Study The Effect of Soluble Beta Glucan of *Saccharomyces cerevisiae* on Protein Concentration of Human Circulating Blood Lymphocytes .

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Abstract:

This study was designed to detection the effect of the soluble beta glucan (ß-glucan) local and commercial extracts (LE , CE) of the *Saccharomyces cerevisiae* on protein concentration of human circulating blood lymphocytes at the concentrations (5 , 50 , 500 , 1000) µg/ml .The results showed significant differences increase on protein content at the level ($P<0.05$) for both effect of beta glucan Local extract , Commercial extract , and the best effect on protein lymphocytes concentration demonstrated at the highest concentration of the LE and CE .The four concentrations of β-glucan (5 , 50 , 500 and 1000 µg/ml) caused significant differences ($p < 0.05$) in protein content of lymphocytes secretion that treated with Local and Commercial extracts which represented (45.90, 63.18, 83.2, and 184.1)µg/ml and (32.27 , 50.45 , 79.54, 164.1) µg/ml respectively , as a compared to their control negative and control positive (58.63 ; 110.45) respectively µg/ml . While the values of both treatment at concentration (5,50,500)µg/ml is lower than control group that treated with (PHA) was represented (110.45) µg/ml . The concentrations 1000 µg/ml of LE, CE have best effect on protein concentration of lymphocytes secretion when compared with the control groups .

دراسة تاثير البيتا كلوكان الذائب لخميرة الخبز (*Saccharomyces cerevisiae*) في تركيز البروتين للخلايا اللمفاوية للإنسان .

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الخلاصة

صممت الدراسة للتحري عن تأثير البيتا كلوكان الذائب للمستخلص المحلي والتجاري لخميرة الخبز *Saccharomyces cerevisiae* على التركيز البروتيني (المحتوى البروتيني) للخلايا اللمفاوية للإنسان للتراكيز المستخدمة (1000, 500 , 50 , 5) ميكروغرام /مل . بينت نتائج الدراسة على خلايا اللمفاوية بعد معاملتها بالبيتا كلوكان الذائب (المستخلص المحلي والتجاري) فروق معنوية عند مستوى ($P<0.05$) للمحتوى البروتيني لإفرازات الخلايا اللمفاوية وأن أفضل تأثير على المحتوى البروتيني للخلايا اللمفاوية كانت عند التراكيز العالية للمستخلص المحلي والتجاري . اذ سببت التراكيز الأربعة المستخدمة فروق معنوية للمحتوى البروتيني للخلايا اللمفاوية المعاملة بكلا المعاملين ,اذ بلغت (45.90, 63.18, 83.2, and 184.1µg/ml), (32.27, 50.45, 79.54, 164.1µg/ml) على حد سواء مقارنة لمجاميع السيطرة السالبة والموجبة (110.45;58.63) على التوالي بينما اظهرت التراكيز , 50 , 5) 500)µg/ml للمستخلص المحلي والتجاري المحضر اقل نسبة مقارنة مع مجاميع السيطرة الموجبة المعاملة ب(PHA) والتي كانت قيمتها (110.45)µg/ml .اظهر التركيز العالي (1000µg/ml) للمستخلص المحلي والتجاري المستخدم في الدراسة افضل تأثير على المحتوى البروتيني المفرز من قبل الخلايا اللمفاوية مقارنتا مع مجاميع السيطرة .

Introduction

Beta glucan is a scientifically proven biological defence modifiers (BDMs) that nutritionally potentiates and modulates the immune response (**1**). through immune response potentiation and modulation, in many instances various therapeutic healing effects generated by the immune cells. For many years glucan have been investigated (History) for these immune enhancing properties (**2,3**) .

β-glucans (beta-glucans) are polysaccharides of D-glucose monomers linked by β-glycosidic bonds. β-glucans are a diverse group of molecules that can vary with respect to molecular mass, solubility, viscosity, and three-dimensional configuration. They occur most commonly as cellulose in plants, the bran of cereal grains, the cell wall of baker's yeast, certain fungi, mushrooms and bacteria (**4**) .

