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Original Article

Assessment of in vivo and ın vitro anti-tumoral effects of *Lycium barbarum* extract on Ehrlich ascites tumor cells: histopathology, DNA damage and AgNOR

Sümeyye Uçar[1*], Harun Ülger[1], Özge Al[1], Mehtap Nisari[1], Gökçe Şeker Karatoprak[2] Fazile Cantürk Tan[3], Ammad Ahmad Farooqi[4], Seher Yılmaz[5]

[1] *Erciyes University, Faculty of Medicine, Department of Anatomy, Kayseri, Turkey*
[2] *Erciyes University, Faculty of Pharmacy, Department of Pharmacognosy, Kayseri, Turkey*
[3] *Erciyes University, Faculty of Medicine, Department of Biophysics, Kayseri, Turkey*
[4] *Institute of Biomedical and Genetic Engineering (IBGE), Islamabad, Pakistan*
[5] *Yozgat Bozok University, Faculty of Medicine, Department of Anatomy, Yozgat, Turkey*

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Abstract

Natural product research has an exciting and glorious past that spans over millennia. Accordingly, natural products mediated inhibition of carcinogenesis by mechanistic modulation of deregulated signaling pathways has revolutionized the field of translational oncology. *Lycium barbarum* has antioxidant and anticarcinogenic effects. The antioxidant activity of the extract and its effect on Ehrlich ascites tumor (EAT) were investigated using in vivo and in vitro techniques. EAT cells were injected into Balb/C mice to create stock mice. EAT cells withdrawn from stock mice were used in equal volumes in the studies. The in vivo study consisted of control and treatment groups (200 mg/kg fractions above and below 50 kDa of extracts). The liver tissues were evaluated for histopathological (H&E), DNA damage (Comet assay), and proliferation (AgNOR staining) status. The in vitro study consisted of control and treatment groups (1500 and 2000 µg/ml of extracts). Cell viability and apoptosis were evaluated. As a result, a decrease in the adhesion of EAT cells, and decreased DNA damage were observed in mice intraperitoneally administered with the fractions of *Lycium barbarum*. The extracts both below and above 50 kDa increased apoptotic death in cancer cells. The extract above 50 kDa was more active than those below 50 kDa. *Lycium barbarum* consumption may be effectual in preventing cancer formation and slowing the progression of cancer.

Keywords: Ehrlich ascites tumor, Lycium barbarum, Apoptosis, AgNOR, Comet.

1. Introduction

Cancer is a heterogeneous disease and its clinical management is highly complicated and challenging. Essentially, mapping of the spatial organization of stromal, malignant and immune cells in different functional states has revealed clinically relevant drug targets. Therefore, identification of therapeutic targets and the conceptual knowledge of their functions is necessary for a durable clinical outcome. Chemotherapy, radiation, surgery, and immunotherapy are all used to treat cancer. Yet, depending on the unique traits and stage of the disease, each of these therapeutic approaches is used for cancer patients [1].

Nowadays, many chemotherapeutic drugs have been developed that are used in various combinations and treatment regimens based on tumor type, risk classification group and genetic structure [2, 3]. 5-fluorouracil (5-FU), a fluoropyrimidine analogue first used to treat cancer in the late 1950s, is still one of the most frequently prescribed chemotherapeutic drugs, with an estimated 2 million individuals receiving it or one of its prodrugs (such capecitabine) per year globally [4]. 5-FU operates through the repression of thymidylate synthase (TS) and the integration of its metabolites into RNA and DNA, and is used as an adjuvant therapeutic component in the cure of many types of cancer, especially colorectal cancer, and in the treatment of advanced disease [5, 6].

Additionally to these process, patients also use complementary and alternative treating methods [7]. Consuming insufficient amounts of fruits and vegetables overall has long been connect with an elevated endanger of stomach cancer. Recent outcome from the Stomach Cancer Pooling (SToP) Project, a global collaboration focusing on the epidemiology research of gastric cancer, suggest that people who consume the most fruits have a much lower chance of developing stomach cancer [8].

Lycium barbarum (Solanaceae), which became popular in Europe and North America at the beginning of the 21st century, is also called wolfberry or goji berry in East Asia and has an important place in traditional medicine [9]. The

* Corresponding author.
E-mail address: sumeyyeucar@erciyes.edu.tr (S. Uçar).
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fruits and leaves of the *Lycium barbarum*, which is considered a medicinal plant, are widely used in diets in various parts of the world [10, 11].

The latest studies have reported that *Lycium barbarum* has immune system-enhancing, antioxidant, anticarcinogen, hepatoprotective, hypoglycemic, hypolipidemic, and neuroprotective effects [12-14]. Polysaccharides, one of the most significant components of *Lycium barbarum* have a molecular weight of 8-241 kDa. Approximately half of the isolated polysaccharides were below 50 kDa [15]. These polysaccharides have been shown to possess a cytotoxic effect on prostate cancer [16], breast cancer (MCF-7)[17], and cervical cancer (HeLa) [18].

In the literature search, there was no study evaluating the effect of *Lycium barbarum* on Ehrlich acid tumor model, therefore this study targets to research the effects of fractions (above and below 50 kDa) of *Lycium barbarum* fruit extracts, cultivated in Kayseri region of Turkey, on an Ehrlich ascites tumor model *in vivo* and *in vitro*. The total phenolic content, total polysaccharide content, glucose amount and 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{+\bullet}$) radical scavenging activities of the extracts were also assessed.

2. Materials and Methods

The study using experimental animals was performed in compliance with the Erciyes University Local Ethics Committee's decision (Decision number: 17/049). The study was conducted at Betül Ziya Eren Genome and Stem Cell Research Center (GENKÖK). 8-10 weeks old Balb/C male mice with weights between 25-30 g was used. The mice were housed in a closed cage system and fed with normal pellets, which, during the trial, had 12 h of light/dark cycles and a constant temperature of 21°C.

2.1. Preparation of *Lycium barbarum* extract

Lycium barbarum grown in the Hisarcık region of Kayseri were collected in summer and the juices were squeezed and centrifuged at 5000 rpm for 15 min. After careful removal of the supernatant, it was filtered with 0.45 and 0.22 µm syringe tips, respectively. Subsequently, fractions above 50 kDa and below 50 kDa were obtained by passing through 50 kDa centrifugal filters (Amicon®Ultra-15 LOT number): R8CA38479. These fractions were lyophilized (Labconco FreeZone 4.5) to obtain a dry powder extract. The powder extract was prepared by dissolving it with distilled water daily.

