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INVESTGIATION OF THE CYTOTOXIC EFFECTS OF WATER EXTRACTS OF *Tribulus Terrestis* L.

Giray Buğra Akbaba, Füreya Elif Öztürkkan

Kafkas University, Kars, Türkiye

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At the beginning of his life, mankind started to use plants for therapeutic purposes. Many drugs used in modern medicine are also derived from plants. Although plants are used in the field of alternative medicine (used in place of medical treatment) today, the correct thing is to use them in the field of complementary medicine. The use of complementary medicine is based on the use of the plant together with medical treatment [1]. If plants are not used consciously for therapeutic purposes, human health may deteriorate while trying to be treated. Because there are many important parameters such as the application dose, whether the right plant is used, its side effects, whether it will trigger other diseases, and its toxic effects. For this reason, scientists who work on the potential of plants to be medicine have important duties. *Tribulus terrestris* L. is a plant known in Turkey as “deve çökerten”, “çoban çökerten” and “çarık dikeni”. The plant is so called because its fruit has horn-shaped spikes. The medicinal and pharmaceutical importance of the plant is based on its steroidal saponin content. There are limited studies on the effect of *T. terrestris* L. extracts, which have been reported to have low cytotoxicity on healthy cells in many studies, on different cancer cells [2]. In this context, in this study, cytotoxic effects of ultrapure aqueous extracts of *Tribulus terrestris* L. obtained by ultrasonic wave-assisted extraction method were investigated on the colon (DLD-1), breast (MCF-7), and prostate (PC-3) cancer cells and human peripheral blood lymphocyte cells (PBMC) were investigated by the MTT Method. Extracts were prepared in the concentration range of 7.81-1000 µg/mL. As the concentration of the plant increased, it was determined that human peripheral blood lymphocyte cell viability increased. In this determined concentration range, the plant caused a moderate cytotoxic effect on the colon (DLD-1), breast (MCF-7), and prostate (PC-3) cancer cells.

Keywords: *Tribulus terrestris* L., MTT method, anticancer, colon cancer, prostate cancer, breast cancer

INTRODUCTION

Herbal medicines have been used since ancient times because of their effectiveness and cultural acceptability. Natural compounds have been widely tested for their anticancer properties due to their minimal side effects. Alkaloids, terpenoids, flavonoids, saponins, tannins, phenolics, etc. are some of the naturally occurring active ingredients found in nature. *Tribulus terrestris* L. (TT) (Zygophyllaceae) is an annual herb used in traditional medicine all over the world [2]. In this study, cytotoxic effects on the colon (DLD-1), breast (MCF-7), and prostate (PC-3) cancer cells and human peripheral blood lymphocyte cells of

E-mail address: gbugraakbaba@hotmail.com

ultrapure aqueous extracts of *Tribulus terrestris* L. obtained by ultrasonic wave-assisted extraction method were investigated by the MTT Method.

MATERIALS AND METHODS

Chemicals and Collection of Plant Material

DMSO, MTT, DMEM, Penicillin-Streptomycin, Fetal Bovine Serum, Phosphate Salt Buffer (PBS), and Trypsin EDTA were obtained from Sigma Aldrich. *Tribulus terrestris* L. aerial parts (leaf, fruit, and stem) were collected from Anı, Kars, Turkey at an altitude of 1410 m at 40°29'46" North 43°33'40" East coordinates. The worldwide distribution of the plant is shown in Figure 1. The collected plant was described by Prof. Dr. Fatma Güneş. The collected plant samples were dried in the dark and ground using a grinder.

Instruments

In this study; Bandelin Sonorex RK 106 Ultrasonic Bath, ISOLAB Balloon Warmer, Panasonic MCO-170AICUVH-PE CO2 Incubator, Hed Lab X BIO MSC CLASS II Biosafety cabinet, Thermo Fisher EVOS FL Inverted Microscope, and BioTek Epoch UV-Vis Spectrophotometer were used.

Plant Extraction

For plant extraction, 10 g of the ground plant sample was weighed and taken into the balloon, and then 50 mL of ultrapure water was added to it. Ultrasonic extraction was continued for 60 minutes. The obtained extracts were filtered with blue band filter paper and the liquid part was dried in a rotary evaporator. A stock solution of 1000 µg/mL was prepared for the MTT test, and other solutions were also prepared using this stock.

MTT Assay

In 96-well plates, 100 µL (~5000 cells/well) of DLD-1 cell suspension prepared according to protocols [4] was added to each well and allowed to incubate for 24 hours. After incubation, 100 µL of each prepared solution (1000, 500, 250, 100, and 10 µg/mL) was added to the wells in aliquots (100 µL of medium + 100 µL of cell suspension was added to the control wells) and incubated for 24 hours. Then, 10 µL of MTT (5 mg/mL) solution prepared in PBS was added to each well and incubated for 4 hours. To dissolve the formed formazan crystals, 100 µL of DMSO was added to all wells and kept in a CO_2 incubator for 18 hours at 37°C. After this period, absorbances were recorded at a wavelength of 570 nm with a microplate reader. Cell viability percentages were calculated with the following formula. All experiments were performed in 3 repetitions. The same procedures were performed separately to observe their effects on lymphocyte, PC-3, and MCF-7 cell lines. The equation "Cell Viability = (Absorbance of test well/absorbance of control well) x 100" was used to calculate cell viability.

