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ORIGINAL RESEARCH

Investigation of Antitumor Activity of Fenugreek Paste Extracts

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Abstract

Objective: Fenugreek paste, which contains fenugreek and spices, is a popular food in Kayseri and the surrounding region of Türkiye. This study aims to evaluate the antitumor effects of an extract derived from fenugreek paste in Balb/C mice bearing Ehrlich ascites tumor (EAT).

Materials and Methods: In the *in vivo* experiments, fenugreek paste extract was administered at concentrations of 200 mg/kg or 400 mg/kg. For the *in vitro* study, extract concentrations of 250, 500, and 1000 µg/mL were used. At the end of the experiment, the volume of abdominal ascites fluid was measured, and cell counts were performed.

Results: Fenugreek paste extract delayed weight gain due to EAT cell proliferation in the treatment groups and caused a significant decrease in the number of cells in the ascites fluid in the 400 mg/kg extract group (47.28×10^6) compared to the control group (67.60×10^6; p=0.041). Histopathological analysis revealed that EAT cells adhered intensely to tissues in the control group but adhered less in the treatment groups. The most significant decrease was observed in the 400 mg/kg fenugreek extract group. After 24 hours of culture, there was a substantial difference in EAT cell viability between the control group (5.9±0.2) and the treatment groups receiving 250, 500, and 1000 µg/mL of fenugreek paste extract (5.7±0.2, 5.7±0.2, and 5.6±0.1, respectively; p=0.013).

Conclusion: The study results suggested that fenugreek paste extract had an antitumor effect on EAT cells.

Keywords: Ehrlich Ascites Tumor, Fenugreek Paste, Fenugreek, Red Pepper, Garlic

INTRODUCTION

According to the World Health Organization (WHO), cancer is one of the leading causes of death worldwide. Although anticancer treatment research and development efforts are well-funded and active, cancer remains a significant threat to human health. As a result, there is a strong interest in cancer research for more effective treatments with fewer side effects[1].

Chemopreventive agents are an innovative field in cancer prevention. They are used in conjunction with pharmacological, biological, and nutritional interventions. Previous studies have shown that non-toxic fruits and vegetables contain phytochemicals [2]. It is well-established that dietary habits and nutrition play important roles in cancer prevention. Numerous epidemiological studies have shown that a diet high in vegetables and fruits reduces the risk of cancer[3]. Recently, studies have focused on naturally occurring biologically active substances, diet, and chemical cancer preventive agents in medicinal plants. Many biologically active substances exhibit chemotherapeutic activity by blocking cell cycle progression and triggering apoptotic cell death[4].

Phytochemicals derived from spices and herbs possess significant anticancer properties. The potential anticancer effect of fenugreek paste, comprising fenugreek[4,5], red pepper[6], garlic[7], and cumin[8], which is consumed in Central Anatolia in Türkiye, has not yet been studied. Fenugreek paste is commonly consumed as a food and used as a mixture in pastry production in Kayseri and the surrounding region. The mixtures obtained from companies in the province of Kayseri that produce fenugreek paste are generally similar in content. The fenugreek paste is composed of approximately 50% water and 50% herbal mixture. The herbal mixture is primarily made up of fenugreek (33%–37%), red pepper (3.5%–22.5%), garlic (5%–7%), and cumin (1%–2.5%), as well as small amounts of black pepper, allspice, ginger, coriander, clove, and cinnamon (each less than 1%). The literature provides the following percentages for the contents of fenugreek paste: The mixture consists of 40% fenugreek seed powder, 7.5% vetch flour, 2.5% wheat flour, 20% flaked red pepper (5% Kayseri, 10% Nazilli, and 5% Kahramanmaraş pepper), 30% garlic, and 0.1% dye (2/3 Pounceau 4R. E 124 + 1/3 Sunset Yellow E 120)[9,10]

Fenugreek (*Trigonella foenumgraecum* L.) is an aromatic herbaceous annual plant belonging to the Papilionaceae, a subfamily of the family Leguminosae. It is widely grown in Mediterranean countries and Asia and is one of the ancient medicinal plants of India and North Africa[11, 12]. The extracts and powders of leaves and seeds are widely used in medicinal preparations. Seeds are the main source of many elements, such as calcium, phosphorus, iron, zinc, and manganese[11, 12, 13]. Fenugreek has reportedly been used in the treatment of many diseases. It has been shown to have apoptosis-promoting and anticancer effects on breast cancer cells[4, 5].

