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Effect of *Tribulus Terrestris* Aqueous Extract on Survival and Growth of Human Peripheral Blood Mononuclear Cells (Hpbmc) and Several Cancerous Cell Lines

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A B S T R A C T

Tribulus terrestris (TT) is a member of the Zygophyllaceae family. Traditional Chinese medicine recommends the use of TT for treatment of a variety of diseases. In the present study we further investigated anti-proliferative and cytotoxic effects of TT extract on normal and several malignant cell lines. For this purpose, TT was collected and after authentication, aqueous extract was prepared. Cytotoxic activity of TT extract at different doses (5-640µg/ml) was determined by LDH assay. Proliferative activity of TT extract was determined by trypan blue staining and cell counting methods. The results showed that IC50 of the extract for cancer cell line is 320 µg/ml and it is higher for hPBMC. On the other hand, we observed proliferative effect of TT extract on hPBMC but not on malignant cell lines.

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Introduction

Despite the enormous progress made in the area of various disease, the treatment of cancer with a very high incidence and mortality rate is still a mystery that faced by mankind. Most drugs used for cancer therapy have side effects on normal cells and need complementary drugs such as stimulating agents and antibiotics. Although stimulating factor is good choice for combination-therapy, adverse effects such as fever, atopic cutaneous reactions, eczema, diarrhea, bone pain and psoriasis limit their uses [1]. Traditional medicine covers three thousands species of plants that has been used to treat cancer. That is why today is toward the use of herbs and their constituents as potential anti-cancer drugs that have proven a direct toxic action on malignant cells.

Tribulus terrestris (TT) belongs to the zygophyllaceae family and is widely distributed in all parts of the world [2]. TT has a significant effect for the treatment of various disease including hypertension, coronary heart disease, kidney troubles, colicky pains, hypercholesterolemia and sexual dysfunction and it has an anti-bacterial and cytotoxic activity [3-5]. Various studies have showed that TT contains compounds such as steroids, saponins, flavonoids, glycosides, phytosterols, alkaloids and non-saturated fatty acids [6-9]. Saponins of TT have anti-cancer effects; however, their exact mechanism is not well understood [10]. In the present study, the effect of TT extract on survival and growth of human peripheral blood mononuclear cells (hPBMC) and several cancerous cell lines was evaluated.

Materials and Methods

Extract preparation

TT was obtained from hillside of Kermanshah Mountain (West of Iran) and authenticated by the Agricultural College of Razi University (Kermanshah, Iran). For aqueous extract preparation, one weight of air-dried and fine powdered thistle, seeds and leaves of TT were suspended in 20 volumes of distilled water and stirred for 24 hours at room temperature. The mixture was filtered and dried using a freeze drier.

Cell culture

Non-adherent cell lines including KG-1, K562, SP20 and Jurkat were purchased from Cell Bank of Pasteur Institute (Tehran, Iran). The culture media for cell lines was RPMI-1640 plus 10 % FCS, 100 units/ml penicillin and 100 µg/ml streptomycin.

Isolation of human Peripheral Blood Mononuclear Cells (hPBMC)

Blood was obtained from healthy donors. Heparinized blood samples were diluted with RPMI-1640 two times and carefully layered on equal volume of Ficoll-Paque solution. The mixture was centrifuged at 1400 ×g for 20 min at 18 ^{0}C. The undisturbed lymphocyte layer was carefully collected using a Pasteur pipet. The lymphocyte was washed carefully with adequate volumes of RPMI-1640 plus 10% FBS, 100 units/ml penicillin and 100 µg/ml streptomycin. PBMC was enriched for lymphocytes by incubating culture plates at 37 ^{0}C for 24 h. Non-adherent lymphocytes were separated from adherent cells (monocyte). Viability of PBMC was estimated by trypan blue exclusion assay that was consistently greater than 95%.