β-glucan refers to yeast-derived, isolated from cell wall of *Saccharomyces cerevisiae* has been shown to act as a potent non-specific immune-activator , and further efforts resulted in the development of a water-soluble, pharmaceutical grade yeast β-glucan whose biological effects have been extensively in different studies *in vivo and in vitro* (**5,6**).

Polysaccharides from fungi have attracted attention in the fields of biochemistry and pharmacology for their immunopotentiation and anti-tumor effects (**7**) . It can stimulate the polymorph nuclear cells (PMNs) to trigger the secretion of cytokines that will modulate the immune system (**8,9**) , due to recognition of these compounds by certain receptors located on the leukocytes and other immune cells that lead to enhance the innate and cell mediate immune responses (**10,11**).

Dendritic Cells (DCs) have been recognized as important mediators of immune response in *vivo*. They are specialized antigen-presenting cells that are highly potent in their presentation of antigen to naïve or quiescent $CD4^+$ and $CD8^+$ T cells. They capture, process, and present antigens in combination with Major Histocompatibility Complex (MHC) class I and II molecules, activating specific Cytotoxic T lymphocytes (CTLs). This ability to stimulate CTLs directly and effectively makes DCs ideal targets to exploit for manipulation of the immune system for immunotherapy purposes (**12**).

The best known effects of glucan consist of the direct stimulation of phagocytosis of professional phagocytes (**13**) . And direct activation of natural killer cells , it stimulate macrophages to secrete cytokines such as TNFα and IL-6 , cytokines are molecules that provide a critical mechanism for communication between the cells of the immune system via activating or inhibiting certain receptors leading to alteration of their effector functions ,these pro-inflammatory cytokines can potentially enhance the activation of adaptive immunity, such as antigen presentation and T cell activation , the administration of β-glucan could link the activation of both innate and adaptive immunity , considered to be the basic effector cells in host defense against bacteria, viruses, parasites and tumor cells (**14,15,16**) . The immunological effects are manifested through its binding to several specific receptors, most of all Complement receptor-3 (CR3) and Dectin-1(**17,18**).

The general aims of the study are designed to :

1 - Preparation of solube β-glucan and the determination of their polysaccharides .

2 - Detection the effect of the soluble β-glucan local extract and commercial of *Saccharomyceses cerevisiae* on protein concentration of peripheral lymphocytes of human.

Materials & methods

Preparation of soluble beta glucan extract (Local extract)

The soluble β-glucan was prepared as follow :

A – Preparation of particulate β-glucan (from baker yeast)

Laboratory extract of beta-glucan was prepared by extraction procedure from baker yeast (*Saccharomyces cerevisie*a) as follow :

According to the (**19** and **20**) the baker's yeast *(S. cerevisiae*) β-glucan material was obtained from the Market, This material was processed from common, active dry yeast (500gm) then added to one liter 0.1 mole of NaOH and stirred for 30 min at 60 °C. The material was then heated to 115 °C at 8.5 pressure /inch for 45 minute by used automatic autoclave (Daihan ,Korea) and then allowed to settle for 72 h. The sediment was resuspended and washed in D.W. by centrifugation (350 g for 20 min). The alkali insoluble solids were combined with 0.1 mole of 1L of acetic acid and heated to 85 °C for 1 h, then allowed to settle at 38 °C. The acid insoluble solids were drawn off and centrifuged as above. The compacted solid material was mixed with 3% H2O2 and refrigerated for 3 hours with periodic mixing. Then the material centrifuged and the pellet (sediment of lymphocytes) washed twice with D.W. followed by two washes in 100% acetone. The harvested solid material was dispersed on drying trays and dried under vacuum at 38 °C for 2 h in the presence of Ca_2SO_4, and then further dried overnight under vacuum at room temperature. This procedure yielded a bright yellow powder .

