Immunomodulation of IraqiLyciumbarbarium Carotene *in vitro*

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Abstract

The contents of *Lyciumbarbarum*active constituents as wild Iraqi plant was extracted then estimated qualitatively and quantitatively by HPLC method. Effect of the extracted carotene was employed in the current study on the normal human blood lymphocytes to estimate the extract effect on IL-6 and IL-8 levels as immune modulating agent.*L.barbarum*considered as a good source for caroteneas it's containedwas 0.287mg/g dried fruits.The current study results about the effect of extracted carotene on normal human lymphocytes and interleukins 6 and 8 levels showed that the compound can enhance cell-mediated immune responsesthrough enhance lymphocytes proliferation with increasing in IL-8 and decrease IL-6 level

Keywords: *Lyciumbarbarumactive, lymphocytes, L.barbarumconsidered, proliferation, caroteneas.*

Introduction

Wild Iraqi *Lyciumbarbarum*is rich with carotenoids that make the fruit possessed a beautiful orang color(1). Lycium was very important medicinal plant that possessed several biological activities, among them; hepatic protection from being damage, immune boosting agent, and can reduce the harmful effects of the chemotherapy drugs and radiotherapy treatment(2), also the regular carotene intake with daily foods as rich carotene vegetables diets might play important rule in reduction of different types of cancer(3). Due to the potent antioxidant properties of such compounds, many researches correlated between their importance in curing several severalchronic disease as well as a potent biological effects on human(2). Moreover the immune modulation effects of beta carotene were suggested to be a major factor in fighting cancer,by boosting the immune system(4). This study projected on extraction of total carotene from Wild*L.barbarum*to insure the immune boosting activity for this components and declare how it can regulate some interleukin levels of a normal human blood lymphocytesculture.

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Material and Method

Extraction the total carotene from the fruit (5):

Ripe *L.barbarum*fruits were dried and powdered by blender.A quantity of one gram dried fruit powdered was homogenized well with 3ml distilled water, to be mixed then with 2ml absolute ethanol with continues agitation. After filtration, total carotene was extracted by 10 ml n-hexaneusing separator fennel. The organic hexane layer was objected to HPLC assay.

Immunomodulation Determination (*in vitro)*(6).

In this study about five ml blood samples was taken from peripheral vein of healthy volunteerswith age of (25-35) year's old whom never using any drugs before 2 weeks. Each blood sample was suspended onto 3ml lymphocyte separation fluid(sp.gr.1.077g/L) in vacuumed tubes separately to be centrifuged then for half an hour at2000 rpm. The isolatedcell of lymphocytes were collected by sterile Pasteur pipetteand transfer each sample into separatedvacuumed tubes after washing the pellets, them suspended in with 5ml RPMI -1640 containing 10% fetal calf serum. All tubes were incubated over night at37^0C in 5% CO_2 incubator.

i-Measurement of the Viable Lymphocytes by MTT Assay (7).

According to Freshney 2012 protocol, the effect of extracted carotene on normal lymphocyte culture was determined by preparing different carotene concentrations (500,250, 125, 62.5,31.25,15.625,and7.8125) µg/ml, then sterilized with disposable millipore filter. Aliquot of 100 µl lymphocyte cell culturewas treatedwith each carotene concentrations in triplicate with control positive that employed as 0.1% PHA solution (phytohemagglutinin), and control negative represented by cells suspended in medium without any treatment.The 96 well plate was incubated for 20 hours, then centrifuged for 5mimutes at 1500rpm. The medium was gentlyaspirated andMTT dye (2mg/ml) to all wells was added. The microtiterplate wasincubated again for about 3- 4 hours. Crystals were formed by living lymphocytes might dissolved with 100µl DMSO and the intensity of purple color for each well was read at 620 nm and recorded within short peroid by ELISA reader.

