exerts a positive action in lactose-intolerant consumers by providing ß-galactosidase in the gut [5]. Besides such natural benefits, another and innovative application of LAB is the antitumor activity to supplement pancreatic and gastrointestinal deficiency in humans [6]. On the strength of those investigations, the present study was proposed for isolation and identification of the species *Lactococcus lactis* that may possess antitumor activity. Extraction of cell wall associated proteins (*CWP*) followed by *in vitro* study of the effect of these (*CWP*) on the growth of *AMN-3* and *Hep-2* tumor cell lines and normal rabbit embryo fibroblast cell lines (*REF*).

Materials and Methods

Samples Collection

Twenty samples of dairy products (bucolic sour yoghurts, pasteurized milk and raw milk) were collected in sterile containers from local markets in Baghdad governorate, followed by propagation the isolates by inoculating test tubes containing MRS broth medium with 1% of each sample and incubated at 37 ºC for 24 hrs under anaerobic conditions.

Isolation of Lactic Acid Bacteria

Lactic acid bacteria were isolated according to Harrigan and MacCance [7], serial dilutions were performed, followed by streaking on MRS agar plates containing 1% calcium carbonate ($CaCO_3$), then incubated at 37ºC for 24 hrs. After that, a loop touch of the growth was transferred to MRS broth and preserved.

Identification of *Lactococcus lactis*

The suspected LAB isolates were identified by the following tests:

Microscopic Examination

A loop full of each isolates culture was fixed on a microscopic slide, and then stained by Gram stain to examine cell shape, Gram reaction, grouping and spore forming phenomena [8].

Biochemical Tests

The biochemical tests used to identify the locally isolate *L. lactis* are gelatinase test, catalase test, acid production and clot formation, production of ammonia from arginine, carbohydrates fermentation, growth in 4% and 6.5% NaCl, growth in different pH, growth at different temperature and growth in (0.1 %) methylene blue [9].

Extraction of Bacterial Cell Wall Proteins

Cell wall proteins of locally isolated *L. lactis* were extracted by growing them in M17 broth and cells harvested by centrifugation at 11000 rpm 20 min. Cells were washed with 30 mM Tris hydrochloride pH 8.1, resuspended in 0.4 ml of cold 20% sucrose –30 mM Tris hydrochloride pH 8.1, and treated with 1/10 volume of lysozyme 1 mg/ml in 0.1 M *EDTA* pH 7.3 for 30 min at 4 °C and disrupted by sonication (six bursts of 1 min each) in ice, using a Sanyo *MSE* Soniprep 150. Debris and unbroken cells by centrifugation at 2,000 rpm for 10 min, an aliquot 1/4 volume of the extract was subjected to ultracentrifugation in a Beckman 70.1 Ti rotor for 2 hrs at 15,000 rpm at 4°C, and the resulting supernatant was used as the cytoplasmic fraction. The total membrane pellet was washed twice with 30 mM Tris hydrochloride pH 8.1, carefully suspended in 20 ml of 0.5% sodium lauryl sarcosinate, and shaken for 1 hr at room temperature. The sample was centrifuged as before, and the supernatant was sterilized through 0.2 µm pore-size filter units. Aliquots were stored at -20 °C until required [10].

Control (II) Preparation

It was prepared by mixing 30 mM Tris hydrochloride pH 8.1, 3 mM *EDTA* pH 7.3 and 0.5% sodium lauryl sarcosinate, sterilized by autoclaving, cooled and followed by the addition of lysozyme 1 mg/ml which was previously sterilized by filtration.

Quantitative Estimation of Proteins

Protein concentration was estimated according to Bradford method by using Commassie blue G-250 and Bovine serum albumin (*BSA*) to determine standard curve and estimate protein in concentrated filtrate [11].

Standard Curve of Bovine Serum Albumin

Bovine serum albumin (*BSA*) solution was prepared by dissolving 0.1 g of *BSA* in a quantity of DW and the volume was completed to 100 ml DW. Different concentrations of BSA (2, 4, 7, 10, 14, 16 and 20 µg/ml) were prepared and plotted [11].