Laboratory studies in rodents are essential for identifying cancer-causing chemicals in humans. Epidemiological studies in laboratory animals have also been one of the best methods for determining carcinogenic potential in long-term studies[14]. Various chemical agents can be administered in different ways (intraperitoneal, intravenous, or subcutaneous) to generate tumor models with multiple characteristics in experimental animals. Solid tumors are transplanted through subcutaneous, intradermal, intramuscular, intraperitoneal, or intravenous injection of cell suspensions. The transplanted tumors are similar in their early formation phases to the spontaneous tumors from which they originate. An example of this model is the Ehrlich ascites tumor (EAT)[15, 16].

Research studies aimed at developing effective cancer treatments and new methods are currently being conducted on tumors formed in experimental animals. Ehrlich and Apolant (1905) first acquired Ehrlich ascites tumor (EAT) from a female mouse with spontaneous breast adenocarcinoma. They subcutaneously transplanted tumor fragments from mouse to mouse, which eventually led to the development of an experimental tumor. In 1932, Loewenthal and Jahn managed to cultivate a liquid-growing form of this tumor in the peritoneum of mice. The tumor was named after the formation of ascites liquid and cells in the peritoneum[17, 18]. Depending on the study's purpose, EAT is used either ascites or in a solid form. The ascite fluid containing tumor cells is injected intraperitoneally into an experimental animal to obtain an ascitic form. On the other hand, a solid form is obtained by subcutaneously injecting the same fluid[15]. The study investigated the effects of fenugreek on EAT cells in both *in vivo* and *in vitro* settings.

MATERIALS AND METHODS

In this study, the appropriateness of animal practices for animal rights and animal experimentation ethics was approved by the Erciyes University, Experimental Animal Local Ethics Committee Decision dated 12.03.2014 No: 14/053. The project that the code TDK-2014-5325 was supported by Erciyes University Scientific Research Projects Unit.

Species, number, and distribution of subjects in the research

The study used 40 male Balb-c mice, eight weeks old, weighing 25-30g. The mice were divided into four groups, each containing ten mice. They were housed in temperature-controlled rooms with a constant temperature of 21°C and a 12-hour light/dark cycle. The mice were fed standard pellet feed.

Experimental procedure

The study utilized cells obtained through intraperitoneal injection of Ehrlich ascites tumor (EAT) cells into Balb/c mice, followed by the reproduction of EAT cells in a culture medium.

Plant material

The fenugreek paste was made by mixing fenugreek (35%), red pepper (5%), garlic (5%), cumin (2%), black pepper (0.5%), cloves (0.5%), coriander

(0.5%), cinnamon (0.5%), ginger (0.5%), and allspice (0.5%). The water, which makes up 50% of the paste, was excluded. A powdery fenugreek mixture was obtained by mixing the remaining ingredients. The appropriate amounts of extracts for the study were determined based on a literature review[16, 19, 20,38]. In this study, we added extracts obtained from a mixture of fenugreek at concentrations of 250, 500, and 1000 µg/mL to the culture medium of EAT cells to evaluate their *in vitro* effects. Additionally, we intraperitoneally injected fenugreek extracts at doses of 200 mg/kg and 400 mg/kg to investigate their impact on liquid tumor cells *in vivo*.

Preparation of fenugreek paste extract

The fenugreek powder (500 g) underwent three 24-hour extractions at 37°C in a shaking water bath using 70% methanol. The extracts were combined and concentrated using a rotary evaporator under vacuum at 37°C–38°C. Subsequently, the extract was lyophilized and stored at −20°C until analysis. Before use, the fenugreek paste extract was dissolved in a 5% ethanol solution.