B- Preparation of soluble beta glucan extract (LE) of *Saccharomyces cerevisiae*

The β-glucan was phosphorylated individually by the improved William method (**20**) .The fraction (4gm) of β-glucan powder was dissolved in (200) ml of Dimethyl sulfoxide Me_2SO containing (72)gm. of urea. With stirrer, about (40) ml of phosphoric acid 85% H_3PO_4 was added drop wise slowly to the above solution at ambient temperature. Then the solution was heated to 100 ◦C , and the reaction was carried out for 6h with stirring. A crystalline precipitate (presumed ammonium phosphate) formed at 1–2 hrs. of reaction. Following heating, the reaction mixture was cooled to ambient temperature and diluted in distilled-water to form a yellow bright solution. Finally, the resulting phosphate derivative was dialyzed (3000 – 5000) Millipore in size against double D.W. for seven days to remove endotoxin (includingMe_2SO,H_3PO_4 and salt).

Carbohydrates Measurement :

Samples containing 1 ml of solvent were mixed with 1 ml of 5% phenol in a test tube cuvette (19-mm path length) ; 5 mL of concentrated sulphuric acid was added rapidly to generate heat to drive the reaction. The reaction mixture was allowed to cool to room temperature, and absorbance was measured at 490 nm in a spectrophotometer (Sequoia-Turner, model 690) against a water blank as described by Dubois (**21**), with some modification.

Determination of LE β-glucan by high performance liquid chromatography (HPLC) Technique :

Procedure was carried according to (**22**); (**23**) .

A- Material :

1 - HPLC colum : Lichrospher C 18 (4.6 mm x 50 mm , 3 µm), particle size , mobile phase : deionized water , with binary delivery pump model LC – 10 A shimadzu (Germany) .
2 - Detection ; Spectraphysics AS3500 auto injector and a Shimadzu RID6A refractive index detector RF shimadzu (Germany) , with Chromeleon 6.80 software.
3 - Flow rate ; 1.2 ml/minute. at 30 C°.

Preparation of sample :

Ten mg (commercial extract capsule) were dissolved in 250 ml to get 40 µg/ml standard , then 10 drops of lichenase (endo-beta (1-3)D-glucan – 4 – glucanhydrolase) were added to hydrolase the beta – glucan to oligosaccharide .
1 – Beta – cellobiosyl – d- glucose DP3.
2 – Beta – cello triose – D – glucose DP4.

And then 20 µl were injected into HPLC analyzer ,The sequences of the eluted material of the standard were as follow , each standard was 40 µl/ml.

Sequences	Subject	Retention time	area	Concentration µg/ml
1	Beta–cellobiosyl –d glucose DP3	2.51	13265	40 µl/ml each
2	Beta-cello triose-D-glucose DP4	3.35	21171	

The separation was occurred on liquid chromatography shimadzu 10 Av-LC equipped with binary delivery pump model LC – 10 A shimadzu , the spectrophotometer .

This procedures was done according to **(24)** ; HPLC colum , Lichrospher C 18 (4.6 mm x 50 mm , 3 µm) was performed using with binary delivery pump model LC – 10 A shimadzu ,These pump was used of high pressure that speed the movement of the molecules down the column ,as well as higer quality chromatographic materials that abled withstand the crushing forces of the pressurized flow , by reducing the transit time on the column (**23,24**). And they were connected with a Spectraphysics AS3500 auto injector and a Shimadzu RID6A refractive index detector controlled with Chromeleon 6.80 software. Serially HPLC columns were connected to a Shodex OHPack SB-LG precolumn and eluted at 30°C with 50 mM Na_2SO_4 (1.2ml/min), and (20 µl) of CE β-glucan (40µg/ml) prepared were injected using a 100 µL loop. Beta glucan molecular were estimated offline by the software WINGPC −6.2 using CE for calibration (the peak of LE ß-glucan as compared with the peak of CE as seen in fig.(2) .

