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CYTOTOXICITY EVALUATION OF *P. KURDICA* ACTIVE COMPOUNDS ON HUMAN LYMPHOCYTES

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Abstract

The discovery and diagnosis of a new drug to stimulate the human immune system has become a necessary importance and a main objective of many researches and studies.

This study focused on the effect of active compounds in *Pistacia atlantica kurdica* plant as a stimulant and anti-tumor agent. *P. kurdica* has an important medical effectiveness. It is rich in bioactive and important compounds for humans. This plant gum has been traditionally used because it contains phenolics compounds to treat many diseases including stomach infections, Colon infection, wound healing, gastrointestinal cancer treatment and others. Quantitative assay (Total phenolics compounds), shown that the gum extract contains about (128 mg of Gallic acid equivalent per g dry weight). (HPLC) was also performed that the gum contained (Rutin, Quercetin, Kaempferol, Catechin). The treatment of normal lymphocytes with the gum phenolic extract showed a highly significant effect in increasing the proliferation of these cells, reach to 300%. This increase in cell differentiation was directly proportional to the increased concentration of gum extract used compared with the control (cells with medium only). The gum phenolic extract caused stimulation in (IL-2 and TNF-α) level, this was studded by ELISA technique at 450nm.

Gum extract resulted in a significant increase in the levels of both (IL-2 and TNF-α) compared with control (cells with medium only). That the change in the level of the interleukins, for 2 hours and 4 hours of treatment, in the 2 hours fond that IL-2 increase the level into 830pg/ml then decrease in the 4 hours to 13.15pg/ml.

TNF-α effects, after the cells were exposed to gum phenolic extract for 2 hours, there was a significant effect between (540.38 pg / ml and 642.31 pg /ml), while after 4 hours the concentration of TNF-α increased significantly, this results agree with previous related studies.

***Key words*:** *P. kurdica,* human Lymphocytes, *Pistacia atlantica kurdica,* Medicinal plants

Introduction

Medicinal plants are Plants that have curative properties or may do beneficial pharmacological effects on the human body. Medicinal plants naturally produced and generally accumulate some secondary metabolites such as alkaloids, flavonoids, steroids, terpenes, saponines, glycosides, tannis and volatile oils, medicinal plants have been used for curative of illness and diseases, since the dawn of history as shown in ancient Chinese scriptures and Egyptian papyrus hieroglyphics shown the uses of medicinal plants. Domestic culture mostly used plants for curing rite, nonetheless other people develop traditional medical system such as Ayurvedic in which herbal therapies are being used. (Motaleb, 2011). The pistachio tree belongs to Pistacia, a genus of eleven species in the Anacardiaceae family distributed in the Mediterranean and Middle Eastern areas. *P. kurdica* (Betoum or Baneh) is a tree which can range to 25 m in height. Greece is one of the most important pistachio producing countries, along with Iraq, Iran, Turkey, and India (Hosseini *et al*., 2013). Baneh tree or wild pistachio with scientific name of *P. kurdica*is a plant from Anacardiaceae family. It is native in north of Iraq (Kurdistan region), Iran especially in the Auramanat area lying in the Kurdistan region in western part of Iran. (Minaiyan *et al*., 2015) It is the most characteristic plant species of the pre-Saharian regions of the Iran (Hosseini *et al*., 2013). This plant is an evergreen shrub that grows native in some southern and central American and eastern Mediterranean countries. *P. kurdica* thrives particularly around the

Zagros mountains in Iran (Khodavaisy *et al*., 2016).

The gum phenolic extract of Pistacia species can be considered as a natural herbal source in the pharmaceutical industry.

Methodology

Materials used in this thesis experiments were provided from Different sources; chemicals are supplied from the local markets. Instrument used in this study were present in Biotechnology Research Centre laboratories in Al-Nahrain University. Kits Were supplied from Elabscience company/china and testes were done in Biology department laboratory in the Biology collage at Babylon University. The Lymphocytes cytotoxicity by MTT assay for viability and the Cytokine profile were carried out in the Biotechnology Research Centre in Al-Nahrain university. The High-performance liquid chromatography (HPLC) assay and total phenolic compounds was accomplished in the Ministry of Science and Technology laboratories.*P. kurdica* gum dissolved with equal volume 1:1 in DMSO (room temperature); then the normal human lymphocytes treated with gum phenolic extract to measuring the cell viability. IL-2 and TNF-α were used to show the cytotoxicity effect of gum phenolic extract immunology.

Result

Lymphocyte proliferation was determined using MTT assay. Results of the effect of different concentrations of *P. kurdica* gum extract on proliferation of normal human lymphocyte are shown in fig 1.

In case of IL-2, after 2 hours exposure, to the gum extract the level of interleukin was increased significantly as increasing in gum extract concentration treated when compared with the negative control. The highest significant effect (830 pg/ml) was at 1000 µg/µl *P. kurdica* gum phenolic extract conc., while at 62.5 µg/µl conc. there was no significant effect in IL-2 level. At the 1.966 µg/µl conc. of *P. kurdica*gum phenolic extract had significant effect reducing IL-2 level to reach 590pg/ml.