2.2. Total phenolic, polysaccharide and glucose content

Per gram of extract, total phenols were calculated as gallic acid equivalents (GAE). A 10.0 mL volumetric flask containing ca. 6.0 mL of H_2O and 100 µL of sample (2–6 mg/mL) was then filled with 500 µL of undiluted Folin-Ciocalteu reagent. After waiting one minute, 1.5 mL of 20% (w/v) Na_2CO_3 was added, and then 10.0 mL of water was added to make the volume. The absorbance at 760 nm was measured after 2 hours of incubation at 25^0C and compared to a calibration curve for gallic acid. The information is shown as the average values of three replicate analyses [19]. The phenol-sulfuric acid technique was used to assess the total polysaccharide content using - α-D glucose [20]. Samples dissolved in distilled water (1mg/mL) and varying concentrations of reference were put into test tubes in 1 mL volume. 1 mL of 5% phenol solution (in water) was added. Then 2.5 mL of concentrated sulfuric acid was added to the mixture. And then, kept at room temperature for 20 min in a shaking water bath and then placed in the tubes and their absorbances at 490 nm were read. A calibration curve was drawn for the glucose standard and compared with the samples. The total sugar content was calculated as D (+) glucose equivalent. The experiments were performed in triplicate, and the mean values for the outcomes were provided. The amount of glucose in the extracts was measured following the instructions of the Spinreact Glucose-LQ kit (Girona, Spain). The extracts were prepared at a concentration of 1 mg/mL. Glucose at a concentration of 100 mg/dL was used as the standard.

2.3. 2,2-Azinobis (3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{+\bullet}$) radical scavenging activity

$ABTS^{+\bullet}$ radicals were identified using the Re et al.[21] After waiting for the $ABTS^{+\bullet}$ aqueous solution and $K_2S_2O_8$ (2.45 mM, final concentration) in the dark for 12–16 hours, the $ABTS^{+\bullet}$ radical (7 mM) was produced. The absorbance was 0.700 (0.030) at 734 nm at room temperature. Extracts were investigated the concentrations of 1.5, 3, 6, and 12 mg/ml. The extract solution (10 µL) and radical solution (990 µL) were combined, and the reaction kinetics were assessed at 734 nm every min. for 30 minutes. Butylated hydroxyanisole (BHA) was used as positive control. The percentage of inhibition assessed versus concentration was equal to Trolox. The triplicate analyses' means were employed.

2.4. Formation of stock mice

EAT cells used in the study were obtained from Erciyes University, Department of Anatomy, Cell Culture Laboratory (Kayseri, Turkey) were frozen in cryotubes including 10% DMSO (dimethyl sulfoxide) and stored at -80°C. 0.1 cc of approximately $1x10^6$ EAT cells i.p. was injected. Swelling in the abdominal region because of the increase in ascites fluid in the peritoneal cavity was accepted as a marker of acid tumor formation in approximately 7-8 days. EAT cells within ascitic fluid injected into the peritoneal cavity of this stock animal were used in equal volumes in the in vivo and in vitro experimental groups.

2.5. In vivo studies

The mice were randomly separated two control and three treatment groups. In the first (day 0), 0.1 mL of distilled water was injected i.p into the negative control group and 0.1 mL of ascites fluid containing $1x10^6$ EAT cells was injected into the other groups. Fractions of *Lycium barbarum* (above and below 50 kDa) were prepared daily at a dose of 200 mg/kg per mouse for i.p. injections. 15mg/kg 5-Fu (5-Fluorourasil) injected to 5-Fu group (0.1 mL) in distilled water.

EAT cells in mice were recorded in general condition, weight, abdominal circumference, feather condition, and defecation disorders. Mice were anesthetized with ketamine (50 mg/kg) and xylazine (15 mg/kg) at the end of day 11. Ascites fluid from the abdominal region was taken with a 10 mL syringe and the cell count was determined. Ascite fluid was placed in a centrifuge tube and the amount of the packet volume was determined and centrifuged at 3000 rpm for 5 min. The acid liquid was withdrawn using an automatic pipette and its amount was recorded. The remaining pellet was recorded as packet volume. Cells

were counted on the thoma slide. 100 microliters of trypan blue from 100 microliters of cell suspension was mixed and spread on the thoma slide. At 40x magnification, dead cells were stained blue and live cells were seen as white and counted. After opening the anterior abdominal wall of the mice, liver was removed and then divided into three parts for hematoxylin-eosin staining, AgNOR, and comet assay.

2.5.1. Histopathological evaluation

The liver tissue was placed in 10% formaldehyde, cervical dislocation was applied and then in the medical waste box discarded. The liver tissues were determined by holding two days in formaldehyde, passed through the alcohol series, and dehydrated. It was then embedded in paraffin blocks before the xylene series for transparency. 5 µm sections of paraffin blocks were stained with hematoxylin-eosin (H & E) and examined in light microscope (Olympus, Japon BX51)[22].

2.5.2. AgNOR detection

5 µm thick slices were taken from paraffin blocks, deparaffinized in xylene, and rehydrated in graduated alcohol solutions before AgNOR staining.[23]. The stained liver cells were observed using a Nikon's Eclipse 80i light microscope, photographed via a DigitalSight DSfi1 camera, and analyzed for mean AgNOR number and total AgNOR area per nuclear area (TAA/NA) using ImageJ software.

2.5.3. DNA damage assessment

The comet assay is an efficient, cost-effective, and swift technique for identifying single and double-strand DNA breaks at the cellular level, particularly when DNA is unwound. These breaks can occur due to various factors such as radiation, toxins, or chemicals. The assay finds wide application in fields like carcinogenesis, genotoxicity, radiotherapy, irradiated food imaging, environmental bioimaging, and various research domains.

Cell suspension preparation: Liver tissue (approximately 0.5 g) was dissected in 2 mL of cold phosphate-buffered saline (PBS) using a clean scalpel. The mixture was then agitated at 500 rpm for 5 minutes and allowed to settle on ice for 10 minutes. The supernatant was used to create a single-cell suspension for the comet assay.

Slide coating: Slides were coated with 0.5% normal melting point agarose and left to dry at room temperature overnight.

Gel casting: 100 µl of the cell suspension was mixed with 0.8% low-melting-point agarose in PBS, spread on pre-coated slides, and covered with a coverslip. The slides were then placed in an icebox for 10-15 minutes to solidify.

Lysis: The coverslipped slides were lysed in freshly prepared cold lysis buffer (1000 mL of TBE, 25 g SDS) for 4 minutes at 40°C. Subsequently, the slides were transferred to a Comet horizontal electrophoresis tank filled with freshly prepared electrophoresis buffer (TBE: 54 g Tris, 27.5 g boric acid, 20 mL EDTA, pH 8.4, at 40°C) and kept for 10 minutes to unwind the DNA strands. Electrophoresis was performed for 2 minutes at 64 V-250 mA at 40°C. The slides were then washed with dH_2O for 5 minutes to remove ions and detergents. Following neutralization, they were stained with 50 µL ethidium bromide (1 µg/mL) and covered with a coverslip. All procedures were conducted in the dark to prevent DNA damage. Measurements were taken at 400× magnification using a fluorescence microscope (Olympus, BX51, Tokyo, Japan).

Image analysis: Using the Comet Assay Software Project (CASP-1.2.2, Windows 2010), 100 randomly selected cells were imaged using seven parameters (head length, tail length, comet length, percent DNA in the head, percentage DNA in the tail, tail moment-TM, and olive tail moment-OTM).

Damage was identified by fragmented DNA that had migrated from the cell head, forming a "comet" pattern, while all non-comet cell heads were considered undamaged.[24].