Cellular toxicity analyzed by lactate dehydrogenase (LDH) assay

For toxicity assay, the cells were treated with 5, 10, 20, 40, 80, 160, 320 and 640 µg/ml of TT extract in 96 well plates. 24 hours after treatment of cells with TT extract, LDH was assayed. Three wells of untreated cells were selected for maximum LDH release (high control) and treated for 45 min by phosphate buffer saline (PBS) containing 1% Triton x-100. Three wells of untreated cells were selected for low control (minimum LDH release). The plates were centrifuged at 200 ×g and 100 µl of clear supernatant from each well was added to 100 µl of LDH assay mixture and incubated at 37 ^{0}C for 30 min. The absorbance of wells was measured by a micro plate reader at 495 nm.

Cell toxicity of extract was determined by the following formula:

$$\textbf{Toxicity \%} = (OD_{test} - OD_{low\ control}) / (OD_{high\ control} - OD_{lowcontrol}) \times 100$$

Cell proliferation assay by trypan blue

The proliferative effect of the extract was assayed using trypan blue exclusion assay. Cell concentration of PBMC and cell lines were adjusted to $1x10^6$ cells/ml before use and 100 µl of the cell suspensions were added into a 96-well flat-bottomed plate. The cells were treated with different concentrations of extract (10 - 640 µg/ml) and incubated in 5% CO2-air humidified atmosphere at 37 ^{0}C for 48 h. Negative and positive controls were the wells treated with culture medium without extract and culture medium containing Concanavalin A (5 µg/ml), respectively. Finally, 10 µl of cell suspensions were mixed with equal volume of 0.4% trypan blue and viable cells were counted using Neubauerhaemocytometer and a bright field microscope.

Proliferation assay by cell counting

Proliferation assay also was performed on hPBMC, KG-1, K562, SP20 and Jurkat by cell counting. Exponentially growing cells were seeded in 25 cm^2 flasks. After 24 hours incubation at 37 ºC and 5% CO_2, the cells were incubated in medium supplemented with different concentrations of TT extract (10-640 µg/mL) and cultured for additional 48 hours. The cells were finally counted against control wells by a cell counter (KX-21 Sysmex Co).

Results

Our aim in this study was to compared the TT aqueous extract effect on human peripheral mononuclear cells (PBMC) and several cancer cell lines including KG-1 (Human myeloid cell line), K562 (Human erythroleukemia cell line), SP20 (Mouse myeloma cell line) and jurkat (Acute T cell leukemia). To evaluate the effect of *Tribulus terrestris* aqueous extract on viability and toxicity of cancer cell lines and human PBMC, the cells were treated with 5-640 µg/ml of aqueous extract for 24h and subjected to LDH assay. Aqueous extract showed toxic effect on cancer cell lines dose dependently and IC50 for cancer cell lines were 320 µg/ml (figure1). Also LDH assay showed cell viability of PBMC more than 90% up to 80 µg/ml but at higher concentration toxicity increase and at 640 µg/ml became 41% (figure2). The result of proliferating assay showed that the extract increases cell number of hPBMC at 10-160 µg/ml compared to positive and negative controls (figure3). On the other hand, the extract had no proliferating effect on cancer cell lines significantly. The results indicated that TT extract at doses 10-80 µg/ml had no significant effect on growth of all cancer cell lines, and even decreased cell number at higher doses because of its toxicity.