Estimation of Extracted Proteins

The same steps followed in standard curve were used to determine the protein in concentrated filtrate by taking 20 µl from each extract.

Viable Cell Count

Cells count were performed according to Freshney [12], using trypan blue dye, dead cells take up the dye making them easily distinguished from viable cells which counted by Neubauer chamber.

Inhibition Assay

The procedure below was depending on cytotoxicity testing mentioned in Freshney [12], which was applied for both *AMN-3* and *Hep-2* cell lines and rabbit embryo fibroblast (*REF*) which were obtained from Iraqi centre for cancer and medical genetic research. Cell suspension was well mixed and treated with both extracts poured into 96 flat bottom well microtiter plate and incubated at 37 °C for 72 hrs in an incubator supplemented with (5%) CO_2. 50 µl/well of neutral red dye were added and incubated again for 2 hrs. The results were read by *ELISA* reader at 492 nm.

Statistical Analysis

The results were statistically analyzed to determine the significance effect among the concentrations of both extracts and their effect on tumor cell line and normal cells. The comparison between groups has based on analysis of variance test (*ANOVA*), while the significance differences based on Duncan's test [13].

Results and Discussion

The colonies of some isolates on MRS agar by pour plate were circular, white to yellow in color some of which were gray, smooth, mucous, bright and convex, Table (1). These characteristics were the same to those observed when pure culture was obtained using streaking method. It was observed that the growth of LAB on MRS agar was heavier than their growth on SL medium this may be due to the high ratio of sodium acetate and high acidity [14].

Also, there were other isolates their colonies characterized by different shapes (fusiform, ovoid and circular) white to pale in color, soft with smooth edges, non bright and some of which were convex. Such cultural characteristics are concerned with those of the colonies of the genus *Lactobacillus* [15].

Microscopic examination after Gram staining showed that some suspected cells were spherical, tend to be ovoid, and grouped in pairs, tetrads and short chains, Gram positive and non spore forming, Table (1), these results are supported by those of El Soda *et al*., [16] when they isolated 237 isolate of *L. lactis* among a total of 2000 isolates of LAB obtained from traditional Egyptian dairy products (different types of raw milk, ras, domiatti and kareish cheese, mish, cream, butter and fermented milk) obtained from different regions in Egypt.

Also, it was observed that the other isolates that may be belong to the genus *Lactobacillus* characterized as long curved rods, arranged in short and long chains (3-8) cells and some of which were single and in pairs, Gram positive and non spore forming.

Depending on the results of the cultural and microscopic tests, nine isolates may be belonging to the genus *Lactococcus* referred to as (Lc1, Lc2, Lc3, Lc4, Lc5, Lc6, Lc7, Lc8 and Lc9) and the other tested isolates may be

belonging to the genus *Lactobacillus*. The abundance of the genus *Lactococcus* in dairy and dairy products is reasonable because they possesses proteinase enzyme system encourage them to grow in milk and its products [17].

Since the present study focused on studying the effect of the crude extract of the genus *Lactococcus* on tumor cell lines, so we restricted the biochemical tests on these suspected isolates ignoring the others.

Table (1)
Morphological and Cultural Taxonomic Characteristics of the suspected isolates.

Characteristics	*Results*
1- Gram stain	G +ve
2- Cell shape	Spherical to oval
3- Grouping	Single, in pairs and short chains
4-Colony shape on MRS and SL agar	Circular, small, regular, convex with smooth edges
5- Motility	Non motile
6- Growth under aerobic conditions	+
7- Growth under anaerobic conditions	+

(+): Presence of Growth.