-Percentage of viable Lymphocytes can be calculated with the following equation:

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

Determination of the Cytokine Level by ELISA Technique[8]

-For this assay two tissue culture plates of 24 wells, were seeded with 1ml of isolated lymphocytes suspended cells($1X10^6$cell/well). One plate was treated with 1ml from the extracted carotene in three selected concentrations (100, 500, 250)µg/ml in triplicate to be incubated for two hours, while the other plate incubated for four hours after treatment. Lymphocyte cells in growth medium alone was represented the negative control.

All wells content were pooled at the end of exposure time the end of each interval times into sterile tubes and centrifuged for 10 minutes at 2000 rpmto separate the pellets from the supernatant of each tube and kept at $-20C^0$ to be estimated by ELISA kits for IL6 and IL-8 assy.

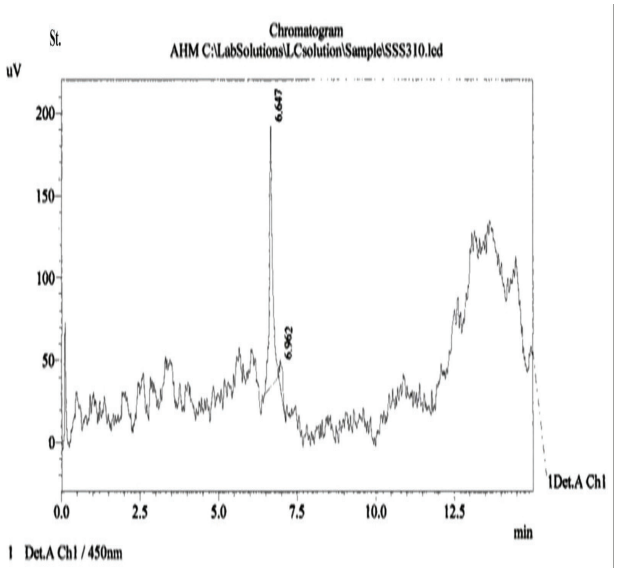
The Cytokine IL-6 and IL-8 Levels

The kit work was done accordingkit protocol of United StateBiological and Biochemical Reagents Trade Company specific for (IL-6 and IL-8) level, that can be calculated through plotting standard curve between concentration versus absorbanceread by ELISA microplate reader (Olympus/Japan) at 450 nm were plotted ,then IL-6 and IL-8 levels for each sample was calculated and then evaluated statistically.

Results

Content of Total Carotene Determination by HPLC

The retention time for the *β*-carotene standard was (6.647 minutes)as shown in figure(1). HPLC chromatogram of the *Lyciumbarbarum*extracted carotene showed a presence of sharp peak with retention time (6.638)minutes, figure(2).

