Original article

Immunomodulation of Polysaccharides Extracted From Wild *Lycium barbarum* Iraqi plant

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ABSTRACT

This study demonstrates the favorable effects of Iraqi wild type *Lycium barbarum* active component as an immunomodulation agent. The fruit of Iraqi *Lycium barbarum* is rich with Polysaccharides, which were investigated qualitatively and quantitatively in the present study. The extracted polysaccharides total content calculated, as glucose was 3.4mg/g dried fruits.

The Immunomodulation effect for the extracted polysaccharides on normal human peripheral blood lymphocytes showed an increasing in lymphocytes proliferation significantly when it tested by MTT assay. The immune stimulating effect of the polysaccharides extract caused alteration in both IL-10 and TNF-α levels. After 2 hours of exposure to the extracted polysaccharides at concentrations (250 and 500 µg/ml), the normal human blood lymphocytes showed an elevation in IL-10 level against TNF-α level while the apposite results developed after 4 hours of exposure and both estimations were done by ELISA technique.

Key words: *Lycium barbarum* ,polysaccharides, Immunomodulation.

Introduction

The discovery and identification of a new drugs, which can potentiate the immune function has become an important goal of researches in immune-pharmacology. The flora of Iraq, the ancient Mesopotamian land of civilization is interesting; about 1500 medicinal plat species, which have been recorded in Iraq, and large number of these plants, are used for medicinal purpose [(1)]. Studies are in progress to understand how these compounds may or may not provide protection against toxic, mutagenic and carcinogenic activities of chemical compounds. *Lycium barbarum*, a well-known Chinese traditional medicine and foodstuff, contained different active components which have many proposed pharmacological and biological effects; including anti-aging activity [(2)], immune modulation and

anti-cancer activity[3] .The polysaccharides is an important bioactive compound in *L. barbarum* fruits, which has been found to have anti-cancer properties. The α-D (1→4) polygalacturonan from *L. barbarum* polysaccharide (LBP3a) is able to induce T lymphocyte proliferation and to promote an increase in interleukin-2 (IL-2) receptors expressed on isolated human peripheral lymphocytes [4].

All studies and researches on *Lycium barbarum* biological active components were done on the Chinese grown plant, while there are little (if not) researches about the Iraqi wild type plant. Therefore, the study of the effects of polysaccharides on the immune cells is of great significance and the present work was aimed to:

1-Identify the polysaccharides component from the fruits of the Iraqi wild *L. barbarum* plant, qualitatively and quantitatively.

2-Investigate the effect of the extracted polysaccharides towards normal human blood lymphocytes culture (by MTT assay).

3- Determine the cytokines level (IL-10& TNF-α) in lymphocytes cultured cells by ELISA technique.

Material and Method

i-Plant Collection:

Ripe orange small fruits from *Lycium barbarum* trees grown as a wild plant were collected from Al-Jadriya district at University of Baghdad, and classified by the herbarium of the Biology Department, collage of Science at University of Baghdad.

ii-Extraction of Polysaccharides from the Fruits: [5]

About 25 g of powdered *Lycium barbarum* fruits were mixed with 300 ml distilled water , then boiled for one hour, cooled, and filtered with piece of guise finally centrifuged for 30 minutes at 1500rpm.The filtrate was collected and cold solution of 95% ethanol was added and allow to stand for 24 hours. The precipitated polysaccharide was collected and washed with cold absolute ethanol then acetone and weighted after drying and kept in refrigerator at 4ºC.

iii- Determination of Total Polysaccharides Content in the Fruit [6]

For total polysaccharide determination, different glucose standard solutions (0.3, 0.25, 0.20, 0.15 and 0.1) mg/ml were prepared from glucose stock solution of 1mg/ml. About 250 mg from the extracted polysaccharides (the precipitate) was dissolved in 50 ml hot water to get solution of (5mg/ml) concentration. The following methods were done to determine polysaccharides content in the fruit.

A. Qualitative Determination

A general Benedict's test was done as primary qualitative determination for polysaccharide[7]. The reagent contained

blue copper(II) ions(Cu+2) which were reduced to copper(I)ions(Cu+1) which precipitated as insoluble red copper(I) oxide in the presence of reducing sugar and heating.

B. Quantitative Determination

For quantitative determination, a phenol-sulfuric method by Dubois *et al* [8]. was applied as follows :

About 0.4 ml from each standard solutions and 0.4 ml from the extracted polysaccharide were transferred each to separated glass tubes, then 0.4 ml of 5% phenol solution was added to all tubes, mixed with 2ml concentrated sulfuric acid. The mixture was shaken for 30 minutes and finally the absorbance was measured at 490 nm. A standard curve was plotted between concentrations verses absorption then from straight line equation the total polysaccharides concentration was calculated as glucose**.**

iv-Immunomodulation Determination(*in vitro)*

To determine *in vitro* immune effects for *Lycium barbarum* extracts; lymphocytes culturing and viable counting was employed in each step; lymphocytes proliferation, and cytokines level(IL-10 &TNF-α) in the supernatant of lymphocyte culture treated cells were involved.