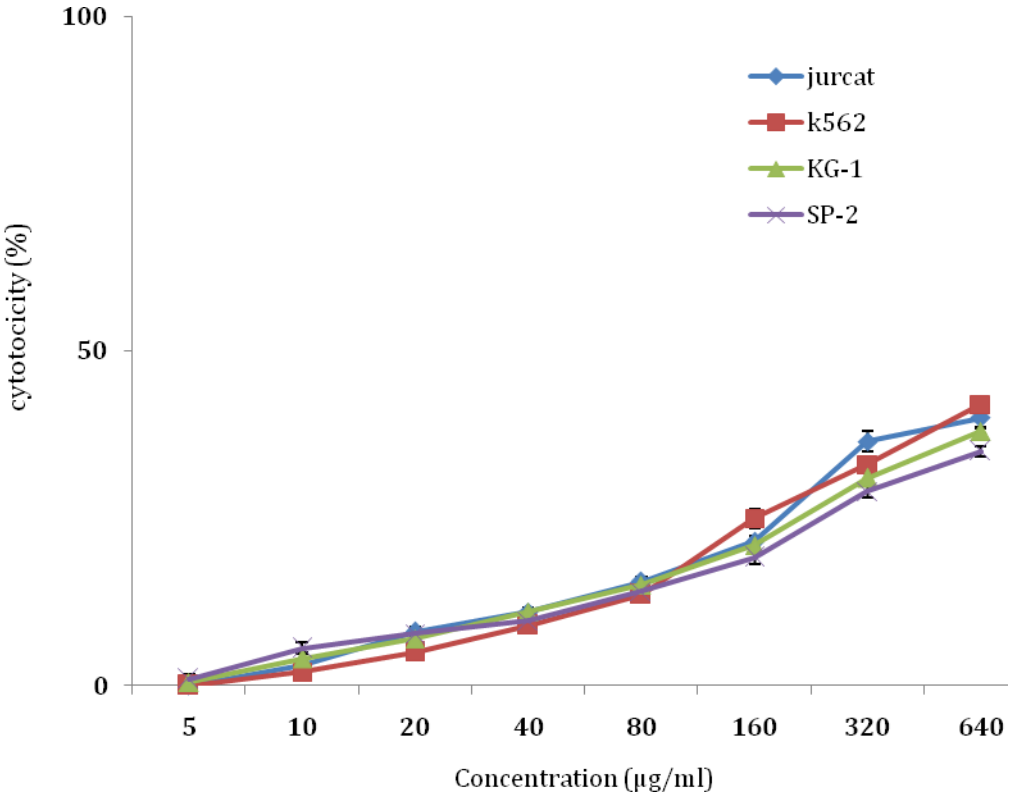

Fig. 1. Toxicity of different concentrations of *Tribulus terrestris* aqueous extract on cancer cell lines determined by LDH assay. Each column displays the mean ± Errors bar from three independent duplicates.

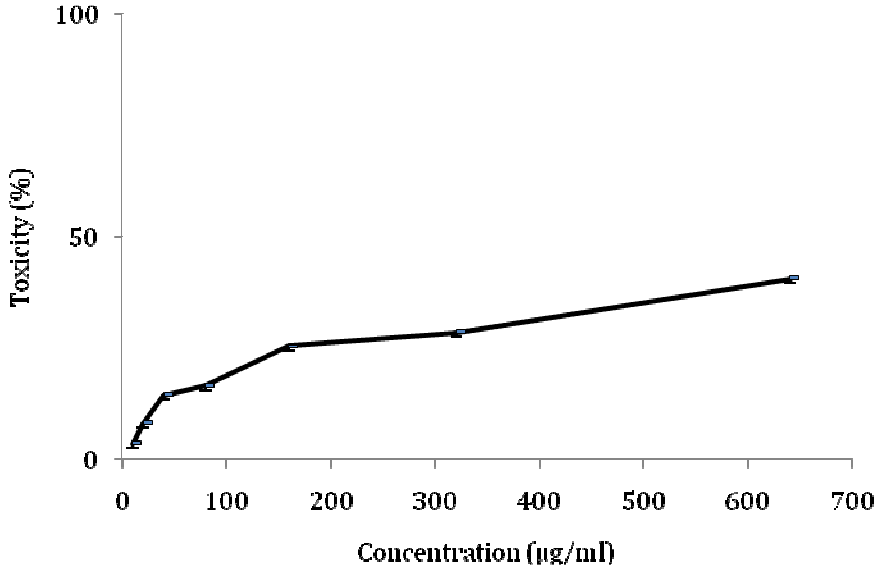

Fig. 2. Toxicity of different concentrations of *Tribulus terrestris* aqueous extract on human PBMC determined by LDH assay. Each column displays the mean ± Errors bar from three independent duplicates.