2.6. *In Vitro* studies: Annexin V and dead cell assay

EAT cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Capricorn Scientific, CP21-4310) supplemented with streptomycin/penicillin (100U/mL; Sigma Life Science, 046M4846V) and 15% fetal calf serum (FCS, Biowest, S181G-500). The cells were maintained in a humidified atmosphere containing 5% CO_2 at 37°C using a Sanyo incubator (MCO-19 A/C(UV)).

For the experiments, $1x10^5$ EAT cells were seeded per well in 24-well plates and incubated for 3, 24, and 48 hours. At the end of each incubation period, the contents of each well were transferred to labeled Eppendorf tubes and centrifuged at 300 g for 5 minutes. The supernatant was carefully removed, and the cells were washed with phosphate-buffered saline (PBS).

Next, 100 µL of the Muse® Annexin V & Dead Cell Kit (Catalog Number: MCH100105) and 1% of 100 µL FBS were added to each tube. Finally, analyzed samples using the Muse® Cell Analyzer (Merck-Millipore) by the manufacturer's directions.

2.7. Statistical evaluation

The Statistical Package for Social Sciences (SPSS, Inc., Chicago, Illinois, USA) for Windows 10.0 was used to conduct the statistical analysis. Shapiro-Wilk, histogram, and q-q graphs were used to assess the normal distribution of the *in vivo* and *in vitro* data. The Levene test was used to assess the homogeneity of the variance. Quantitative factors in more than two groups were analyzed using one-way ANOVA. The Tamhane T2 test was applied to several comparisons. For repeated measurements of time-based comparisons of in vitro data, variance analysis was performed. The Tukey test was applied to numerous comparisons. A significance level of 0.05 was used.

3. Results

3.1. Total phenolic, total polysaccharide, and glucose content

The amount of glucose in the 50 kDa above fraction was higher than 50 kDa below fraction, the phenolic compound and the total sugar content were approximately the same for both fractions (Table 1).

3.2. $ABTS^{+\bullet}$ radical scavenging activity

A rise in antioxidant activity was detected with increas-

Table 1. Total phenol, total sugar and glucose content.

Lycium barbarum extract	Total Phenol ($mg_{GA}/g_{extract}$)	Total sugar (mg/mL)	Glucose (mg/dL)
Above 50 kDa	33.012±4.72	36.55±1.54	62.14±2.28
Below 50 kDa	31.40±3.46	36.86±0.68	51.81±8.60

Results are presented as mean ± SD.

ing concentration, and the fractions with a concentration of 12 mg/mL had similar effects with the standard BHA (Butylated hydroxyanisole) (Figure 1). However, none of the extracts were found as active as BHA, which was studied at low concentrations.

3.3. *In vivo* findings

When the daily weight gain of mice was analyzed, it was observed that there was no difference in the negative control and the mice in the 5-Fu group while the weight of the other groups increased. According to the weight changes between the groups (the difference between consecutive days), it was seen that there was a important rise in the negative and positive control according to the 5-Fu groups from the 6th day and a important rise in the *Lycium barbarum* groups ($p < 0.05$)

Weight gain was less in the *Lycium barbarum* groups compared to the positive control group, and this reduce was significant on the 11th day ($p < 0.05$). According to the results of abdominal circumference changes, an increase was observed in the negative control and 5-FU groups as per the positive control and *Lycium barbarum* groups. The difference between the consecutive days was found to be significant since the 6th day ($p < 0.05$). Measurements of intraperitoneal ascite fluid revealed the absence of ascitic fluid in negative control mice and in the 5-Fu group mice. While there was no change in the supernatant values in positive control groups and *Lycium barbarum* groups important reduction ($p < 0.05$) was observed in total volume, pellet volume, dead cells, and living cells in groups above 50 kDa and below 50 kDa (Table 2).

3.3.1. Histopathological evaluation of tissues

Examination of tissue preparations revealed that there was no change in the normal histological structures of the tissues, but invasive EAT cell groups were seen in the peritoneum covering the tissues. EAT cells were observed to have different sizes and shapes, ranging from large nuclei to eosinophilic cytoplasm. Compared to the positive control group, a lower amount of EAT cells adhering to the connective tissue surrounding the liver (Figure 2) was observed in *Lycium barbarum* (especially in the *Lycium barbarum* group above 50 kDa).

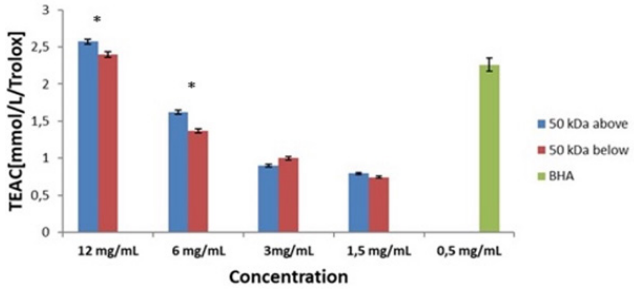

Fig. 1. Determination of antioxidant effect by ABTS (Trolox Equilibrium Antioxidant Capacity-TEAC) method.* The extract above 50 kDa was found to be more active at 6 and 12 mg/mL concentrations than the extract below 50 kDa.

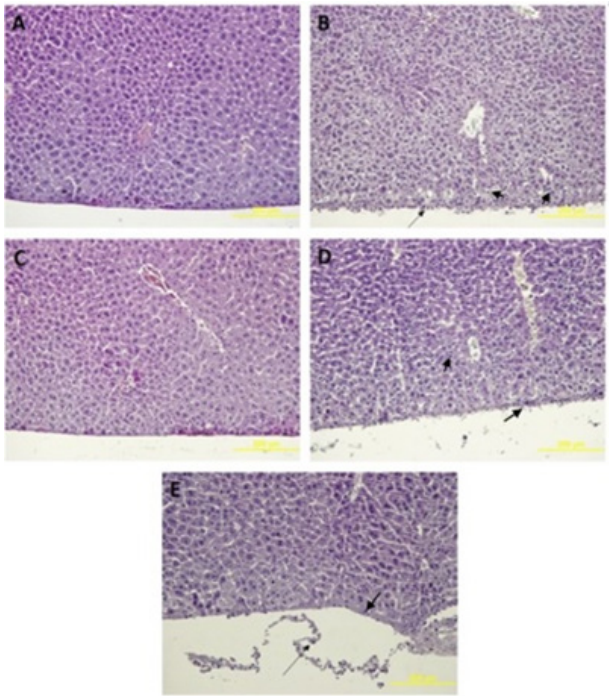

Fig. 2. Histologic appearance of liver tissue of hematoksilen & eosin staining. **(A)** Negative control group, **(B)** Positive control group, **(C)** 5-Fu group **(D)** Above 50 kDa *Lycium barbarum* extract group, **(E)** Below 50 kDa *Lycium barbarum* extract group, (H&E, 20X, Olympus, Tokyo, Japon). The black arrow demonstrates EAT cell.

Table 2. Analysis of cells of ascitic fluid withdrawn from periton.