A-Lymphocytes Culturing and Viable Counting [9,10]

-About forty milliliters of venous blood were taken from healthy volunteers, their ages in the range of (25-35) year's old, never taken medication at least 10 days ago.

-Each ten milliliters was transferred into vacuumed tubes containing 0.2% EDTA as anticoagulant with continuous gentle shaking.

-The human peripheral blood was diluted PBS (pH=7.2) in 1:1 ratio.

-About 5 milliliters of the diluted cell suspension was layered onto three milliner of Ficoll-isopaque separation fluid (lymph prep; specific gravity=1.077g/l, placed into vacuumed tubes(10 ml capacity).

-The tubes were centrifuged at 2000 rpm for 30 minutes.

-The mononuclear cells were collected with sterile pasture pipette, transferred into 10ml vacuumed tubes, suspended with 5ml RPMI 1640, and centrifuged for 10minutes at 2000 rpm. The step was repeated twice.

-The isolated lymphocyte cells were collected again and suspended in RPMI-1640 medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, and 100µg/ml streptomycin, then transferred into appropriate tissue culture plate and incubated for 18 hours at 370C in 5% CO2 incubator.

B-Determination of cell viable counting for the isolated lymphocytes

The cell count and viability were determined according to Freshney procedure [11]. Trypan blue 1% solution freshly prepared in PBS was used. Dead

cells unlike viable cells took up the dye within seconds which could be easily distinguished under light microscope.

-About 10µl from both Trypan blue stain and lymphocyte cell suspension were mixed for 30 seconds, then 10 µl from the mixture was applied gently into both grooves edge at the two sides of a haemocytometer chamber, underneath the cover slip.

-Under light microscope 40X objective lens all cells were counted in 1mm2 ,then a separate counting of viable(transparence) and non-viable(blue) cells was done.

-Cell concentration(cell/ml), total cell count and %viable cell count were calculated:

% viable cell= number of living cells/ total number of cells.

-The viable counting with Trypan blue result should be more than 90% viable cells count.

C-Measurement the Proliferative Cultured Lymphocytes by MTT Assay [11]

-About 100µl of the suspended cells was seeded in each of the 96 well microtiter plate, about(106cell/well).The plate was incubated at least for 2 hours in a CO2 incubator.

-Serial concentrations from extracted polysaccharide was prepared from stock solution (1000µg/ml)to get (500,250, 125, 62.5, 31.25, 15.625, and 7.8125) µg/ml, then sterilized with 0.22 µm Millipore filter.

-About 100 µl from each concentration of the extract was added in triplicate to the lymphocytes seeding plate .Control positive was employed as 10 µl of 0.1% PHA solution (phyto-hemagglutinin), while negative control represented by untreated lymphocytes suspended in medium.

-The plate was incubated in a CO2 incubator for 24 hours at 37^0c.

-About 50 µl of MTT dye(2mg/ml) was added to all wells, then incubate for further 4 hours.

-The MTT-formazan crystals which formed only by live cells were dissolved with100µl DMSO added to all wells.

-Absorbance at 620 nm was recorded immediately by ELISA reader.

-Viable cell Lymphocytes as a percentage was calculated as follow:

[Absorbance of the test /Absorbance of negative control]X 100.

-A comparison between the results of the extracted polysaccharide at different concentrations was statically calculated to choose the most effective dosages of each extract that may cause lymphocytes proliferation that to be used in further experiment as immunostimulants.

D- Determination Cytokine Concentration by ELISA Kit [12]

The work was done following the instruction of US Biological TNF-α and IL10 kit protocol/Biochemical &Biological Reagents ,United State Biological. Catalog No(T9160-01).The

supernatants of treated lymphocytes at different concentrations of extracted carotene (previous steps) were applied in this test. At the end of experiment a standard curve for each cytokine different concentrations was plotted with their absorbance at 450nm, then all test reading were calculated according to straight line equation obtained from the standard curve and both TNF- α and IL-10 level of the treated lymphocytes in the supernatant were obtained and evaluated statistically.