Biochemical characteristics shown in Table (2) demonstrated that each of the nine isolates were catalase negative since no bubbles were observed after addition of hydrogen peroxide, gelatinase negative, and the isolates (Lc1, Lc4, Lc5 and Lc8) produced ammonia from arginine due to the presence of arginine deiminase (AD) which is one of three enzymes that comprise the AD system, AD catalyzes the conversion of L-arginine to citrulline, with the concomitant production of ammonia [18]. Also these four isolates have grown in 0.1% methylene blue with reduction of dye with tenuous growth in 4% NaCl while the growth was lacking in 6.5% NaCl and 45°C and produced acid and formed clot in litmus milk causing the lowering of pH from 6.5 to 4.5, while other isolates (Lc2, Lc3, Lc6, Lc7 and Lc 9) did not produce ammonia from arginine due to the stability of the orange color of the medium, and lacked the ability to grow in 4% NaCl as well as inability to grow in 0.1% methylene blue, also, all isolates taken part in their inability to grow at 45°C and pH 9.5 but have grew at pH 9, facultative anaerobic since it had the ability to grow under aerobic and anaerobic conditions and can grow at (10-40°C), these results are in agreement with taxonomic characteristics of the genus *Lactococcus* which were mentioned by Elsoda, *et al*., [16].

In order to differentiate the nine isolates of *Lactococcus* species, carbohydrates fermentation test was performed. The isolates were different in their ability to ferment the carbohydrates source used. The isolates (Lc2, Lc3, Lc6 Lc7 and Lc9) which fermented (glucose, sucrose, lactose, mannose, mannitol, galactose, maltose and raffinose) but varied in their ability to ferment arabinose and xylose were identified as *Lactococcus raffinolactis*. While the isolates (Lc1, Lc4, Lc5 and Lc8) vary in their ability to ferment xylose while unable to ferment arabinose and raffinose but have fermented the other used sugars were identified as *L. lactis* [19].

According to the results above, the overall result was nine isolates of *Lactococcus*; five of them (Lc2, Lc3, Lc6 Lc7 and Lc9) were identified as *Lactococcus* sp. *Raffinolactis* and the others (Lc1, Lc4, Lc5 and Lc8) were identified as *Lactococcus* sp. *lactis*. Our study focused on studying the effect of the crude extracts of the genus *Lactococcus* sp. *lactis* ignoring the species *raffinolactis.*

Table (2)
Biochemical tests of the locally isolated Lactococcus Isolates.

Isolate	Catalase Test	Gelatinas Test	NH_3 product. from Arginin	Growth in litmus Milk	Growth at			Growth in 1% methylene blue	Acid Production from									
					10 ºC	40 ºC	45 ºC		Glu	Suc	Lac	Mans	Manl	Arab	Xyl	Gal	Mal	Raf
Lc1	−	−	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	−
Lc2	−	−	−	+	−	+	−	−	+	+	+	+	+	−	+	+	+	+
Lc3	−	−	−	+	−	+	−	−	+	+	+	+	+	−	+	+	+	+
Lc4	−	−	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	−
Lc5	−	−	+	+	+	+	−	+	+	+	+	+	+	−	−	+	+	−
Lc6	−	−	−	+	−	+	−	−	+	+	+	+	+	−	+	+	+	+
Lc7	−	−	−	+	−	+	−	−	+	+	+	+	+	+	+	+	+	+
Lc8	−	−	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	−
Lc9	−	−	−	+	−	+	−	−	+	+	+	+	+	+	−	+	+	+

Glu: glucose. Suc: sucrose. Lac: lactose. Mans: mannose. Manl: mannitol.
Arab: arabinose. Xyl: xylose. Gal: galactose. Mal: maltose. Raf: raffinose.

Extraction and Purification of Extracted Proteins

The quantity of extracted *CWP* was 18.382 µg/ml. partial purification of the proteins was carried out by precipitation with ammonium sulphate. In addition to purification, this step leads to considerable lose in the concentrations of extracted protein which was 16.493 µg/ml.