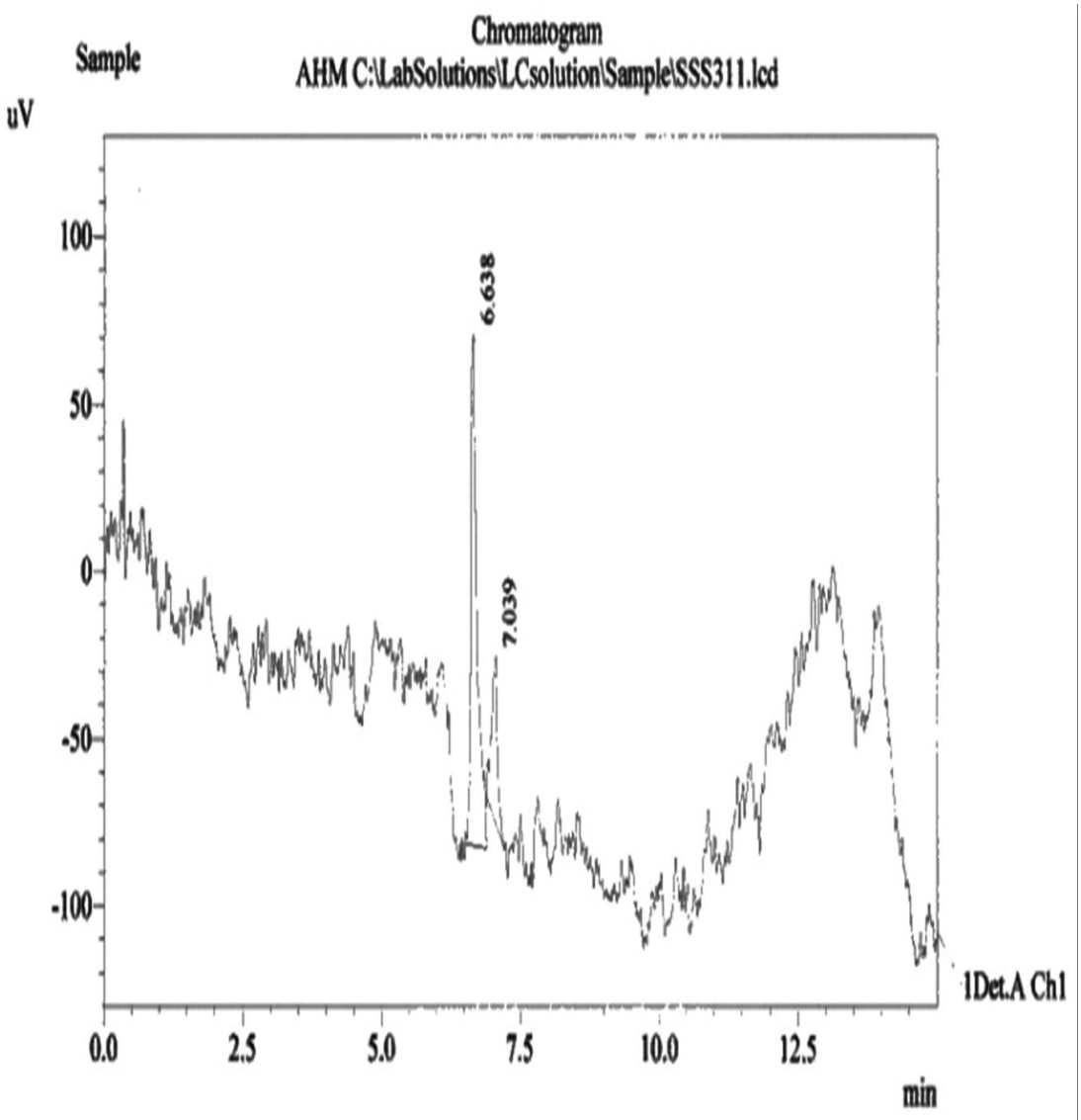


PeakTable

Detector A Ch1 450nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.647	1145	159	90.127	90.525
2	6.962	125	17	9.873	9.475
Total		1271	176	100.000	100.000

Figure(1) HPLC Chromatogram of the standard *β*-carotene



PeakTable

Detector A Ch1 450nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.638	1162	154	76.226	75.752
2	7.039	362	49	23.774	24.248
Total		1524	203	100.000	100.000

Figure (2) HPLC Chromatogram of the extracted carotene.

Total extracted carotene concentration can be calculated thoughdata applied for area under the curve at retention time6.638 minutes of the extract andm6.647 minutes of the standard with the folloeing equation:

$$\frac{\text{Peak area of extracts}}{\text{Peak area of standard}} \times \text{Standard solution concentration} \times \text{total volume of extract} = \text{total carotene (mg) in 1g dried fruit powder}$$

That is

$$\frac{154}{159} \times 0.0018 \frac{\text{mg}}{\text{ml}} \times 165\text{ml} = 0.287\ \text{mg}$$

MTT Result

Lymphocyte Proliferation determination by MTT assay:

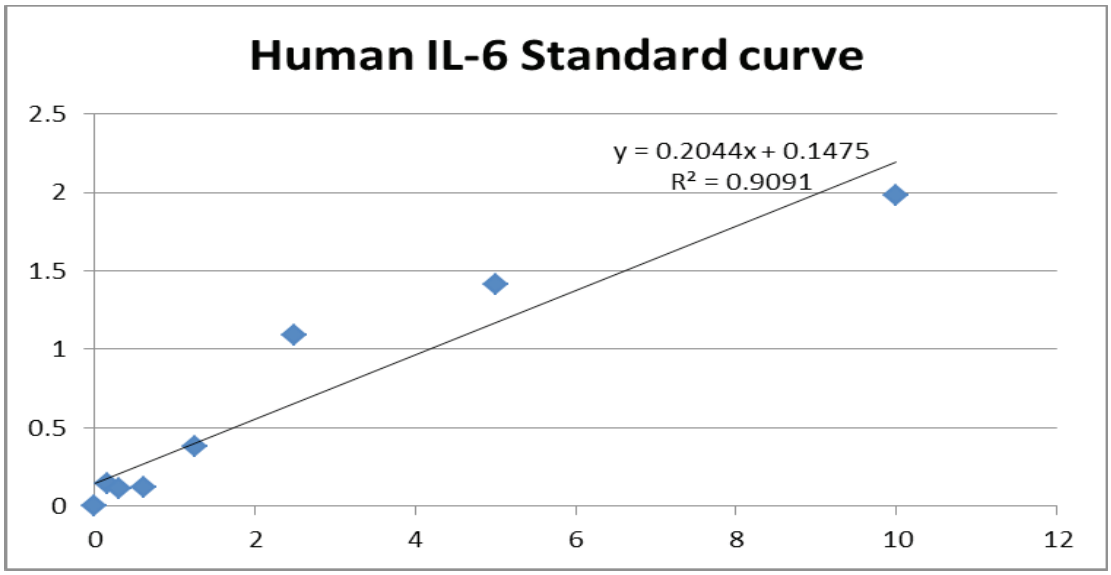
All Results were summarized in Figure(1) showed that the seven concentrations of extracted carotene affect normal human Lymphocyte by proliferation in corresponding with negative control.

Levels of IL-6

Table (1) showed the average absorption of standard solutions for IL-6 , from which a standard curve, figure (2), was plotted to get the straight line equation

Table(1) Standard IL-6 concentration and average absorption

IL-6 standard concentration(pg/ml)	Average absorption
0	0
0.156	0.144
0.312	0.112
0.625	0.117
1.25	0.38
2.5	1.093
5	1.411
10	1.978