Statistical Analysis:

The Statistical Analysis System- SAS (2004) was applied for all results to show effect of difference concentration and other factors in studied parameters. The least significant difference (LSD) test and Duncan test at the comparative between means in this study

The Results

***i-Lycium barbarum* Active Components**

There is no study about Iraqi wild type *Lycium barbarum* and its active components had been done before. The polysaccharides are the main constituents of Iraqi *Lycium barbarum* was estimated qualitatively and quantitatively in the current study.

ii- polysaccharides content in the fruits:The final precipitate yield was one gm from25g dried powdered fruits. Only 250 mg were dissolved in 50ml hot distilled water for quantitative analysis of total polysaccharide calculated as Glucose.

iii-Total Polysaccharides Content in fruits of *Lycium barbarum* A. Qualitative Determination:A red precipitate was formed as a result of the Benedict's general test

Figure (1) Benedict's test give red precipitate with the total polysaccharides extracted from *Lycium barbarum* fruits

B. Quantitative Determination:

Spectrophotometric absorption of the extracted *L. barbarum* were done to determine the total polysaccharides by reading the absorbance of different concentrations of D-glucose standard solution. All readings were plotted for standard curve to estimate the total polysaccharides in the extracted fruits sample, which gave an absorbance of(1.2nm) Figure(2).

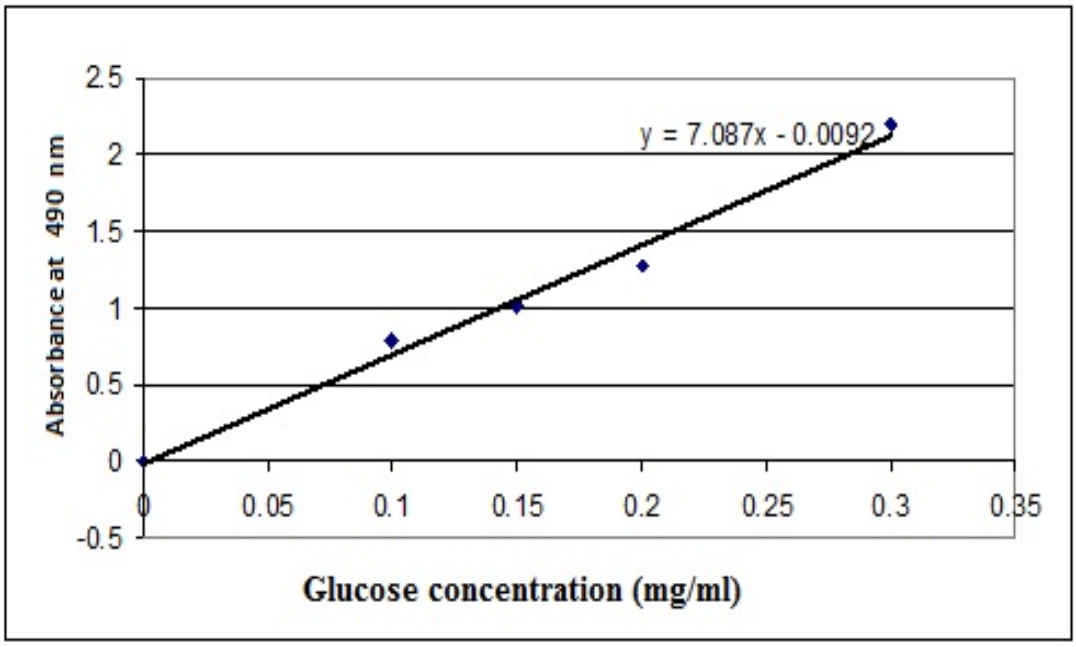


Figure (2) Standard curve for glucose as determined by spectrophotometer, R^2=0.986

The results showed that the concentration of total polysaccharides in the extracted fruits were 85 mg (3.4mg/g dried fruit).

Extraction and isolation of polysaccharides even in low concentration is simple, as they are soluble in hot water and the easiest method is first produce a hot water extract of herb using more than one extraction to get most of polysaccharides into solution and then force the polysaccharides out of the solution by adding alcohol in which they are not soluble, then the liquid is separated off and the residue is dried to produce the finished polysaccharides [13].The yield of this procedure is 3.4mg/g of the dried fruits from the wild Iraqi type, while references showed (5-8) % polysaccharide content in cultivated type as in Chinese desired and more content up

to 10-15% can be obtained with optimized condition of extraction [14].

iv-Extracted Components from *Lycium barbarum* as Immunomodulator (*In vitro*)

In order to trace immune-modulation and the mechanism for regulation of the immune system; Lymphocyte proliferation (by MTT assay), and cytokines (IL-10 and TNF-α) level were employed. Results of the effect for polysaccharides extracted from *L.barbarum* on these parameters were analyzed statistically.

Effect of extracted Components from *Lycium barbarum* on Lymphocyte Proliferation:

This work was held at Al-Nahrain Biotechnology Center Laboratories. Lymphocyte proliferation was determined using MTT method. Results of the effect of different concentrations of purified extracts of *L.barbarum* on proliferation of normal human lymphocyte are shown in Table (1).