Effect of Cell Wall Protein (*CWP*) on AMN-3 cell line

The cytotoxic effect of the *CWP* in the growth of *AMN-3* cell line has started with high significance difference ($P \leq 0.001$) at the concentration 0.625 µg/ml toward the higher concentrations when compared with the control I (*AMN-3* cell line treated with serum free medium "*SFM*") during the incubation period 24 hrs, Table (3). When the incubation period was 48 hrs, cytotoxic effect of this crude extract with high significance difference ($P \leq 0.001$) has started at the concentration 0.625 µg/ml toward the higher concentrations when compared with control. While during the incubation period 72 hrs, it was found that the cytotoxic effect of this crude extract has began with high significant difference ($P \leq 0.001$) at the concentration 0.312 µg/ml toward the higher concentrations when compared with control I and with high significant effect ($P \leq 0.0002$) when compared with control II (*AMN-3* cells treated with lysozyme 1 mg/ml).

Table (3)
The effect of CWP on AMN-3 cell line.

Group	*Conc. (µg/ml)*	*O.D. Mean ± (SE x 10 $^{-3}$) at different incubation periods*		
		After 24 h	*After 48 h*	*After 72 h*
Treated	0.312	0.46267 ± 5.363 [a]	0.45400 ± 4.041 [a]	* 0.40400 ± 3.510 [a]
	0.625	* 0.44100 ± 5.294 [b]	* 0.40100 ± 8.082 [b]	0.38600 ± 5.132 [a]
	1.25	0.38500 ± 6.108 [c]	0.36700 ± 3.215 [b]	0.33800 ± 0.027 [b]
	2.5	0.38000 ± 7.551 [c]	0.31100 ± 5.132 [b]	0.32200 ± 5.132 [c]
	5	0.34100 ± 7.765 [d]	0.30300 ± 3.602 [b]	0.22400 ± 4.584 [d]
	10	0.28700 ± 7.095 [e]	0.26100 ± 5.132 [c]	0.24200 ± 0.035 [e]
Control I		0.46500 ± 3.054 [a]	0.45600 ± 4.162 [a]	0.43500 ± 3.510 [a]
Control II		0.42800 ± 4.041 [a]	0.42800 ± 4.041 [a]	0.42800 ± 4.041 [a]

*different letters= significant differences (P<0.05) between mean.

Effect of Cell Wall Protein (*CWP*) on Hep-2 Cell Line

At incubation period 24 hrs, the cytotoxic effect of *CWP* with significant difference (P≤0.047) was noted at concentration 1.25 µg/ml toward the higher concentrations when compared with the control I (*Hep-2* cell line treated with *SFM*) and high significant difference (P≤0.001) when compared with the control II, Table (4). The cytotoxicity with high significant difference (P≤0.01) during the incubation period 48 hrs has started at the concentration 0.625 µg/ml accompanied with an increase in cytotoxicity toward the higher concentrations when compared with the control I and high significant difference (P≤0.02) when compared with the control II. whereas at incubation period 72 hrs, cytotoxic effect with high significant difference (P≤0.001) has begun at the concentration 0.625 µg/ml toward the higher concentrations when compared with control I and high significant difference (P≤0.002) when compared with the control II.

Table (4)
The effect of CWP on Hep-2 cell line

Group	*Conc. (µg/ml)*	*O.D. Mean ± (SE x 10 $^{-3}$) at different incubation periods*		
		After 24 h	*After 48 h*	*After 72 h*
Treated	0.312	0.39000 ± 5.132 [a]	0.36333 ± 5.785 [a]	0.36100 ± 5.132 [a]
	0.625	0.38000 ± 6.714 [a]	* 0.35500 ± 5.132 [b]	* 0.32800 ± 3.787 [b]
	1.25	* 0.37800 ± 4.041 [b]	0.34800 ± 6.246 [c]	0.28900 ± 5.132 [b]
	2.5	0.35567 ± 5.455 [b]	0.30000 ± 6.656 [d]	0.28833 ± 3.481 [b]
	5	0.32567 ± 0.018 [c]	0.28800 ± 5.132 [d]	0.26067 ± 0.011 [c]
	10	0.31733 ± 0.035 [d]	0.22500 ± 5.132 [e]	0.16500 ± 5.686 [d]
Control I		0.39200 ± 3.602 [a]	0.36700 ± 5.565 [a]	0.36300 ± 4.041 [a]
Control II		0.35700 ± 3.602 [a]	0.35700 ± 3.602 [a]	0.35700 ± 3.602 [a]

*different letters= significant differences (P<0.05) between mean.