Figure(3) Interleukin-6 standard curve

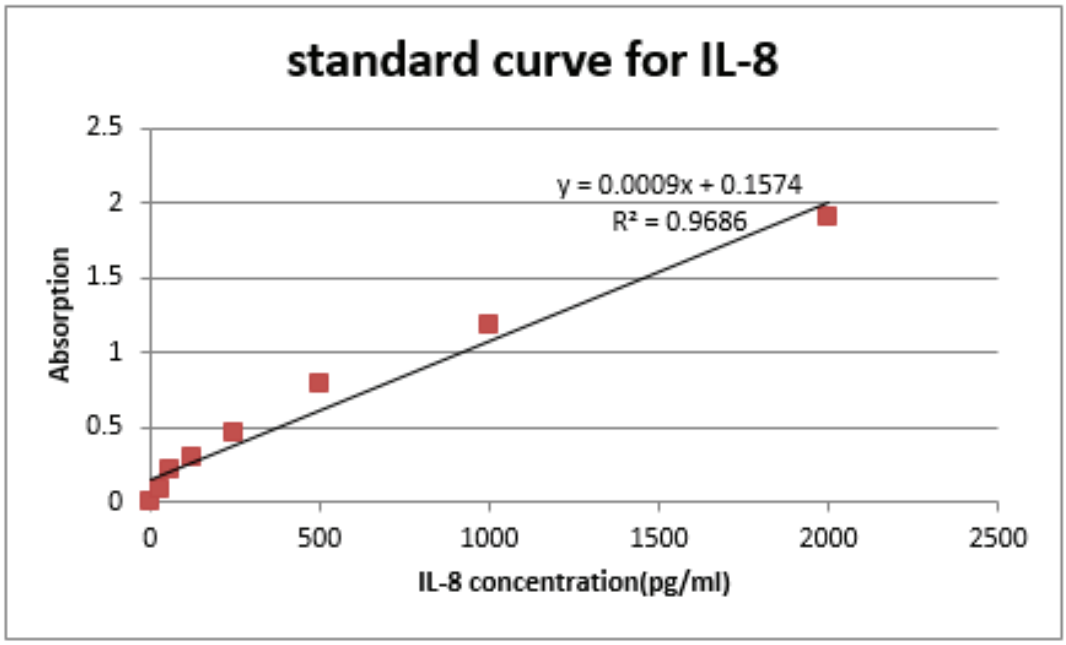
Y=0.2044X+0.1475

{X=Y-0.1475/0.2044 pg/ml} calculation ofthe IL-6 level.

Levels of IL-6 from lymphocytes treated with three concentrations of extracted carotene were shown in table(2) after two intervals 2 and 4 hours of exposure.

Interleukin 6 (IL-6) encoded by the *IL6* geneis; produced from different cells such as smooth cells in blood vessels. The IL-6 main action was indicated as a pro- inflammatory cytokine and myokine[9], which mediated by inhibition the secretion of TNF-α and IL-1 with activation of IL-10[10].Adecrease in IL-6 level secreted from treated lymphocytes with carotene in different concentration and 2 or 4 hours exposure, were shown in the current study. Dietary carotenoids and retinoidsplayed an obvious roles in boosting human's innate and acquired immunity against inflammation[11]. The currentaresearch could be a preliminary *invitro* study involve the beneficial effect of *L.barbarum* carotene, in spite thatin astudy concluded that the carotene decrease IL-6 level due to its suppression of transcription of this interleukin[12].

Levels of IL-8:



Figure(4) Interleukin-8 standard curve

Figure(4) showed a standard curve for IL-8 and the straight line equation

Y=0.0009X+0.1574

{X=Y-0.1574/0.0009} calculating IL-8 level (pg/ml)

After application of IL-8 equation, the levels of treated lymphocytes with three concentrations from the extracted carotene were determined for 2 and 4 hours intervals, table(2).

Table(2) Level of IL-8 for Lymphocytes treated with *Lycium* carotene

Lyciumcarotene Extract concentration(mg/ml)	**IL-8 Level(pg/ml) After 2hr.exposure**	**IL-8 Level(pg/ml) After 4hr. exposure**
125	2922.8888	3167.333
250	2951.7777	2856.2222
500	2986.8888	3062.8888
Control	2799.000	2799.550

Results showed that IL-8 level elevated for blood lymphocytes treated with different concentrations and two intervals time of exposure. Interleukin 8 secreted by macrophages and epithelial orendothelial cells, is an important mediator of the innate immune system (9).IL-8 acted as *neutrophil chemotactic factor*by induction of chemo-taxis for neutrophils and granulocytes, in order to migrate toward infection. Moreover, it acted to stimulate phagocytosis at site of inflammation as in bronchitis and respiratory viral diseases [13]. *Lyciumbarbaum* carotene play umportent role in treating different diseases via improvement of body immunity[14].

The current results were agreed with a study by Lin and co about the effect of edible carotene on normal peripheral blood lymphocytes[15].

Conclusion

The contents of *Lyciumbarbarium* active constituents as wild Iraqi plant was considered a good source for carotene which contained 0.287mg/g dried fruits.The current study focused on the effect of extracted carotene on normal human lymphocytes and interleukin 6 and 8 levels. The study general conclusion insured that this compound can enhance cell-mediated response for immune system.*Lyciumbarbarum* carotene possess major role in boosting human immunity that highlighted their importance as food as well as a promise medical component.

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Conflict of Interest: There is no conflict of interest among the authors.

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Ethical Clearance: This study is ethically approved by the Institutional ethical Committee.

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