Table (1) Effect of purified flavonoid and polysaccharides extracted from *L.barbarum* Normal human lymphocytes treated for 24 hours

Conc.(µg/ml)	% viable Lymphocytes treated by the Extracted components		T-test Value
	Falvonoid	Polysaccharide	
3.91	12.57 ± 0.90 A	115.47 ± 3.63 A	8.93 *
7.812	13.14 ± 0.34 A	83.01 ± 1.60 D	10.47 *
15.625	13.50 ± 4.09 A	85.72 ± 5.27 D	19.52 *
31.25	13.91 ± 0.75 A	99.65 ± 4.23 C	14.37 *
62.5	11.16 ± 0.27 A	102.49 ± 1.96 Bc	10.04 *
125	13.19 ± 0.24 A	110.05 ± 3.04 Ab	13.58 *
250	12.99 ± 0.68 A	112.40 ± 2.97 Ab	9.85 *
500	15.07 ± 0.76 A	106.79 ± 2.36 Abc	7.38 *
LSD Value	4.677 ns	10.006 *	---
PHA (+ve control) = 193.61% ± 12.6 for all readings. The (-ve control) = 100 % ± 12.6 for all readings.			
Mean with different letters at the same column represented significant differences.			

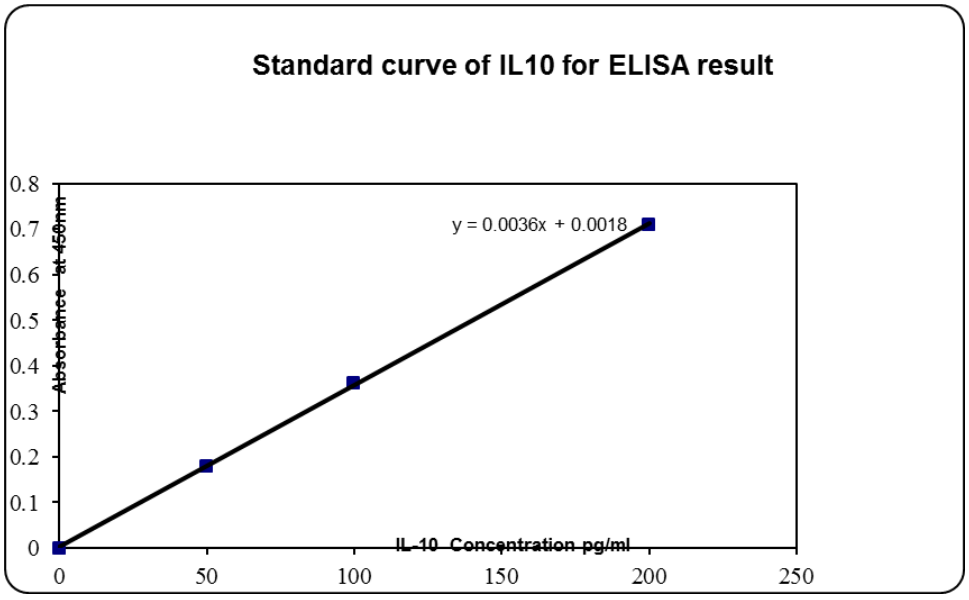
Results indicated that a significant differences (P≤0.05) among the polysaccharides and purified flavonoid as well as within each extract at different concentrations as compared with results of the mitogenic (PHA) as a positive control and the untreated lymphocytes as negative control. As shown in Table(1), the purified flavonoid suppress lymphocyte proliferation with no significances between all concentrations, while the extracted polysaccharides showed stimulating effect for the immune-system by increasing lymphocytes proliferation, specially at high concentrations(500, 250, and 125 µg/ml) in respect to control result. The proliferative activity by the mitogenic (PHA) appeared to be with a high influence than polysaccharides on normal lymphocytes. The results agreed with studies about the effects of flavonoids on immune cell functions [15,16]. These studies showed that the aglycon part of well-known flavonoids possessed inhibitory effects on human lymphocytes [17] and the derivatives of flavone and flavonol which have 2,3-unsaturated bond and at least 1 hydroxyl group present in the flavonoid structure Figure(1-3) showed the suppressive activity, but when various glycosidic substitutions to A- and/or C-ring of the flavonoid aglycones found , this substitutions will eliminate the suppressive activities of their aglycones, regardless of sugar compositions and positions of substitutions[16]. The flavonoids possessing 5-hydroxyl,5-methoxyl and 6-methoxyl groups and those with cyclohexyl instead of phenyl substitution (i.e.2-cyclohrxyl-benzopyran-4-one), showed the greatest inhibition [15]activity to normal lymphocytes. Evidence indicated that selected flavonoids, depending on structure, can affect (usually inhibit) secretary processes, mitogensis and cell-cell interaction including possible effect on adhesion molecule expression and function, moreover certain flavonoids may affect gene expression of cytokines and their effects and cytokine receptors. This due to their capacity to stimulate or inhibit protein phosphorylation that regulate cell function or by counterbalancing the effect of cellular protein tyrosine phosphatases [17].As a result of the relatively poor prognosis and response to conventional chemoradiotherapy, there is a great need for new effective agents. Renewed attention in recent years to natural therapies has stimulated a new wave of research interest in traditional practices. Herbs have become a target for the search for new anticancer drugs. About half of the drugs used in clinical practice come from natural products[18].Various *in vitro* studies about the mechanism of the plant cytotoxicity were differ from one cell culture to another depend on whether whole plant extract was used or any of the plant component. In fact, many nutritive and nonnutritive phytochemicals with