Variables	Negative control (n=10)	Positive control (n=10)	5-FU (n=10)	Above 50 kDa (n=10)	Below 50 kDa (n=10)	*p*
Total volume(mL)	---	$8.28±4.08^{b}$	---	$4.14±1.85^{a}$	$4.94±2.74^{ab}$	0.012
Supernatant(mL)	---	5.14±3.81	---	3.14±1.80	3.79±2.31	0.276
Pellet (mL)	---	$3.14±1.35^{b}$	---	$1.00±0.41^{a}$	$1.15±0.62^{a}$	<0.001
Live cell (in one square)	---	$81.30±19.69^{b}$	---	$47.70±11.08^{a}$	$62.20±18.20^{a}$	0.001
Dead cell (in one square)	---	$2.20±1.03^{b}$	---	$8.90±5.45^{a}$	$5.40±2.36^{a}$	0.001
Average number of cells in 1 mL ($x10^{4}$)	---	1300.80^{b}	---	763.20^{a}	995.20^{ab}	0.001

Results are presented as mean ± SD. For each parameter, in a column, the data with different superscript letters are significantly different from each other ($p < 0.05$).

3.3.2. AgNOR staining results

We evaluated proliferation in the liver using AgNOR staining. When performing AgNOR scoring, the NOR proteins area was first measured one by one and summed to find the total agnor area value. The nuclear area was then measured and recorded. Then, the total AgNOR area was compared to the nuclear area. Liver tissue revealed a important rise in AgNOR number and TAA/NA of positive control when compared with negative control. Proliferation revealed a significant decrease in 5-Fu, above and below 50 kDa groups compared with positive control (Figure 3) displays illustrations of cells that exhibit AgNOR staining. (Table 3) display the average AgNOR number and TAA / NA values.

3.3.3. DNA damage findings

The comet assay has become very popular over the last two decades and is probably one of the most used assays today for the assessment of DNA damage and repair. We evaluated DNA damage in the liver using neutral comet assay. Liver tissue revealed a important rise in DNA damage of positive control when compared with negative control. DNA damages were revealed a significant decrease in 5-FU, above 50 kDa and below 50 kDa compared with positive control (Table 4, Figure 4).

3.4. In vitro findings: annexin v & dead cell kit findings

The analysis of in vitro results indicated no important difference between the groups in terms of the ratio of live cells to early apoptotic cells after three hours of culture, statistically. A significant decrease of live cell rates in above-50 kDa groups (1500, 2000 µg/mL) was observed at the end of 24 and 48 h of culture (p<0,05). When each group was examined individually according to time, it was seen that the above-50 kDa and 2000 µg/mL groups had the highest level of decrease in live cell rate at the end of 48 h. During the examination of early apoptotic cell rates after 24 and 48 h, it was showed that percentage of early apoptotic cells raised (p<0,05) in above-50 kDa (1500 µg/mL, 2000 µg/mL) and below-50 kDa (1500, 2000 µg/mL) groups. When each group was examined individually according to time, it was seen that above-50 kDa and 1500 µg/mL and 2000 µg/mL groups had the highest level of rise of a percentage of early apoptotic cells (p<0,05).

Results related to the percentage of late apoptotic cells were examined and it was observed that at the end of 3 hours of culture, late apoptotic cells increased (p<0,05) in below-50 kDa groups. At the end of 24 and 48 hours of culture, late apoptotic cells increased (p<0,05) in above-50 kDa (1500, 2000 µg/mL) groups. When each group was

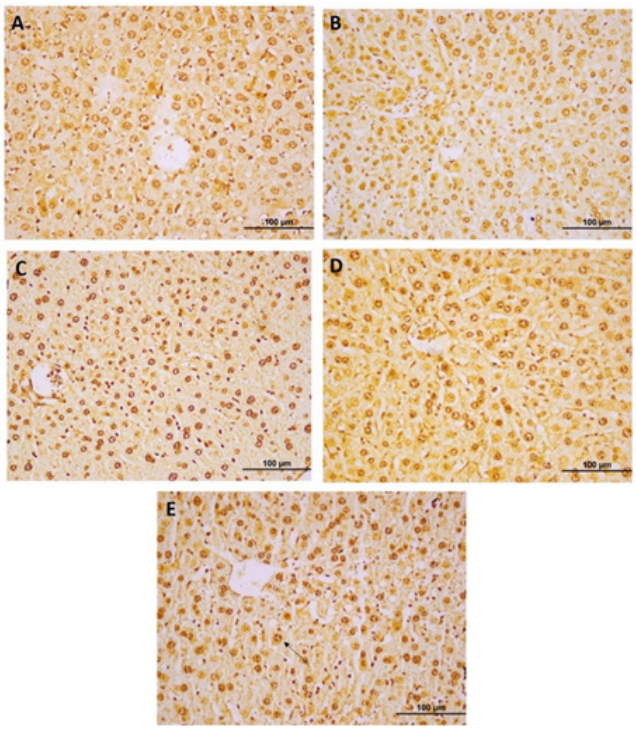

Fig. 3. Image examples of the AgNOR staining liver cells. (A) Negative control group, (B) Positive control group, (C) 5-Fu group, (D) Above 50 kDa Lycium barbarum extract group, (E) Below 50 kDa Lycium barbarum extract group (40X, Eclipse 80i, Nikon, Tokyo, Japon) Black arrow= Demonstrates AgNOR staining region.

Table 3. Average AgNOR number and TAA/NA. area.

Variables	Negative control (n=50)	Positive control (n=50)	5-FU (n=50)	Above 50 kDa (n=50)	Below 50 kDa (n=50)	*p*
AgNOR number	2.14±0,67^{a}	2.80±1.12^{b}	2.42±0.93^{ab}	2.34±0.98^{b}	2.72±0.99^{b}	p<0,05
TAA/NA	0.08±0,03^{a}	0.12±0.03^{b}	0.09±0.03^{a}	0.08±0.23^{c}	0.11±0.04^{d}	p<0,05

Results is presented as mean±SD. Amongst the groups that share the same letter, there is no statistically significant difference (p< 0.05). AgNOR: Argyrophilic nucleolar organizer region TAA/NA: total AgNOR area per nuclear.

Table 4. Effect of *Lycium barbarum* on the cellular liver DNA damage in the experiment groups.

Liver	Negative control	Positive control	5-FU	Above 50 kDa	Below 50 kDa
L-head (µm)	206.1±12.24^{a}	203.4±26.94^{a}	162.1±19.28^{b}	133.0±23.46^{c}	133.1±23.40^{c}
L-tail (µm)	19.98±4.74^{a}	93.94±25.55^{b}	34.86±15.3^{c}	23.12±11.99^{a}	23.16±11.98^{a}
L-comet (µm)	226.1±14.34^{a}	297.3±41.64^{b}	197.0±28.56^{c}	156.1±28.93^{d}	156.3±28.83^{d}
Head DNA (%)	97.62±0.49^{a}	89.74±2.09^{b}	94.30±1.58^{c}	95.02±2.41^{cd}	94.56±1.98^{cd}
Tail DNA (%)	2.38±0.49^{a}	10.26±2.09^{b}	5.70±1.58^{c}	4.78±1.93^{c}	5.44±1.98^{c}
TM	1.0±0.0^{a}	9.76±4.35^{b}	2.18±1.41^{c}	1.44±0.88^{ca}	1.50±0.89^{ca}
OTM	2.2±0.45^{a}	11.65±3.48^{b}	3.98±1.88^{c}	2.52±1.28^{a}	2.6±1.26^{a}

L Head: Length Head; L Tail: Length Tail; L Comet: Length Comet; Head DNA: % DNA in the head, Tail DNA: % DNA in the tail; TM: Tail Moment; OTM: Olive Tail Moment. Mean±SD in a column that has a different superscript letter indicates significance. Similar letters on the same line illustrate how groupings are similar, while dissimilar letters highlight how groups are different (p< 0.05).