Calculation :

$$\text{Concentration of samples (µg/ml)} = \frac{\text{area of sample}}{\text{area of standard}} \times \text{conc. of standard} \times \text{dilution factor}$$

Commercial extract (CE) (Pure Pharmaceutical Grade β-glucan)

Imunic (10 mg/capsule) was purchased, Istanbul Turkey, and 1 mg/ml from imunic was prepared by (**20**) method .

Tissue Culture Media

1 - The Ross well Park Memorial Institute (RPMI)-1640 Medium was prepared according to the instructions produced by manufacturing company, as follows:

RPMI – 1640 medium powder, 10.4 g/L was dissolved in approximately 700 ml of double distilled water and then the other component were added:-

Sodium bicarbonate	2 gram
Streptomycin	0.5 ml
Benzyl penicillin	0.5 ml
Nystatin	0.25 ml

The volume was completed to 1000 ml with distilled water, and then the solutions put on the magnetic stirrer, in order to facilitate dissolving all particles completely. The pH was adjusted to 7 and then the medium was sterilized by filtration with 0.22 µ Millipore and was stored at -20^{o}C.This medium is called serum free medium and was used as a maintenance medium (**25**) .

Growth media (20%) was prepared as described in serum free medium but, in growth medium, 100 or 200 ml respectively of newborn bovine calf serum replaced 200 ml of distilled water. Sterilization was carried out by filtration with sterile 0.2 µm Millipore filter and stored at -20^{o}C **.**

Bovine Serum Albumin solution "BSA"

It was prepared by dissolving 10mg of Bovine Serum Albumin in 10ml of D.W to make up initial concentration (1mg/ml), from which series dilutions should be done just before used (**26**).

Preparation of Lymphocytes suspensions :

Preparation of lymphocytes suspension was carried out according to (**27**) method as follows:-

1 - Five ml of peripheral blood was drawn from healthy individual by heparinized syringe immediately prior to be used.

2 - Blood was diluted with equal volume of Phosphate Buffer Saline (PBS, PH 7.2).

3 - The diluted blood was added to a glass test tube containing 5ml lymphoprep solution (Filcon solution) without allowing it to be mixed (by tilting the tube and gently dropped the blood on the sidewall of the tube).

4 - The tube was centrifuged at 1500rpm for 30min at 18˚c by using cooling centrifuge.

5 - The content of the tube was separated into 4 layers, from upper layer; plasma, lymphocytes, lymphoprep solution, and the lower layer of RBCs.

6 - The plasma layer was discarded by Pasteur pipette, then the lymphocyte layer was aspirated and transferred to a sterilized tube, the volume was completed to 5ml by Serum Free Medium (SFM).
7 - The tube was centrifuged at 1500 rpm for 10min at 18˚c, the supernatant was discarded and the cells were resuspended with SFM.
8 - Step 7 was repeated three times, at the last one the pellet was resuspended after culturing with complete growth media CGM (RPMI-1640 with 20 % serum) and transferred to tissue culture vessel (25cm²), and incubated for 1h at 37˚c to eliminate the adherent cells.
9 - Twenty µl of cell suspension was immediately transferred to the edge of the haemocytometer chamber, and left for 2min, then the number of lymphocytes was counted in one large square (1mm²) and the concentration of cell suspension was **calculated as follows**:

$$\mathbf{c = n \times 10^4}$$

Where **n** = number of counted lymphocytes, **c** = cells/ml, $\mathbf{10^4}$ = inversion of volume index.

The cell suspension was diluted to appropriate concentration (1×10^6 cells/ml) by adding Complete growth media.