diversified pharmacological properties have shown promising responses for the prevention and treatment of various cancers, including different types of cancer. Most of the animal studies done on *L.barbarum* explained the anti-cancer effects of the plant were through immune enhancements and prevent the development of complications or even tendency to carcinogenesis by increasing numbers of $CD4^+$and $CD8^+$ T cells to relieve the immunosuppression and enhance the anti-tumor function of the immune system[19]. T lymphocytes play a central role in adaptive immunity [4] . At the same time, the percentage of cells in G0/G1 phase was increased, thus because T cells spontaneously arrest in G0 and may remain quiescent for long period of time until exposed to specific antigen or mitogens that initiates a cascade of biochemical events leading the resting T cells to enter the cell cycle then proliferating and differentiating .For this reason the plant active components had been used as immune stimulant or immune adjuvant.[4,20]In China, many types of polysaccharides , among them *lycium* polysaccharides(LBP) and *Astragalus* polysaccharides(APS) are widely used as an immune adjuvant; having been identified as a class of macromolecule that can profoundly affect the immune system, stimulate cell proliferation, induce the expression of surface antigens on lymphocytes, affect the expression of cytokines, and promote the production of antibodies [21]. In a study, it was reported that LBP and APS possess effective immunostimulatory effects when used in vaccination programs against Foot and mouth disease virus (FMDV), Newcastle disease virus (NDV) and Infectious bursal disease virus (IBDV) [22]. The appropriate concentration and antiviral action of APS on the propagation of H9N2 AIV (Avian influenza subtype H9N2 belongs to the low pathogenic avian influenza virus (AIV) group; considered to be a common cause of disease epidemics)in chick embryo fibroblasts (CEF) was investigated .*Astragalus* polysaccharide (APS) effectively increases the expression of IL-2, IL-4, IL-6, IL-10, LITAF and IL-12, promotes cell growth, and improve humoral immunity, and boosts both T cells and B cells .*L. barbarum* polysaccharides (LBP) can stimulate moderate immune responses therefore could potentially be used as a substitute for oil adjuvant in veterinary vaccines. Ling and his colleagues results showed that the isolated polysaccharides, combined with a DNA vaccine encoding the major outer membrane protein (MOMP) of *Chlamydophilaabortus*, induced protection in mice against challenge[23]

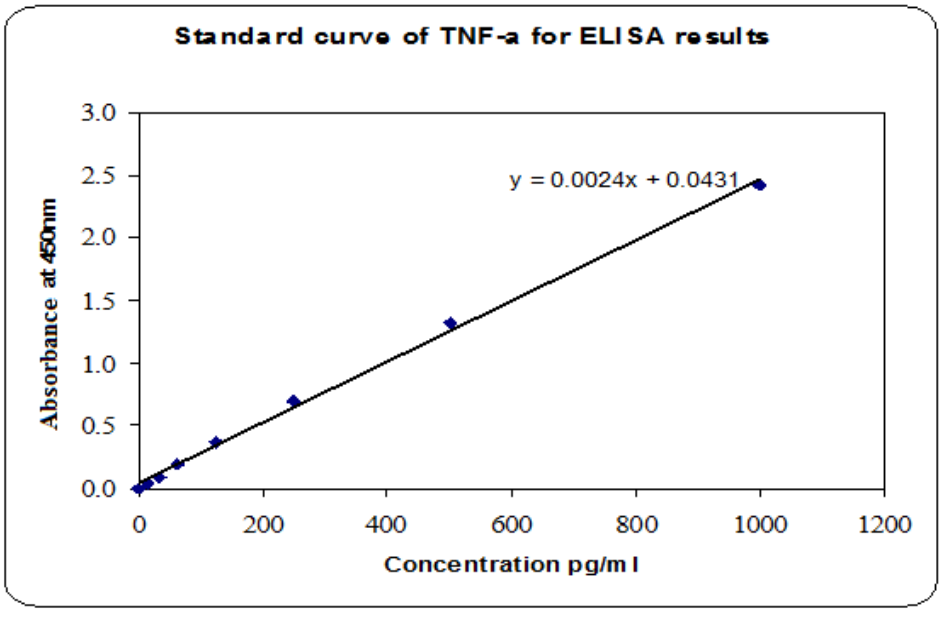
Effect of Extracted Components *L.barbarum* on Cytokine Levels (IL-10 and TNF-α)