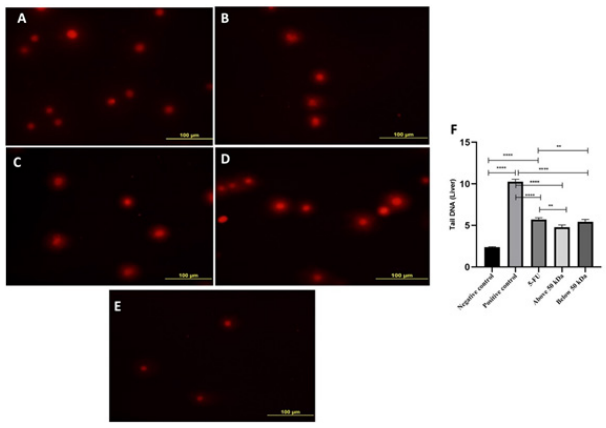

Fig. 4. DNA damage in liver tissue using comet assay. **(A)** Negative control tail DNA 2.38 %, **(B)** Positive control tail DNA 10.26%, **(C)** 5-fu tail DNA 5.7%**(D)** Above 50 kDa tail DNA 4.78%, **(E)** Below 50 kDa tail DNA 5.44% **(F)** Statistical comparison between groups of the percentage of tail DNA in liver tissue using the comet assay. (Ethidium bromide boyama x400, Olympus, Japan).

examined individually according to time, it was observed that the above-50 kDa (1500, 2000 µg/mL) groups had the highest percentage of late apoptotic cells ($p<0,05$). Based on the results regarding the total amount of apoptotic cells, after 3, 24, and 48 hours of culture, there was a important difference between the groups ($p<0,05$), and the percentage of total apoptotic cells increased in above-50 kDa (1500, 2000 µg/mL) groups. In both concentration groups above 50 kDa, the highest percentage increase in apoptotic cells was found in total (Table 5 and Fig. 5).

4. Discussion

Lycium barbarum, which grows in various parts of the world, is consumed as a medicinal plant [25]. It has been investigated in vivo and *in vit*ro that the polysaccharides (*Lycium barbarum* polysaccharide - LBP) found in *Lycium barbarum* in particular on Ehrlich ascites tumor (EAT) have been investigated, and their antiproliferative and apoptotic effects have been found. Although there are many studies in the literature where the chemical components of *Lycium barbarum* are determined, it is stated that the chemical content of fruits varies depending on the growing environment. Benchennouf et al. stated that the total phenolic content of *Lycium barbarum* fruits grown

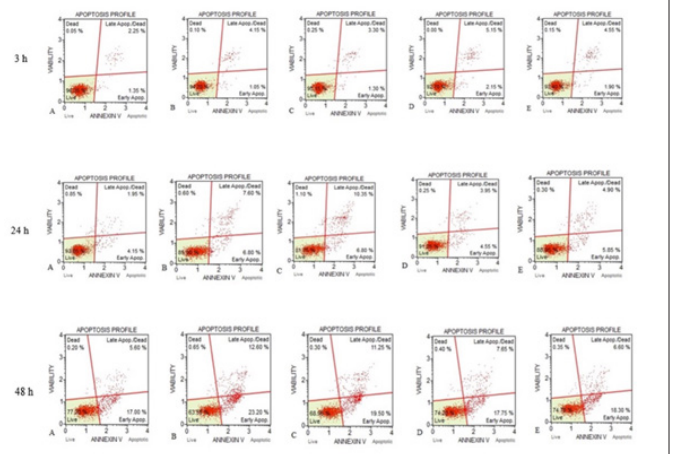

Fig. 5. Raw graphs of apoptosis 3, 24, 48 hours. **(A)** Control. **(B)** Above 50 kDa 1500 µg/ml, **(C)** Above 50 kDa 2000 µg/ml, **(D)** Below 50 kDa 1500 µg/ml, **(E)** Below 50 kDa 2000 µg/ml.

Table 5. In vitro cell culture results.

	Groups						
	Hour	Control	Above 50 kDa 1500 µg/mL	above 50 kDa 2000 µg/mL	below 50 kDa 1500 µg/mL	below 50 kDa 2000 µg/mL	p
Live cell	3	94.17 ± 1.07^{A}	95.56 ± 1.31^{A}	96.56 ± 0.99^{A}	90.32 ± 10.5^{A}	93.04 ± 0.99^{A}	0.151
	24	90.8 ± 4.69^{bA}	85.41 ± 2.34^{abB}	81.56 ± 3.65^{aB}	90.26 ± 1.57^{bA}	86.34 ± 4.66^{abB}	<0.001
	48	75.71 ± 1.17^{bB}	63.46 ± 3.11^{aC}	67.45 ± 3.75^{aC}	75.68 ± 2.71^{bB}	76.57 ± 2.98^{bC}	<0.001
	P*	0.009	0.000	0.000	0.009	0.000	
Early apoptotic cell	3	2.47 ± 1.44^{A}	1.68 ± 1.37^{A}	0.98 ± 0.37^{A}	1.19 ± 0.27^{A}	1.76 ± 0.54^{A}	0.061
	24	6.27 ± 2.69^{abAB}	7.65 ± 1.55^{abB}	9.63 ± 3.84^{aB}	5.84 ± 1.06^{abB}	7.17 ± 1.86^{abB}	0.038
	48	16.15 ± 2.94^{aB}	20.91 ± 2.77^{bC}	19.90 ± 2.35^{bC}	18.32 ± 2.23^{aC}	17.05 ± 1.53^{acC}	0.005
	P*	0.13	0.000	0.000	0.000	0.000	
Late apoptotic cell	3	3.29 ± 0.54^{aA}	2.68 ± 0.46^{aA}	2.41 ± 0.69^{aA}	4.49 ± 0.66^{bAB}	5.08 ± 0.65^{bA}	0.001
	24	2.69 ± 2.47^{bA}	6.37 ± 1.16^{abB}	8.23 ± 1.25^{aB}	3.39 ± 0.97^{bA}	6.04 ± 4.03^{abA}	0.001
	48	7.93 ± 1.59^{bB}	15.19 ± 1.79^{cC}	12.14 ± 1.72^{aC}	5.70 ± 0.84^{bB}	6.03 ± 1.46^{bA}	<0.001
	P*	0.002	0.000	0.000	0.001	0.570	
Total apoptotic cell	3	5.75 ± 1.11^{bcA}	4.36 ± 1.35^{abA}	3.39 ± 0.95^{aA}	5.68 ± 0.67^{bA}	6.83 ± 0.96^{bcA}	<0.001
	24	8.95 ± 4.46^{bA}	14.02 ± 2.22^{abB}	17.86 ± 3.74^{aB}	9.23 ± 1.73^{bB}	13.21 ± 4.62^{abB}	<0.001
	48	24.08 ± 1.43^{abB}	31.73 ± 11.68^{abC}	32.04 ± 3.67^{aC}	24.01 ± 2.84^{abC}	23.08 ± 2.95^{bC}	0.010
	P*	0.009	0.000	0.000	0.000	0.000	