Our findings consistent to those gained by Degnan *et al*., [21] who showed that *Streptococcus pyogenes* (group A *Streptococcus*) cell extracts (CE) at concentrations above 0.5 µg/ ml among the used concentrations (0, 0.008, 0.04, 0.2, 0.5, 1.0, and 5.0 µg/ ml) consistently caused potent inhibition of T-cell proliferation *in vitro* during three days incubation period. On other hand, Streptococcal acid glycoprotein (SAGP); which possesses between 31.5 and 39.0% amino acid identity with arginine deiminase

(AD) which has been well documented as having anti-proliferative activity against a range of tumor cell lines, including murine fibrosarcoma Meth A cells, human *HL60* cells, murine embryonic cells (BALB/3T3), *HeLa* cells, and murine leukemic L1210 cells [22]. Also, the action of AD will lead to a depletion of L-arginine in growth media, and may be in the absence of L-arginine cells are simply unable to synthesize new proteins, thus inhibiting growth and proliferation. Since *L. lactis* is a genus of group A streptococci so their inhibitory effect in the growth of tumor cell lines may be attributed to this active compound (SAGP) [21]. Also, bacterial products seem to restrict access of lipopolysaccharides (LPS) to CD14 receptors on monocytes/macrophages this is associated with lowering of NF kappa B signalling in immune cells and hostile of TNF-α secretion. Although intestinal macrophages do not express CD14 under basal conditions, their expression is upregulated under inflammatory conditions underlining the potential beneficial effect of probiotic bacteria under these conditions [23]. Another interpretation of our result that purified lactococcal cell walls have antitumour activities in that the cell wall induces activation of phagocytes to destroy growing tumor cells. Bifidobacteria probiotics reduced colon carcinogenesis induced by 1,2-dimethylhydrazine in mice when used with fructo-oligosaccharides and inhibited liver and mammary tumors in rats [24]. This effect may be attributed to the active compounds involved in construction of bacterial cell wall. Lipoteichoic acids of Gram positive bacteria such as bifidobacteria and LAB possess high binding affinity for epithelial cell membranes and can also serve as carriers for other antigens, binding them to target tissues, where they provoke an immune reaction, also, there are other bacterial metabolic products which possess immunomodulatory properties include: endotoxic lipopolysaccharide, peptidoglycans, and lipoteichoic acids [3].

Effect of the *CWP* of the Locally Isolated *L. lactis* on Normal Rabbit Embryo Fibroblast (*REF*) Cell Line

Statistical analysis has showed that there is no significant difference ($P \leq 0.039$) for each concentration of these crude extracts when compared with both control I and control II indicating that these crude extracts have no lucid effect on the growth of *REF* cell line after incubation period for 72 *hrs*. The compounds of the secondary metabolites characterized by their selectivity in their effect on tumor cells, so a comparison was made between the effects of *CWP* on *AMN-3* and Hep-2 tumor cell lines and their effect on normal cells (*REF*) at incubation period 72 *hrs*, considering the later as control. It was observed that there is an obvious cytotoxic effect for these extracts in the growth of these cell lines comparing with control (*REF*) with variation in inhibition intensity of each crude extract, Fig.(2).

It was observed that there is an obvious cytotoxic effect for these extracts on both tumor cell lines (*AMN-3* and *Hep-2*). In contrast, there was no effect or slight effect without significance on the growth of *REF* cell line, this selectivity may be attributed to the metabolic behavior possessed by cancer cells rather than in normal cells such as metabolic nature to form new blood vessels, the shape and nature of the receptors presents on the surface of cancer cell and the ability to bind with different compounds [24]. In addition, in cancer cells DNA strands are relaxant and the whole molecule is unstable due to the distance between the hydrogen bonds (H-bonds) that bind the two strands together this facilitates the binding of intra- and extra-compounds with DNA strands while in normal cells DNA molecule is cohesive and H-bonds are close to each other this prevent the binding of other foreign compounds [25].