In order to trace cytokines (IL-10 and TNF-α) level in the supernatant of the treated lymphocytes with polysaccharides at different concentrations and exposure time (2 and 4 hours) as well as control culture and standard solutions, ELISA was used and standard curve for both interleukins was plotted separately Figure (3).

(A)



(B)



Figure(3):Standard curve of IL-10 (A) and TNF-α (B) analyzed by ELISA, R^2=0.98 And 0.93 respectively

Table (2) Effect of different concentrations of polysaccharides and exposure time (2 , 4 hours) on lymphocytes IL-10 &TNF concentrations

Concentration (µg/ml)	IL-10 Level (pg/ml)		LSD Value	TNF Level (pg/ml)		LSD Value
	2 hr.	4 hr.		2 hr.	4 hr.	
125	140.00 ±7.54c	152.86 ±7.43b	29.41 NS	786.0± 28.71a	338.00 ±8.73c	83.32 *
250	244.20 ±9.37a	129.20 ±7.60c	33.51 *	532.0± 19.62c	359.33 ±8.83c	59.77 *
500	222.4± 8.21ab	74.10 ±7.55d	30.98 *	666.4± 24.62b	493.16 ±10.11b	73.89 *
Control	200.00 b	220.00 a	12.67 *	676.0 b	650.0 ba	17.43 *
LSDValue	23.75*	21.27*	----	69.48*	26.12*	----

* ($P \leq 0.05$).
The means with different small letters at the same column represented significantly difference.

Table (2) showed that whenever IL-10 level increased, a clear decrease in TNF-α level was noticed, for the polysaccharides treated lymphocytes after (2 and 4) hours exposure time. The greatest effect of polysaccharides treated lymphocytes for 2hours exposure caused increasing in IL-10 level to be (244.2pg/ml), at polysaccharides concentration 250µg/ml in comparison to control (200pg/ml), at the same time a significant decreased in TNF-α level to lowest value (532pg/ml) was shown at the same concentration. The highest TNF-α level after 2 hour treatment was (786pg/ml) at 125µg/ml polysaccharides concentration in relative to control value(676pg/ml), at the same time this concentration affected lymphocytes IL-10 level to be at lowest its value(140pg/ml) as shown in Table(2). However, after4 hours exposure, an increase in TNF-α level was combined with decreased in the IL-10 level. Treatment of normal human lymphocytes with different polysaccharides concentrations for 4 hour exposure, showed difference in their effects on both cytokine levels. As shown in Table(2) a large decrease in IL-10 level was dependent on increasing in polysaccharides concentration while in contrast, TNF-α level increased with the increasing of polysaccharides

concentrations with or without significances results.

Both cytokines showed different levels as time of polysaccharides exposure increased. There was a direct relation between polysaccharides concentrations and IL-10 level after 2 hours exposure, and a reverse relation between them after 4 hour exposure, the opposite relation was found for TNF-α level and polysaccharides concentration with the duration of exposure Table(2). Similar results were obtained in a study by *Lei et al on* the effect of *Aralia chinesis* and *Tripterygium wilfordii* on serum TNF-α, IL-4 and IL-10 level in rats with adjuvant-induced arthritis. Both plants had significantly increased IL-4, IL-10, levels and markedly reduced TNF-α level compared with control group[24]. The induction of cytokine is a key event in the initiation and regulation of an immune response. Many compounds are now used routinely to modulate cytokine production, and therefore the immune response, in a wide range of diseases, such as cancer.

Interleukin-10 and tumor necrosis factor- α are two important cytokines in antitumor immunity. In a study by [25] showed that the effects of *L.*barbarum polysaccharides protein complex (LBP3p) on the expression of interleukin-2 and tumor necrosis factor-α in human peripheral blood mononuclear cells were investigated by reverse transcription polymerase chain reaction (RT-PCR).The LBP3p significantly enhanced interleukin-2 mRNA expression at 8 hours, peaking at 12 hours and returning to baseline levels at 15 hours. At24 h, a marked decrease was observed. With respect to tumor necrosis factor mRNA expression, human peripheral blood mononuclear cells exposed to LBP3p showed a significant increase as early as 2 hours after treatment. The greatest increase was observed at 4 h after treatment, returning to baseline levels at 8 hours, being undetectable at 24 hours [25]. Interleukin-10 (IL-10) is a pleiotrophic cytokine that has an important role in regulating the immune response [26]. This cytokine potently inactivates macrophages, inhibiting the expression of proinflammatory cytokines [e.g., tumor necrosis factor α (TNF-α) and IL-6] and disabling antigen presentation/T cell activation, by inhibiting expression of major histocompatibility complex class II, B7-1, and B7-2 [27]