Results is presented as mean±SD. The resemblance between the groups and the various letters is shown by using the same letters in the same row. The same capital letters in the same column reflect similarities across groups throughout time, whereas different letters indicate difference significance between groups ($p< 0.05$), p * = 3, 24, 48 hours shows the significance between.

in Greece ranged from 14. 13 and 109.72 mg GAE/g [26]. Islam et al. showed the total phenolic content of 4 different *Lycium barbarum* collected from China, respectively 2, 17; 2.87; 3.12; 4.48 mg GAE/g [27]. Also, Vulic et al. determined the total phenolic content of *Lycium barbarum* collected from Serbia as 716.91 mg GAE/g [28]. In this study, the total phenolic content of the fractions (below and above -50 kDa) obtained from *Lycium barbarum* fruit grown in Turkey are defined to be respectively 33.012 and 31.40 mgGAE/$g_{extract}$.

Water-soluble polysaccharides are the most common compounds in *Lycium barbarum* fruit, and glucose is predominantly contained in these polysaccharides. On the other hand, Zhang et al. explained the glucose amount of 2 different *Lycium barbarum* collected from China as 6.2 and 35.2 mg/dL [29]. In our study, glucose amounts of *Lycium barbarum* fractions above and below 50 kDa were found to be 62.14 mg/dL and 51.81 mg/dL, respectively. According to the results, the *Lycium barbarum* fruit grown in Kayseri/Turkey is understood to be richer in terms of the total phenolic and total glucose amounts. The results of a recent study revealed that *Lycium barbarum* fruits scavenge 90% of DPPH radical at a concentration of 40 mg/mL [13]. In our study, strong scavenging activity was shown on ABTS radical at a concentration of 12 mg/mL. Its antioxidant activity has also been proven in *in vitro* studies.

In recent studies, *Lycium barbarum* has been tested *in vivo* on different cancer models and its curative effects have been reported. Mice with H22 tumor (*in vivo* murine hepatoma H22) were administered *Lycium barbarum* polysaccharide (LBP)-3 250 mg/kg via gavage for 10 days. As a result, LBP-3 has been shown to significantly reduce tumor severity and also does not damage liver and kidney tissues [30]. Among these studies, there is no Ehrlich ascites tumor model, and the results of this model are presented in this research for the first time.

In recent studies, it was seen that AgNOR staining had not been done before in the effects of *Lycium barbarum* on cancer. In this respect, this study was a first. Besides, many studies are evaluating the activity of *Lycium barbarum* extracts in various cancer cell lines. In the H22 cell line, 400, 800, 1200, and 1600 µg/mL concentrations of 4 *Lycium barbarum* fractions were evaluated by Deng et al. The most active fraction, LBP-3 has been reported to cause over 60% inhibition in tumor cells at a dose of 1600 µg/mL after 48 h. They also reported that LBP-3 both increased apoptosis and stopped the cell cycle in the S phase [30]. In these studies, it was observed that *in vitro* LBP doses ranged from 1-2800 µg/mL. In our *in vitro* study with EAT cells, concentrations of 1500 and 2000 µg/mL were studied for both fractions based on the concentrations in the literature. When the relationship between *Lycium barbarum* and apoptosis was investigated in the results of Annexin V & Dead Cell Kit Test, it was investigated that there was no significant difference between the groups after 3 hours of culture. At the end of 24 and 48 h, it was observed that the *Lycium barbarum* caused a important reduce in the viable cell count and an rise in the number of early and late apoptotic cells in the above 50 kDa group compared to the control group. These results confirmed that *Lycium barbarum*'s effect on EAT cells may be due to the induction of apoptosis. Medicinal plant extracts have been previously shown to be effective in solid tumor studies [31-34] and Ehrlich ascites [35] in our previous works. Based on the data we have obtained, it has been concluded that *Lycium barbarum* consumed as a nutrient can be beneficial in slowing the formation and progression of cancer, which is one of the biggest health problems of today, like other medicinal plants.

Our comet assay results confirmed histopathological findings in liver. These results were confirmed with increased DNA damage %, tail length, and tail moment and other parameters in positive control group.

LBP shows promise as an anticancer drug because of its high activity and low toxicity. While its anticancer features are known, its mechanisms of action are not yet completely understood. A study on the MCF-7 cell line revealed that LBP successfully inhibits breast cancer cell proliferation and promotes ferroptosis through the xCT/GPX4 pathway. This novel property of LBP in inducing ferroptosis suggests it could be a possible therapeutic option for breast cancer [36].

Research indicates that *Lycium barbarum* exhibits antioxidant and cytoprotective effects on normal cells. Conversely, it demonstrates apoptotic and antiproliferative effects on various cancer cell lines, including HL-60, PC3, DU145, and HeLa cells [16, 18, 37].

In a study investigating the anticancer mechanism of *Lycium barbarum* fruit, calcium emerged as a potential signaling molecule in apoptosis pathways, likely due to upstream MAP kinase activation. This implies that LBP could elevate cellular calcium ion concentrations, significantly impacting the signal transduction pathway of apoptosis and the chemosensitivity of tumor cells to anticancer agents. While the direct relationship between LBP and apoptosis remains unclear, these findings suggest that certain active components in *Lycium barbarum* may mediate apoptosis [38, 39].

The effects of *Lycium barbarum* on cancer were investigated for the first time in this study AgNOR staining method is a method used in many cancer studies to evaluate proliferation such as MDA-MB-231 cells in cell culture and in many in vivo studies. In the literature review, DNA damage has not been examined with Comet analysis before in *Lycium barbarum* studies. However, in similar studies, comet analysis was used to measure DNA damage [32, 40, 41].

5. Conclusion

The effects of different molecular-sized fractions of *Lycium barbarum* grown in Turkey on EAT cells have been studied and it has been shown that the fraction especially above 50 kDa- is more effective at the 2000 mg/mL concentration. Total phenolic, total polysaccharide, total glucose, and ABTS radical scavenging activity were also evaluated and the radical scavenging properties of the fractions were determined. *Lycium barbarum* demonstrated a strong anticancer effect in EAT cells, demonstrating its apoptotic effects by lowering the cell viability percentage and increasing DNA damage in the comet assay. *Lycium barbarum* was determined to induce apoptosis in the *in vitro* model of the EAT cell. The detailed evaluation of *Lycium barbarum* with studies on different tumor models will shed light on its anticancer activity. Interdisciplnary research collaborations are necessary to facilitate large-scale discoveries in molecular oncology.

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Conflict of interest statement
The authors declare that they have no conflict of interest.