Determination of total protein content in intracellular lymphocytes and secretions :

The total protein content in those secretions and intracellular protein content of lymphocytes was determined by Bradford method (**26**) as follows:-
1 - Four tenth ml of lymphocytes suspension (1×10^6cells/ml) was seeded in each well of 24-well microtiter plate, the plate was incubated at 37˚c until confluent monolayer was formed.
2 - The medium was discarded and 0.4 ml serum free media (SFM) contained Local and commercial extracts were added in appropriate replicate wells (three replicates for each concentrations (5 , 50 , 500, 1000) µg / ml) , leaving few wells as control negative (-) that were treated with serum free media (SFM) ,and mitogenic agent PHA(these consider as control positive (+)) , the plate was sealed and incubated at 37˚c for 72hrs .
3 - At the end of exposure time the medium was aspirated by tilting the plate to 45 degree and inserting the pipette tip in the angle between the base and side of the well and transferred to eppendroff tubes.
4 - The secretions were centrifuged at 1500rpm for 10min to precipitate the associated cells and the supernatant was referred to cell-line secretion.
5 - Serial dilutions of Bovine Serum Albumin (BSA) in D.W (1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 50, 25, and 10 µg/ml) should be prepared for the construction of standard curve as seen as in figure (1).
6 - One tenth ml of protein solution (lymphocytes secretions) was added to a tube containing 1.8 ml coomassie blue reagent(This reagent was prepared by dissolving 0.1g of coomassie blue-G250 powder in 50ml of ethanol (95%), then add 100 ml orthophosphoric acid (85%) and complete to 1L with D.W and filtered throughout Whitman filter No.1 and stored in dark bottle at room temperature until used), then mixed and let stand for 10min.
7 - One tenth ml of SDS (detergent agent) solution was added to the lymphocytes cells (sediment) and incubate for 30 minute , 1.8 ml coomassie blue reagent was added, then mixed and let stand for 10min.
8 - The optical density O.D. on a spectrophotometer at 595nm was read against suitable blank 1 (i.e., coomassie reagent , each of Commercials ,Glucan extracted and/or SFM, D.W) for lymphocytes secretions , blank 2 (coomassie reagent with 0.1ml of SDS) for sediment.

- **Calculation** :

The concentration of protein was calculated by using the following linear equation extracted from standard curve of BSA (figure 1):-
Y = 0.0011 X + 0.0925 .

Where ***Y*** is the optical density of the tested sample, and ***X*** is the concentration of protein in the sample.

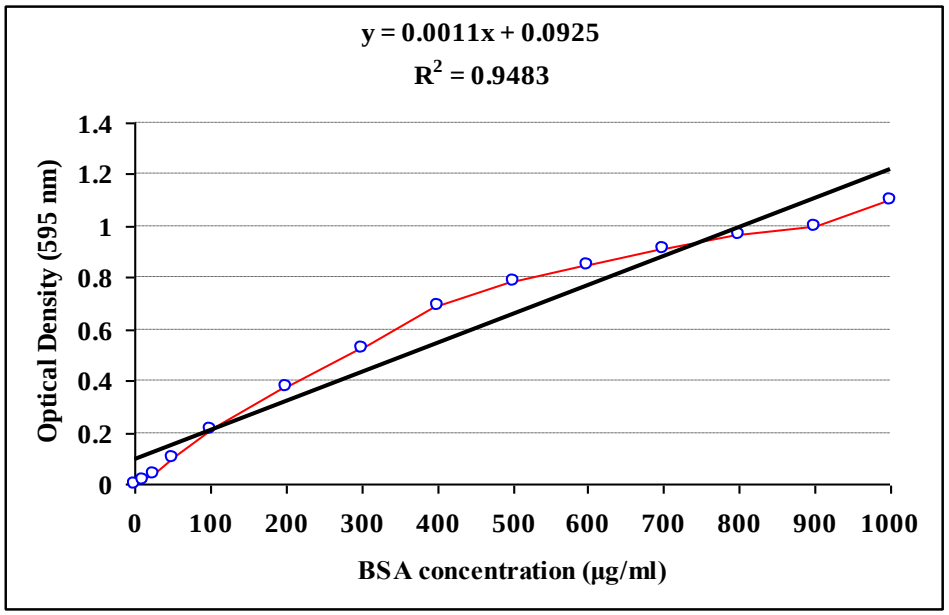

Figure (1): Standard calibration curved of BSA for Bradford Protein assay (measured of protein concentration in solution) .