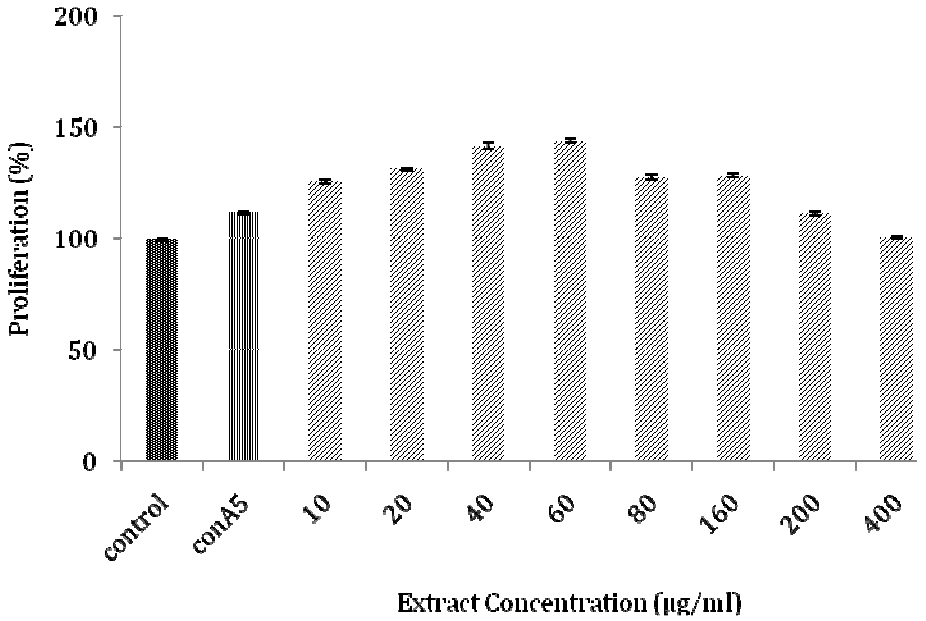

Fig. 3. proliferation effect of different concentration of *Tribulus terrestris* aqueous extract and Con A (5Mg/ml) on human PBMC after 48h culture. Each column displays the mean ± bar ± from three independent duplicates.

Discussion

The results of the present study showed that aqueous extract of *Tribulus terrestris* affect survival of normal and cancerous cell lines at a dose dependent manner and IC50 was different between normal and abnormal cells. The anticancer effects of *Tribulus terrestris* on mice sarcoma categories (ASC), breast cancer (Bcao-37), liver cancer (BEL-7402) and SK-mel, KB, BT-549, SK-OV-3 tumor cell lines have been reported and these studies confirm our results [11-14].TT is a rich source of saponins that its anti-proliferative effect has been proved on Hela-60 cell line. By this fact, anti-proliferative effect of TT, showed in this study, may depend on saponin constituents [15].

On the other hand, our results indicated that TT extract has proliferative effect on PBMC but not on cancer cell lines significantly. One of the major anticancer treatments, that used alone or in combination with other therapy, is chemotherapy. Major problem of chemotherapy is bone marrow toxicity and immune system suppression. Drugs with proliferative effect on hematopoietic progenitors could reduce these side effects. One strategy to reduce cancer-therapy toxic effects on normal cells, are stimulating drugs such as cytokines or stimulating factors. These agents could stimulate hematopoiesis, but have adverse effects like fever, atopic cutaneous reactions, eczema, diarrhea, bone pain and psoriasis [1]. Therefore, herbal drugs with anti-cancer effect, proliferating potential and low toxicity are promising for modulation of defense system in chronic diseases and cancer.

With these two aspects of TT effect, cytotoxic for cancer cell lines and proliferative effect for PBMC, we could propose to use this extract as complementary treatment for induction of PBMC in cancer chemotherapy. However, the idea needs more quantitative evidences and *in vivo* studies for confirmation.

Conclusions

The results of this study revealed that aqueous extract of *Tribulus terrestris* have proliferative potential for hPBMC in contrast to toxic effect for cancer cells. Due to the critical role of PBMC in defense, TT can be a candidate to induce PBMC number/function. Nonetheless, more quantitative

investigations are needed to clarify the curative role of TT extract in cancer treatment, *in vivo*.

Conflict of interest

Authors certify that no actual or potential conflict of interest in relation to this article exists.

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