Ethics statement
During this study, the conformity of the applications on animals to animal rights and animal experiments was approved by the decision of Erciyes University, Experimental Animal Local Ethics Committee, dated 10.05.2017 and numbered 17/049. This study was accepted as successful as Sümeyye UÇAR's Master Thesis at Erciyes University, Institute of Health Sciences.

Author's contribution
Sümeyye Uçar, Harun Ülger: conceived the ideas of the study and writing manuscript. **Özge AL, Mehtap Nisari:** Roles/Writing-original draft. **Gökçe Şeker Karatoprak, Fazile Cantürk Tan;** Performed data collection and analysis. **Seher Yılmaz, Ammad Ahmad Farooqi**: Writing - review & editing.

References

1. Lewandowska AM, Lewandowski T, Rudzki M, Rudzki S, Laskowska B (2021) Cancer prevention – review paper. AEEM 28 (1): 11-19. doi: 10.26444/aaem/116906
2. Frei E, 3rd, Holland JF, Schneiderman MA, Pinkel D, Selkirk G, Freireich EJ, Silver RT, Gold GL, Regelson W (1958) A comparative study of two regimens of combination chemotherapy in acute leukemia. Blood 13 (12): 1126-1148. doi:
3. Yang W, Soares J, Greninger P, Edelman EJ, Lightfoot H, Forbes S, Bindal N, Beare D, Smith JA, Thompson IR, Ramaswamy S, Futreal PA, Haber DA, Stratton MR, Benes C, McDermott U, Garnett MJ (2013) Genomics of Drug Sensitivity in Cancer (GDSC): a resource for therapeutic biomarker discovery in cancer cells. Nucleic Acids Res 41 (Database issue): D955-961. doi: 10.1093/nar/gks1111
4. Ezzeldin H, Diasio R (2004) Dihydropyrimidine dehydrogenase deficiency, a pharmacogenetic syndrome associated with potentially life-threatening toxicity following 5-fluorouracil administration. Clin Colorectal Cancer 4 (3): 181-189. doi: 10.3816/ccc.2004.n.018
5. Benson AB, Venook AP, Al-Hawary MM, Arain MA, Chen YJ, Ciombor KK, Cohen S, Cooper HS, Deming D, Farkas L, Garrido-Laguna I, Grem JL, Gunn A, Hecht JR, Hoffe S, Hubbard J, Hunt S, Johung KL, Kirilcuk N, Krishnamurthi S, Messersmith WA, Meyerhardt J, Miller ED, Mulcahy MF, Nurkin S, Overman MJ, Parikh A, Patel H, Pedersen K, Saltz L, Schneider C, Shibata D, Skibber JM, Sofocleous CT, Stoffel EM, Stotsky-Himelfarb E, Willett CG, Gregory KM, Gurski LA (2021) Colon Cancer, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 19 (3): 329-359. doi: 10.6004/jnccn.2021.0012
6. Longley DB, Harkin DP, Johnston PG (2003) 5-fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer 3 (5): 330-338. doi: 10.1038/nrc1074
7. Laurent V, Saillard J, Thierry M, Lepelletier A, Fronteau C, Huon JF (2021) Anticancer agents and phytotherapy: Interactions that are often unrecognized. J Oncol Pharm Pract 27 (2): 322-328. doi: 10.1177/1078155220920363
8. Ferro A, Rosato V, Rota M, Costa AR, Morais S, Pelucchi C, Johnson KC, Hu J, Palli D, Ferraroni M, Zhang ZF, Bonzi R, Yu GP, Peleteiro B, López-Carrillo L, Tsugane S, Hamada GS, Hidaka A, Zaridze D, Maximovitch D, Vioque J, Navarrete-Munoz EM, Aragonés N, Martín V, Hernández-Ramírez RU, Bertuccio P, Ward MH, Malekzadeh R, Pourfarzi F, Mu L, López-Cervantes M, Persiani R, Kurtz RC, Lagiou A, Lagiou P, Boffetta P, Boccia S, Negri E, Camargo MC, Curado MP, La Vecchia C, Lunet N (2020) Meat intake and risk of gastric cancer in the Stomach cancer Pooling (StoP) project. Int J Cancer 147 (1): 45-55. doi: 10.1002/ijc.32707
9. Potterat O (2010) Goji (Lycium barbarum and L. chinense): Phytochemistry, pharmacology and safety in the perspective of traditional uses and recent popularity. Planta Med 76 (1): 7-19. doi: 10.1055/s-0029-1186218
10. Dong JZ, Lu DY, Wang Y (2009) Analysis of flavonoids from leaves of cultivated Lycium barbarum L. Plant Foods Hum Nutr 64 (3): 199-204. doi: 10.1007/s11130-009-0128-x
11. Mocan A, Zengin G, Simirgiotis M, Schafberg M, Mollica A, Vodnar DC, Crişan G, Rohn S (2017) Functional constituents of wild and cultivated Goji (L. barbarum L.) leaves: phytochemical characterization, biological profile, and computational studies. J Enzyme Inhib Med Chem 32 (1): 153-168. doi: 10.1080/14756366.2016.1243535
12. Ming M, Guanhua L, Zhanhai Y, Guang C, Xuan Z (2009) Effect of the Lycium barbarum polysaccharides administration on blood lipid metabolism and oxidative stress of mice fed high-fat diet in vivo. Food Chem 113 (4): 872-877. doi: https://doi.org/10.1016/j.foodchem.2008.03.064
13. Eroglu Ozkan E, Yilmaz-Ozden T, Gulsoy Toplan G, Mat A (2018) Phenolic content and biological activities of Lycium barbarum L (Solanaceae) fruits (Goji berries) cultivated in Konya, Turkey. TJPR 17: 2047-2053. doi: 10.4314/tjpr.v17i10.22
14. Liu Y-J, Gao K-X, Peng X, Wang Y, Wang J-Y, Hu M-B (2024) The great potential of polysaccharides from natural resources in the treatment of asthma: A review. Int J Biol Macromol 260(Pt 1): 129431. doi: 10.1016/j.ijbiomac.2024.129431.
15. Wang Y, Chen H, Wu M, Zeng S, Liu Y, Dong J (2015) Chemical and Genetic Diversity of Wolfberry. In: Chang RC-C, So K-F (eds) Lycium Barbarum and Human Health. Springer Netherlands, Dordrecht, pp 1-26. doi:10.1007/978-94-017-9658-3_1
16. Luo Q, Li Z, Yan J, Zhu F, Xu RJ, Cai YZ (2009) Lycium barbarum polysaccharides induce apoptosis in human prostate cancer cells and inhibits prostate cancer growth in a xenograft mouse model of human prostate cancer. J Med Food 12 (4): 695-703. doi: 10.1089/jmf.2008.1232
17. Du X, Zhang J, Liu L, Xu B, Han H, Dai W, Pei X, Fu X, Hou S (2022) A novel anticancer property of Lycium barbarum polysaccharide in triggering ferroptosis of breast cancer cells. J Zhejiang Univ Sci B 23 (4): 286-299. doi: 10.1631/jzus.B2100748
18. Zhu CP, Zhang SH (2013) Lycium barbarum polysaccharide inhibits the proliferation of HeLa cells by inducing apoptosis. J Sci Food Agric 93 (1): 149-156. doi: 10.1002/jsfa.5743
19. Singleton VL, Orthofer R, Lamuela-Raventós RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Method Enzymol 299: 152-

178.

20. DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric Method for Determination of Sugars and Related Substances. Anal Chem 28 (3): 350-356. doi: 10.1021/ac60111a017
21. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26 (9-10): 1231-1237. doi: 10.1016/s0891-5849(98)00315-3
22. Ocak, M., Ateş, Ş., Kahveci, S., Okan, A., Doğanyiğit, Z., Uçar, S., Yılmaz, S. (2024). Evaluation of the anticarcinogenic effects of Rutin on brain tissue in mice with Ehrlich ascites carcinoma by micro-computed tomography and histological methods. *Asia Pac J Clin Oncol*, 10.1111/ajco.14058. Advance online publication. https://doi.org/10.1111/ajco.14058
23. Lindner L. E. (1993). Improvements in the silver-staining technique for nucleolar organizer regions (AgNOR). J Histochem Cytochem. 41(3): 439–445. https://doi.org/10.1177/41.3.8429207
24. Sariözkan S, Türk G, Güvenç M, Yüce A, Özdamar S, Cantürk F, Yay AH (2016) Effects of Cinnamon (C. zeylanicum) Bark Oil Against Taxanes-Induced Damages in Sperm Quality, Testicular and Epididymal Oxidant/Antioxidant Balance, Testicular Apoptosis, and Sperm DNA Integrity. Nutr Cancer 68 (3): 481-494. doi: 10.1080/01635581.2016.1152384
25. Toh DWK, Lee WY, Zhou H, Sutanto CN, Lee DPS, Tan D, Kim JE (2021) Wolfberry (Lycium barbarum) Consumption with a Healthy Dietary Pattern Lowers Oxidative Stress in Middle-Aged and Older Adults: A Randomized Controlled Trial. Antioxidants (Basel) 10 (4). doi: 10.3390/antiox10040567
26. Benchennouf A, Grigorakis S, Loupassaki S, Kokkalou E (2017) Phytochemical analysis and antioxidant activity of Lycium barbarum (Goji) cultivated in Greece. Pharm Biol 55 (1): 596-602. doi: 10.1080/13880209.2016.1265987
27. Islam T, Yu X, Badwal TS, Xu B (2017) Comparative studies on phenolic profiles, antioxidant capacities and carotenoid contents of red goji berry (Lycium barbarum) and black goji berry (Lycium ruthenicum). Chem Cent J 11 (1): 59. doi: 10.1186/s13065-017-0287-z
28. Vulić JJ, Čanadanović-Brunet JM, Ćetković GS, Djilas SM, Tumbas Šaponjac VT, Stajčić SS (2016) Bioactive Compounds and Antioxidant Properties of Goji fruits (Lycium barbarum L.) Cultivated in Serbia. J Am Coll Nutr 35 (8): 692-698. doi: 10.1080/07315724.2016.1142404
29. Zhang Q, Lv X, Wu T, Ma Q, Teng A, Zhang Y, Zhang M (2015) Composition of Lycium barbarum polysaccharides and their apoptosis-inducing effect on human hepatoma SMMC-7721 cells. FNR 59 (1): 28696.
30. Deng X, Li X, Luo S, Zheng Y, Luo X, Zhou L (2017) Antitumor activity of Lycium barbarum polysaccharides with different molecular weights: an in vitro and in vivo study. Food Nutr Res 61 (1): 1399770. doi: 10.1080/16546628.2017.1399770
31. Nisari M, Bozkurt Ö, Ertekin T, Ceylan D, İnanç N, Al Ö, Güler H, Unur E (2020) Rhamnetin improves antioxidant status in the liver of Ehrlich solid tumor bearing mice. Med Sci Discov 7 (5): 494-500. doi: 10.36472/msd.v7i5.380
32. Nisari M, Yilmaz S, Eroz R, Ertekin T, Bircan D, Ulger H (2017) The detection of curcumins' antitumoral effects via argyrophilic nucleolar organizing region-associated protein synthesis in mice with ehrlich's ascitic carcinoma. Bratisl Lek Listy 118 (1): 61-65. doi: 10.4149/bll_2017_012
33. Ghobadi R, Rostami Ahmadvandi H, Zeinodini A, Akbarabadi A (2021) Nutritional Properties and Benefits of Camelina Oil and Meal. Agrotech Ind Crops 1(2): 71-76. doi: 10.22126/atic.2021.6441.1009
34. Rahmani AF, Sakandari MN, Mirzaei A, SÖZMEN U (2024) Investigating the physiological responses and the expression of genes effective in steviol glycosides production in stevia plant. Agrotech Ind Crops doi: 10.22126/atic.2024.3067
35. Ceylan D, Aksoy A, Ertekin T, Yay AH, Nisari M, Karatoprak G, Ülger H (2018) The effects of gilaburu (Viburnum opulus) juice on experimentally induced Ehrlich ascites tumor in mice. J Cancer Res Ther 14 (2): 314-320. doi: 10.4103/0973-1482.181173
36. DU, X., Zhang, J., Liu, L., Xu, B., Han, H., Dai, W., Pei, X., Fu, X., Hou, S. (2022). A novel anticancer property of Lycium barbarum polysaccharide in triggering ferroptosis of breast cancer cells. J Zhejiang Univ Sci B. 23(4): 286–299. https://doi.org/10.1631/jzus.B2100748
37. Gan, L., Wang, J., Zhang, S. (2001). Inhibition the growth of human leukemia cells by Lycium barbarum polysaccharide. Wei Sheng Yan Jiu, 30(6): p. 333-5.
38. Tang, W. M., Chan, E., Kwok, C. Y., Lee, Y. K., Wu, J. H., Wan, C. W., Chan, R. Y., Yu, P. H., Chan, S. W. (2012). A review of the anticancer and immunomodulatory effects of Lycium barbarum fruit. Inflammopharmacol 20(6): 307–314. https://doi.org/10.1007/s10787-011-0107-3.
39. Zhang, M., Chen, H., Huang, J., Li, Z., Zhu, C., Zhang, S. (2005). Effect of lycium barbarum polysaccharide on human hepatoma QGY7703 cells: inhibition of proliferation and induction of apoptosis. Life Sci 76(18): 2115–2124. https://doi.org/10.1016/j.lfs.2004.11.009
40. Ceyhan, R. N., Nisari, M., Nisari, M., Uçar, S., Mehmet Koca, F., Kerek, G., Özcanlı, T., İnanç, N. (2024). The detection of pomegranate (*Punica granatum* L.) peel apoptotic effects using AgNOR staining in MDA-MB-231. Toxicol Res 13(1): tfae015. https://doi.org/10.1093/toxres/tfae015
41. Scolastici, C., Alves de Lima, R. O., Barbisan, L. F., Ferreira, A. L., Ribeiro, D. A., Salvadori, D. M. (2008). Antigenotoxicity and antimutagenicity of lycopene in HepG2 cell line evaluated by the comet assay and micronucleus test. Toxicol in vitro. 22(2): 510–514. https://doi.org/10.1016/j.tiv.2007.11.002