The anti-inflammatory activity of IL-10 is augmented by enhancing the release of soluble(s) TNF receptors (R) and IL-1R antagonist. In contrast to its activities on macrophages, IL-10 induces the proliferation of mast cells, B and T cells, and enhances T cell responses to IL-2 [28]. A major focus of IL-10 research has been to identify the mechanism by which IL-10 mediates suppression of cytokine synthesis. This remains a controversial field; specifically, the ability of IL-10 to

inhibit lipopolysaccharide (LPS)-induced gene expression has been shown to be transcriptionally mediated via the inhibition of the nuclear factor-κB pathway. However, further evidence also suggests that IL-10 can act through a post-transcriptional mechanism via destabilizing mRNA, in the case of TNF-α and the chemokine KC. This effect requires the AU-rich elements in the 3′ untranslated region . Furthermore, these reports suggest that the effects of IL-10 are indirect and that IL-10 is inducing a gene whose product is responsible for mediating the destabilization of mRNA [29]

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In one study used microarray analysis to identify IL-10-inducible genes in the presence and absence of the powerful pro-inflammatory stimulus LPS. These studies have identified 19 inducible genes for IL-10. Three of these genes, IL-1ra, SOCS3, and CD163, have previously been shown as being regulated by IL-10; however, the other 16 represent novel IL-10-inducible genes first identified in a study by Kaur *et al* [30]. The result of the present study indicated that *Lycium* polysaccharides acted to reduce tissue inflammation – in part by inhibition of TNF-α gene expression and promote IL-10 production and expression. Although *in vitro* studies did not necessarily predict results *in vivo* out comes, such studies have provided insights into molecular targets, and the relative contributions of these activities as a potent immune-modulator agent which need *in vivo* elucidation and more investigation for their action and mechanism must be subjected to a further studies

Conclusion:

The Immunomodulation effect for the Lycium polysaccharides may play a role through reducing tissue inflammation – in part by inhibition of TNF-α gene expression and promote IL-10 production and expression.

Reference

1. Townsend VC, Gest E. Flora of Iraq (Monocotyledones), vol. 8.Ministry of Agriculture Agrarian Reform.1985, Baghdad, Iraq.

2. Chang R, So K. Use of anti-aging herbal medicine, Lycium barbarum against aging associated disease. Cellular and Molecular Neurobiology 2008, vol.28, pp:643-662.

3. Zhu CP, Zhang SH. Lycium barbarum polysaccharide inhibits the proliferation of HeLa cells by inducing apoptosis. Journal of the Science of Food and Agriculture 2013, vol.93,pp:149-156.

4. Chen Z, Kwong BHT, Ha SC. Activation of T lymphocytes by polysaccharide-protein complex from L.barbarum. International Immunophamacology 2008, vol.8, pp:1663-1671.

5. Wang C, Chang S, Stephen B, Chen, B. Isolation of caroteniods, flavonoids and polysaccharides from Lyciumbarbarum and evaluation of antioxidant activity. Food chemistry 2010,vol. 120, pp:184-192.

6. Chia CW, Shyh CC, Bing, HC. Chromatographic determination of polysaccharides in Lycium barbarium Linnaeus. J. Food chemistry 2009, vol.(116),pp: 595-603.

7. Edwin J, Sheeja EJ. Textbook of Pharmacognosy and Phytochemistry.vol. 4, pp:181. CBS Publishers & Distributors 2009, NewDelhi,India.

8. Dubois M, Gill KA, Hamilton JK, Rebers PA, Smith F. Clourimetric method for determination of sugar and related substances .J. Analytical chemistry 1958,vol.28,pp:350-356.

9. Boyum A. Separation of lymphocytes from blood and bone marrow. Scand. J. Clin. Lab. Invent 1988. vol.21,suppt.97.

10. Rafael F, Vaclav V. Advanced Methods in Cellular Immunology 2000, vol. 3.CRC-Press LLC.U.S.A.-SAS. Statistical Analysis System, User's Guide. Statistical. Version 7th ed. 2004. SAS. Inst. Inc. Cary. N.C. USA.

11. Freshney RI. Culture of Animal Cell. Sixth Edition. 2012.Willy-Liss,New York.

12. De-waal, M, Moree, KM. The cytokine Handbook ,3rd edn.1988 Thompson, Academic Press, London

13.Harunobu A, Norman, R. F.A review of botanical characteristics, photochemistry, clinical relevance in efficacy and safety of Lycium barbarum fruit. Food Research International 2011, vol. 30,pp:3-27.