Figure 1. World distribution of T. Terrestis (in left) [3], T. Terrestis plant (in right).

RESULTS AND CONCLUSIONS

When the effects of ultrasonic wave-assisted water extracts of *T. Terrestis* on lymphocyte cell viability are examined, it is seen that cell viability is close to 100% at a concentration of 1000 µg/mLIt was determined that the aqueous extracts of the plant caused negligible cytotoxicity on lymphocyte cells in the concentration range of 7.81-1000 µg/mL (Figure 2). Ultrasonic wave-assisted aqueous extracts of *T. Terrestis* reduce the viability of DLD-1 colon cancer cells below 50% at all concentrations (Figure 3). It was determined that as the concentration decreased, DLD-1 cell viability decreased. Aqueous extracts of the plant are also cytotoxic on MCF-7 cells. However, it is not possible to say that there is a constant increase or decrease in cell viability depending on the concentration change. The plant extracts are more cytotoxic at lower concentrations, similar to the effect on colon cancer cells. The effects of *T. Terrestis* on PC-3 prostate cancer cells are shown in the graph in Figure 5. Similar to its effect on colon cancer cells, PC-3 cell viability was found to decrease as the concentration decreased. Aqueous extracts of the plant are more cytotoxic at low concentrations. The plant caused a cytotoxic effect on the colon, breast, and prostate cancer cells. However, it causes the most cytotoxic effect on colon cancer cells. Consistent with our study results, it has been reported in many previous studies that the plant does not cause cytotoxic effects on healthy cells. In addition, in a limited number of studies, it has been reported that the plant extracts obtained with solvents such as ethanol and methanol are cytotoxic. In this study, the effects of the plant extract taken with the help of ultrasonic wave-assisted extraction on DLD-1, PC-3, and MCF-7 cancer cells and healthy human blood lymphocytes were investigated for the first time. As emphasized in many scientific studies, according to the results of this study, the use of plant extracts without knowing the required concentrations can lead to fatal results. It is recommended to carry out further studies using different methods, different cell lines, and different concentrations for the use of plants in medicine.

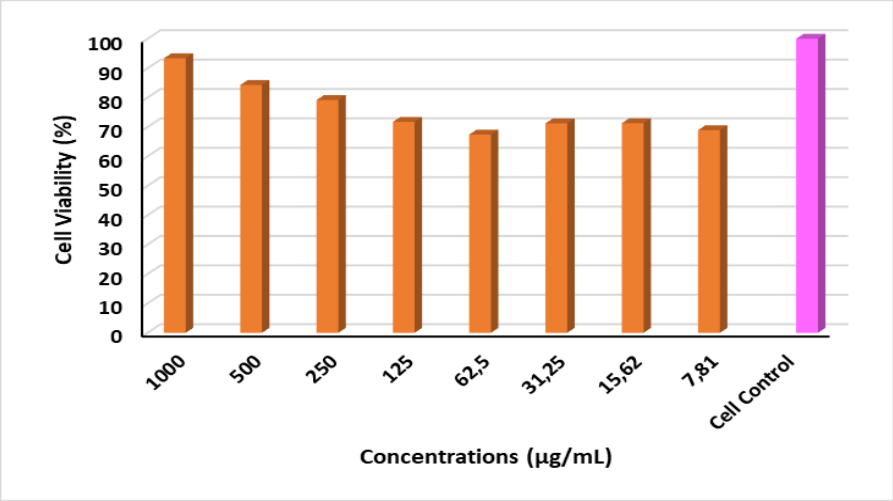

Figure 2. Effects of aqueous extracts of *T. Terrestis* on lymphocyte cells.