Results

Local extract (LE) :

The dried particulate of LE of *Saccharomyces cerevisiae* , in 500g of backer's yeast was given 67g (13.4%) of β-glucan, a bright yellow product , which became powder upon drying.

Chemical detection of active compounds:

Samples containing carbohydrate developed a red-orange colour rather than the amber colour typical of the phenol-sulphuric acid assay. Intensity of the red colour increased with increasing the concentration and compared with imunics (Commercial extract) and the measurement of β-glucan concentration was carried out according to carbohydrate calibration curve , absorbance at 490 nm (the wavelength of maximum absorbance for glucose and starch). High performance liquid chromatography (HPLC) method was used for determination of LE ß-glucan as compared to CE as seen as (fig.2) .

Effects of β-glucan on lymphocytes culture

- **Long-term effect on protein content in lymphocytes culture**

The results in tables (1and 2) shows the significance of differences between the protein content of **lymphocytes secretion** of treated groups and non-treated (control) was determined , in which four concentrations of soluble β-glucan (5 , 50 , 500 and 1000 µg/ml) caused significant ($p < 0.05$) increased in protein content of lymphocytes secretion that treated with Local and Commercial extracts which represented (45.90±1.22, 63.18±1.22, 83.2±1.22, and 184.1±1.63)µg/ml and (32.27±0.40 , 50.45±2.04 , 79.54±2.45, 164.1±1.63)µg/ml respectively as seen as in table (1), as a compared to their negative control and positive control (58.63±0.81 ; 110.45±2.04). There are a high significant increase at ($p < 0.05$) in protein concentration of lymphocytes . The value of both treatment at concentrations (5,50,500)µg/ml is lower than control group that treated with (PHA) was represented (110.45±2.04) respectively as seen as in table (1). While the results shown that the concentration (1000 µg/ml) of LE, CE have best effect on protein concentration of lymphocytes secretion when compared with the control groups .

The protein content in the secretions of lymphocytes culture was estimated in µg/ml according to the standard calibration curve of bovine serum albumin (figure1), their values were blotted in it .The figure showed that the protein content value in the secretions of lymphocytes cultures was gradually increased with the increasing of β-glucan concentrations in both treatment.

Table (2) referred that protein concentration of intracellular lymphocytes .The protein content value of treated group exhibited significant increase in a concentration dependant manner (587.72±2.80 , 596.72±4.08 , 622.4±2.30 , 730.5±4.08)µg/ml for LE, (558.63±3.26, 570.45±4.04, 585.9±2.04, 677.8±1.22)µg/ml for CE at the concentrations (5,50,500,1000)µg/ml respectively. The protein concentration of intracellular lymphocytes content was represented a high value at concentration (1000)µg/ml than control wells which were (622.7±2.30, 647.72±2.30) respectively.

Table 1: The change of protein content of lymphocytes secretion after 72hrs of exposure to different concentrations of soluble β-glucan (Local & Commercial extracts).

Concentration of soluble β-glucan (µg/ml)	Protein content of the secretion of lymphocytes culture (µg/ml)	
	Local extract β-glucan	Commercial β-glucan
5	45.90±1.22 f	32.27±0.40 f
50	63.18±1.22 d	59.45±2.04 d
500	83.2±1.22 c	79.54±2.45* c
1000	184.1±1.63 a	164.1±1.63 * a
Control –ve	55.63±0.81 e	
Control +ve (PHA)	110.45±2.04 b	

Small different letters denoted that significant differences between concentrations at level (P≤ 0.05 , 0.01) .
***Significant different at level P ≤ 0.01 .**

Table (2): The change in protein content of intracellular lymphocytes after 72hrs of exposure to different concentrations of soluble beta glucan .