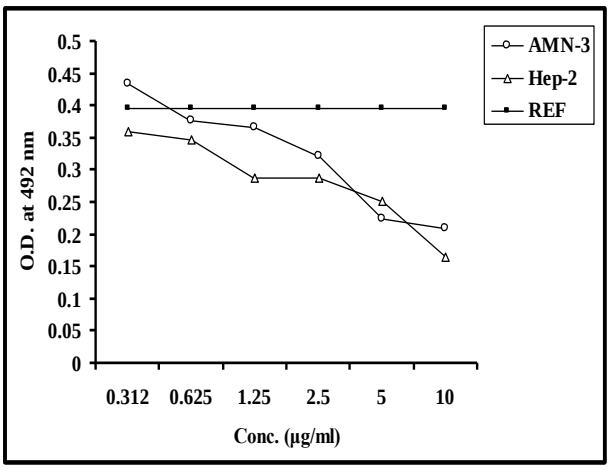

Fig. (3) The cytotoxic effect of CWP crud extract on AMN-3 and Hep-2 tumor cell lines and its effect on normal rabbit embryo fibroblast (REF) cell line after incubation period for 72 hrs.

Conclusions

Cell wall proteins of *L. lactis* possess conspicuous cytotoxic effect in growth inhibition of *AMN-3* and *Hep-2* tumor cell lines *in vitro* depending on both concentration and time. On the other hand, CWP have no cytotoxic effect with observed significance differences in the growth of normal rabbit embryo fibroblast (*REF*) cell line.

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الخلاصة

صُمّمت هذه الدراسة لبيان فعالية مركبات الأيض الثانوي المنتجة بواسطة أحد النبيتات الطبيعية في الجسم البشري وهي مكورات حامض اللاكتيك*Lactococcus lactis* بشكلها الخام وتأثيرها على نمو الخلايا السرطانية خارج الجسم الحي. جُمعت عشرون عينة غذائية شملت منتجات الألبان (اللبن الرائب الريفي والحليب المبستر والحليب الخام)، وعزلت منها تسع عزلات تعود للجنس *Lactococcus* تم عزلها باستخدام الوسط الزرعي MRS السائل ثم أُخضعت العزلات للفحوصات المجهرية والزرعية فضلا عن الاختبارات الكيموحيوية. تم تنمية البكتيريا في الوسط الزرعي M17 السائل عند درجة حرارة ٣٧°م ولمدة ٢٤ ساعة لغرض استخلاص بروتينات جدارها الخلوي باستخدام طريقة الصعق بالموجات الصوتية وتقدير كميات البروتين المستخلصة بالمقارنة مع المنحني القياس لألبومين المصل البقري. كما تم اختبار الفعالية السمية الخلوية لتراكيز مختلفة لكل مستخلص خام من مستخلصات مكورات حامض اللاكتيك على الخطين الخلويين السرطانيين AMN-3 وHep-2 وبثلاث فترات حضن (٢٤، ٤٨ و ٧٢ ساعة) بالاضافة الى اختبار سمية هذه المستخلصات الخام على الخط الخلوي الطبيعي لجنين الأرنب REF ولفترة حضن واحدة فقط وهي ٧٢ ساعة. أظهرت النتائج وجود أربع عزلات تعود للجنس *Lactococcus* النوع *lactis*. كانت النتيجة وجود تأثير سمي واضح وبمعنوية عالية لتلك المستخلصات على نمو الخلايا السرطانية وخلال فترات الحضن الثلاث، وقد لوحظ أن شدة السمية تزداد بزيادة التركيز وفترة الحضن لذا فان التأثير السمي لتلك المستخلصات الخام لمكورات حامض اللاكتيك يعتمد على التركيز وفترة الحضن، في حين لم يكن هناك تأثير واضح وذو معنوية لنفس المستخلصات في نمو الخلايا الطبيعية REF. قد يكون للمركبات الأيضية لمكورات حامض اللاكتيك *Lactococcus lactis* بعض التخصص في التأثير السمي على نمو الخلايا السرطانية دون الطبيعية.