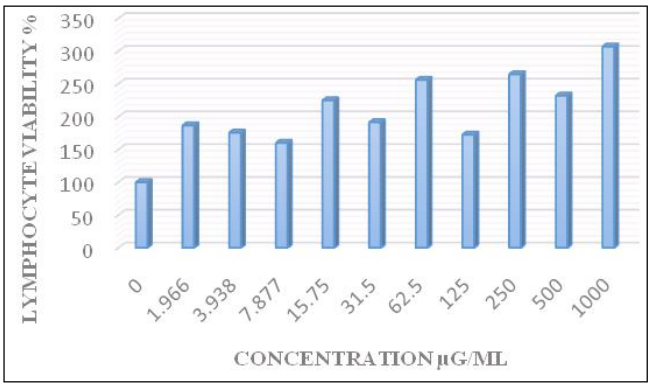

Fig. 1: Viability of treated lymphocytes with gum phenolic extract

As shown in table 1. When the lymphocytes treated with *P. kurdica*gum phenolic extract at different concentration, IL-2 level was significantly decreased after 4 hours exposure. In Case of TNF-α Level; the exposure time and increasing in gum phenolic extract concentrations gave different effects as shown in tab 1. After 2 hours exposure using1000 µg/µl and 62.5 µg/µl conc. of *P. kurdicagum* extract there were significance (540.38 pg/ml and 642.31 pg/ml) in TNF-α level, while the exposure time factor has no significant effect on both. As shown in table 1. After treatment with gum phenolic extract for 4 h, TNF-α Level was significantly increased in human lymphocytes.

Discussion

These results refer that IL-2 expression in human lymphocytes exposed togum phenolic extract showed a significant increase as early as 2 hours after the treatment. While agreatest decline was observed after 4 h after of treatment. However, because of the short half-lives of IL-2 and TNF-α in serum, systemic administration of high doses of IL- 2 and TNF-αis needed, resulting in severe side effects, such as vascular leak syndrome, edema, anemia, fever and hypotension (Villikka and Pyrhonen, 1996). The induction of cytokine is a key event in the initiation and regulation of an immune response. Many compounds are now use droutinely to modulate

Table 1: Effect of different concentrations of gum phenolic extract and exposure time (2, 4 hours) on lymphocytes IL-2 &TNF-α concentrations.

Concentration (µg/ml)	IL-2 Level *pg/ml*		LSD Value	TNF-α Level *pg/ml*		LSDValue
	2 Hrs	4Hrs		2 Hrs	4Hrs	
1000	830± 2.00 a	13.15 ± 0.24 a	102.67 NS	540.38± 26.84ab	632.69± 42.71 a	102.67 NS
62.5	710± 0.50 ab	3.65 ± 0.11 b	79.36 NS	642.31± 37.92 a	596.15± 32.64 a	79.36 NS
1.966	590± 0.40b	1.39 ± 0.01 b	115.82 *	494.23± 21.09b	630.77± 36.81 a	115.82 *
Control	710± 0.30 ab	1.07 ± 0.00 b	107.73 *	496.15± 17.62b	617.31± 36.76 a	107.73 *
LSD Value	135.78*	5.34 *	—	137.64 *	87.92 NS	—

* (P<0.05). Means with different small letters at the same column represented significantly difference.

cytokine production, and therefore the immune response, in a wide range of diseases, such as cancer. The results of TNF-α agree with a study by (Yaseen, 2014); a significant increase as early as 2h after treatment for TNF-α secretion, then a greatest increase was observed at 4h after treatment, returning to baseline levels at 8 h, being undetachable at 24 h. The use of cytokines has a long history in immunotherapy, with interferon-a being the first cytokine used in tumor immunotherapy in 1957. Cytokines can regulate the immune response and are secreted by immune effector cells as well as a large variety of other cells, including tumor cells. Several cytokines are capable of mediating tumor regression in some malignancies. IL-2 &TNF-α are two of the most extensively studied cytokines for tumor immunotherapy purposes. IL-2 stimulates the proliferation of cytotoxic T lymphocytes, helper T lymphocytes, natural killer cells, lymphokine-activated killer cells and macrophages, all of which can participate in immunological antitumor mechanisms (Mizuno *et al.*, 2000). TNF-α is a peptide, which plays a pivotal role in host defense (Beutler, 1995). It may act on monocytes and macrophages in an autocrine manner to enhance various functions, such as could significantly upregulate the expression of IL-2 &TNF-α at both mRNA and protein levels in a dose-dependent manner in human lymphocytes.

References

Hosseini, F., A. Adlgostar and F. Sharifnia (2013). Antibacterial activity of Pistacia atlantica extracts on Streptococcus mutans biofilm. *Int. Res. J. Biological Sci.*, **2(2)**, 1-7.

Khodavaisy, S., S. Rezaie, F. Noorbakhsh, E. Baghdadi, S. Sharifynia and F. Aala (2016). Effects of Pistacia atlantica subsp. kurdica on Growth and Aflatoxin Production by Aspergillus parasiticus. *Jundishapur Journal of Microbiology*, **9(7)**.

Minaiyan, M., F. Karimi, and A. Ghannadi (2015). Anti-inflammatory effect of Pistacia atlantica subsp. kurdica volatile oil and gum on acetic acid-induced acute colitis in rat. *Research Journal of Pharmacognosy,* **2(2)**: 1-12. þ

Mohammed, A. Motaleb (2011). Selected Medicinal Plants of Chittagong Hill Tracts.