Concentration of soluble beta glucan (µg/ml)	Protein content of the sediment of lymphocytes culture (µg/ml)	
	Local extract β-glucan	Commercial extract β-glucan
5	587.72±2.80 * d	558.63±3.26 f
50	596.72±4.08 * d	570.45±4.08 e
500	622.4±2.30 * c	585.9±2.04 d
1000	730.5±4.08 * a	677.8±1.22 a
Control –ve	622.7±2.30 c	
Control +ve (PHA)	647.72±2.30 b	

Small different letters denoted that significant differences between concentrations at level (P≤ 0.05 , 0.01) .
***Significant different at level P ≤ 0.05 .**

Discussion

The extract of *Saccharomyces cerevisiea* yielded crude extract (13.4%) was in agreement with **(28)** ; The yield was (14.4%) of extract β-glucan from *Saccharomyces cerevisiae* , difference may be due to some has been lost in this yield during processing of extraction (Fractionated and hydrol-ysis), depending on the type of preparation to each one. The local extract (LE) β-glucan in the study showed fine bright yellow powder and sticky extract.

According to results in concern with changes in protein content, both the Local extract & Commercial of *Saccharomyces cerevisiae* exhibited dual effects of immunomodulatory activity ,enhancing lymphocyte proliferation at low concentrations(5µg/ml), and increasing in there proliferation with due to the effect at high concentrations (1000µg/ml) .This immunomodulatory effect was stronger on stimulated lymphocytes **(14,29)** .Based on *in vitro* studies, β-glucans act on several immune receptors including Dectin-1, complement receptor (CR3) and TLR-2/6 and trigger a group of immune cells including macrophages, neutrophils, monocytes, natural killer cells and dendritic cells.

As a consequence, both innate and adaptive immune response can be modulated by β-glucans and they can also enhance opsonic and no opsonic phagocytosis **(4,30)** .

β-glucan contains certain constituents capable of modulating the function of lymphocytes in expressing the synthesis or release of several cytokines, that can induce human peripheral blood mononuclear cells proliferation **(14 , 30).** It can also enhance phenotypic and functional maturation of monocyte derived dendritic cells with significant IL-12 and IL-10 production. Similar findings were found by Lin *et al*. using PS-G, in addition, treatment of dendritic cells with *Poria cocos* β-glucan phosphate extract (PS-G) resulted in enhanced T cell-stimulatory capacity and increased T cell secretion of interferon-γ and IL-10 **(30,31).** This action is at least mediated in part through the Dectin-1 receptor. The potency of such immunomodulating effects differs among β-glucans and purified polysaccharides of different size and branching complexity. In general, bigger size and more complex β-glucans such as those derived from *Ganoderma lucidum* have higher immunomodulating potency **(31).**

The suggestions may explain the proliferative effect of β-glucan on lymphocyte; the first one is the ability of glucan or its constituents to act as mitogenic agent, or to act as immuno-regulatory factors that modulate the secretion of certain cytokines particularly those needed in proliferation process **(32,33).**

The mitogenic factor (PHA) which stimulates lymphocyte division and this result denoted in increasing in proliferation of lymphocytes due to the mitogen (PHA) than the β-glucan results, and the competition effect between mitogenic factor (PHA) and β-glucan in present study not doing it .In previous study As a result of interaction with reproduction of lymphocyte *in vitro* **(33,34)** found that addition of a commercial preparation of nettle leaf extracts to whole human blood resulted in an inhibition of phytohaemoagglutinin-stimulated production of T helper cell 1 (Th1)-specific interleukin-2 (IL-2) and interferon-gamma (IFN-g) in culture in a dose-dependent manner up to 50%and 4%, respectively. They may interact with metabolism source on cell membrane .

Conclusion :

The effect of the soluble beta glucan (ß-glucan) local and commercial extracts (LE , CE) of the *Saccharomyces cerevisiae* on protein concentration of human circulating blood lymphocytes at the concentration (1000) µg/ml have best effect on protein concentration of lymphocytes secretion when compared with the control groups .

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Figure (2) : HPLC chromatographs of soluble ß-glucan preparation (A) CE ß-glucan ,(B) LE ß-glucan

A

B