14.Guihao Y, Yuli, D. Optimization of extraction technology of Lycium barbarum polysaccharides by Box-Behnken statical design. Carbohydrate polymers 2008,vol.74,pp:603-610.

15. Hirano T, Oka K, Kawashima E, Akiba M. Effects of synthetic and naturally occurring flavonoids on human peripheral-blood lymphocytes. Life Sci. 1989.vol.45,pp:1407-1411

16. Siess MH, Le-Bon AM, Canivenc-Lavier MC, Suschetet, M. Mechanism involved in the chemoprevention of flavonoids. Biofactors 2000, vol.12, pp:193-199

17. Nair MP, Mahajan S, Reynolds JL, Aalinkeel R, Nair H, Schwartz SA, Kandaswami C. The Flavonoid Quercetin Inhibits Proinflammatory Cytokine (Tumor Necrosis Factor Alpha) Gene Expression in Normal Peripheral Blood Mononuclear Cells via Modulation of the NF-κβ System. Clinical and Vaccine Immunology2006, vol. 13, pp:319-328.

18. Butler MS. Natural products to drugs: natural product derived compounds in clinical trials. Nat Prod Rep. 2005.vol.22, pp:62–195.

19. Lu G, Sheng HZ, Xiang LY, Hui, B.X. Immunomodulation and antitumor activity by a polysaccharide-protein complex from L.barbarum. International immunopharmacology 2004. vol. 4, pp:563-569.

20. Liu J, Zhong Y, Li H, Zhang N, Ma W, Cheng, G. Enhancement of Astragalus Polysaccharide on the immune responses in Pigs innoculted with foot-and mouth disease virus vaccine. Int. J. Biol. Macromol,2011. vol. 49,pp:362-368.

21. Zhang N, Li J, Cheng G, Zhu X, Liu, F, Zhang, Y, Liu, Z, Xu J. Effects of Astragalus polysaccharide on the immune response to foot-and-mouth disease vaccine in mice. Carbohyd. Polym. 2010, vol.82,pp:680-686

22.Kallon S, Li X, Chen C, Xi Q, Chang S, Xue C, Ma J, Xie Q, Zhang Y. Astragalus polysaccharide enhances immunity and inhibits H9N2 avian influenza virus in vitro and in vivo. Journal of Animal Science and Biotechnology 2012, vol. 4, pp:1-20.

23. Ling Y, Li S, Yang J, Yuan J, He C. Co-administration of the Polysaccharide of Lycium barbarum with DNA Vaccine of Chlamydophilaabortus Augments Protection. Immunological Investigations 2011,vol.40, pp:1–13.

24. Lei Y, Jianjun F, Haiwang J, Xuechong Z. Effect of Aralia chinesis on serum TNF-a, IL-4 and IL-10 level in rats with adjuvant-induced arthritis. Bangladesh Journal of Pharmacology 2012. vol. 7, pp:125-130.

25. Lu G, Sheng HZ, Qiong L, Hui BX. Apolysaccharide-protein complex from Lycium barbarum upregulates cytokine expression in human peripheral blood mononuclear cells. European journal of pharmacology 2003,vol.471,pp:217-222.

26. Moore KW, Malefyt R, Coffiman L, Garra, A. Interleukine -10 and interlukine-10 receptor. Annual Review of immunology 2001, vol. 4, pp: 233-250.

27. Buelens C, Willems F, Delvaux A, Pierard G, Delville JP, Velu, T, Goldman, M. Interleukin-10 differentially regulates B7-1 (CD80) and B7-2 (CD86) expression on human peripheral blood dendritic cells Eur. J. Immunol. 2000.Vol. 25,pp: 2668-2672.

28. Gerard C, Bruyns C, Marchant A, Abramowicz D, Vandenabeele P, Delvaux, A, Fiers W, Goldman M, Velu T. Interleukin 10 reduces the release of tumor necrosis factor and prevents lethality in experimental endotoxemia,J. Exp. Med. 2000.vol. 177,pp: 547-550.

29. Schottelius AJ, Mayo MW, Sartor RB, Baldwin AS. Interleukin-10 signaling blocks inhibitor of kappa-B kinase activity and nuclear factor kappa-B DNA binding, J. Biol. Chem.2000.vol. 274,pp:31868-31874

30. Kaur K, Sharma AK, Singal PK. Significance of changes in TNF-α and IL-10 levels in the progression of heart failure subsequent to myocardial infarction. Journal of the American Physiological Society(AJP-Heart)2006,vol.291,pp:H106-H113.

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