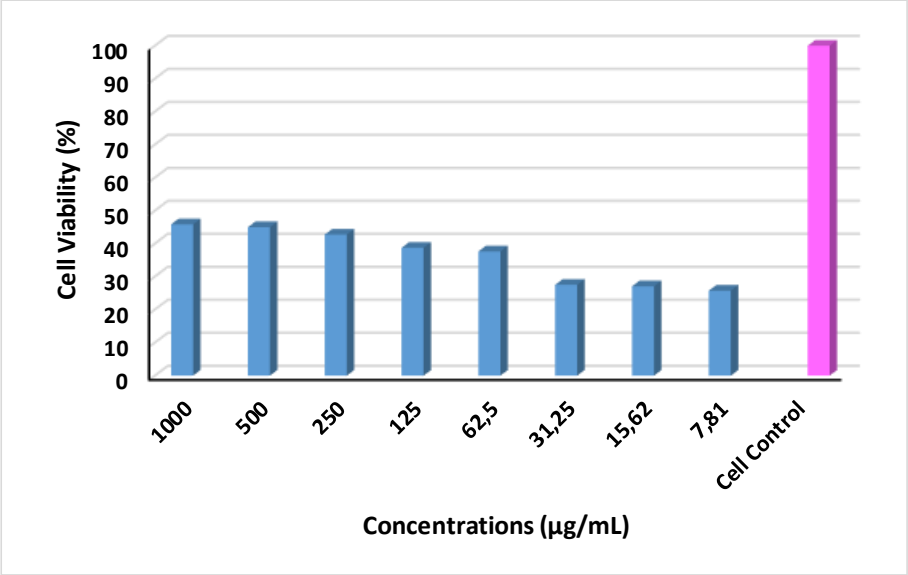

Figure 3. Effects of aqueous extracts of *T. Terrestis* on DLD-1 cells

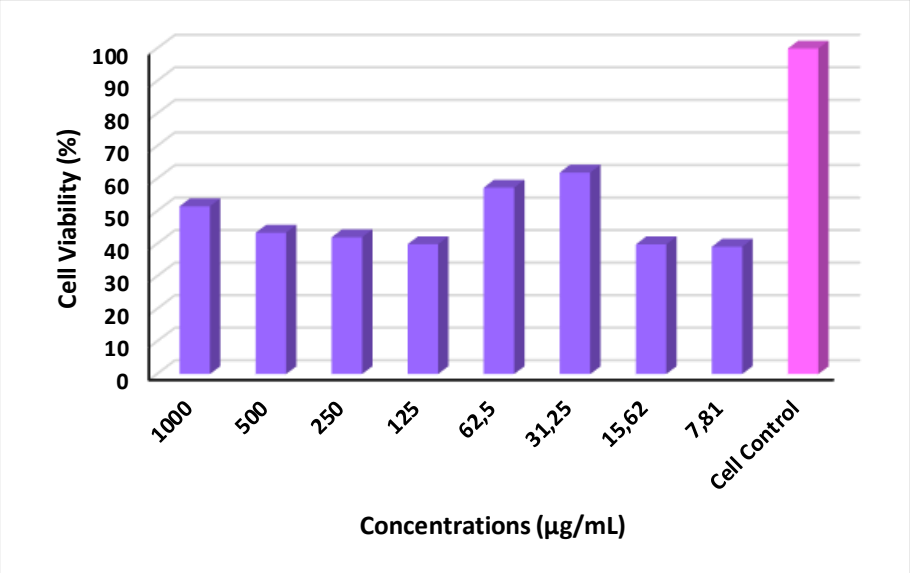

Figure 4. Effects of aqueous extracts of *T. Terrestis* on MCF-7 cells

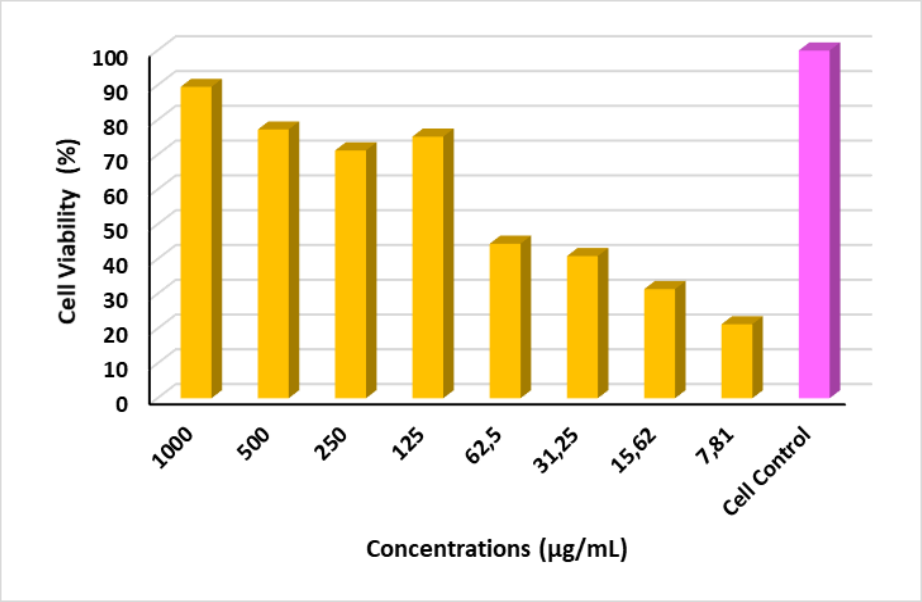

Figure 5. Effects of aqueous extracts of *T. Terrestis* on PC-3 cells

REFERENCES

[1] Alp, M.; Alp, A.S. Medisinal Kimyaya Kısa Bir Giriş, 1st ed. Akademisyen Kitabevi, Ankara, 2019.
[2] Bedir, E.; Khan, IA. New Steroidal Glycosides from the Fruits of Tribulus terrestris. Journal of Natural Products, 2000, ppş 1699– 1701.
[3] https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:873489-1.