Calculation of total phenolic content

The extract's total phenolic content was determined using the Folin-Ciocalteu method and expressed as gallic acid equivalents (GAEs)[21]. To a 10 mL container containing 6 mL of distilled water, 100 µL of sample solution and 500 µL of Folin-Ciocalteu reagent were added. After one minute, 1.5 mL of 20% aqueous Na2CO3 was added, and the mixture was diluted to 10 mL with water. The control consisted of the extract-free reagent mixture. After incubating at 25°C for 2 hours, we measured the absorbance at 760 nm and compared it with the GAE calibration curve. We calculated the total phenolic content of the substance as GAEs. We performed three parallel experiments, and the results are presented as mean values.

***In vivo* studies**

Experimental groups

Group 1, Negative control (- control): This group did not develop cancer and was fed a regular diet for 7 days. Normal saline (NS) was administered via intraperitoneal injection for 7 days.

Group 2, Positive Control (+ control): The mice in this group were intraperitoneally injected with 0.1 mL ascites fluid containing 1×10^6 EAT cells in the abdomen on day 0. From day 0, mice were intraperitoneally injected with 0.5 mL of NS for 7 days.

Group 3, 200 mg/kg: The mice in this group were intraperitoneally injected with 0.1 mL ascites fluid containing 1×10^6 EAT cells in the abdomen on day 0. From day 0, mice were intraperitoneally injected with 200 mg/kg of fenugreek paste extract for 7 days.

Group 4, 400 mg/kg: The mice in this group were intraperitoneally injected with 0.1 mL ascites fluid containing 1×10^6 EAT cells in the abdomen on day 0. From day 0, mice were intraperitoneally injected with 400 mg/kg of fenugreek paste extract for 7 days.

The study evaluated the effects of fenugreek paste extract on tumor development by monitoring daily nutrient and water consumption, weight gain, and tissue pathology. The animals' weight was measured daily to track tumor development in relation to their body weight before tumor inoculation. Additionally, macroscopic evaluations were conducted to assess general morphological appearances, including hair loss, defecation disorders, and anal lesions. On day 8, ascites fluid was collected from the abdominal cavities of all groups of mice under general anesthesia, and the anticancerogenic effect of fenugreek paste extract was assessed by cell counting. Additionally, intraperitoneal organs were taken for histopathological evaluation. For this purpose, the tissues were stained with hematoxylin and eosin.

***In vitro* studies**

Cell culture experiments

For the *in vitro* study, Dulbecco's Modified Eagle's Medium was used, which contained 10% fetal bovine serum, 100 U penicillin, and 100 mg/mL streptomycin. The fenugreek paste extract was dissolved in 0.02 mL of solvent and administered in three doses. The amount of medium and the EAT cell count were kept equal for each group. EAT cells from the stock animal were counted to determine 2.5×10^6 cells (Table 1). A total of 24 wells were seeded for each group. Accordingly, 104166 cells were seeded into each well (2.5×10^6 cells/24). The experimental groups were treated with EAT cells and extracts obtained from the fenugreek mixture at concentrations of 250, 500, and 1000 µg/mL, while only EAT cells were added to the medium in the control group. The culture dishes were then incubated for 3 and 24 hours.

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Table 1. The amounts of fenugreek extract, cells, and medium used in cell culture were recorded for each group, with 24 wells.

	Extract	Number of cells	Medium
Control	-	$2.5x10^6$ cells (1 mL)	4 mL (medium)
Group 1	1250 µg fenugreek + 0.02 mL solvent	$2.5x10^6$ cells (1 mL)	3.98 mL (medium)
Group 2	2500 µg fenugreek + 0.02 mL solvent	$2.5x10^6$ cells (1 mL)	3.98 mL (medium)
Group 3	5000 µg fenugreek + 0.02 mL solvent	$2.5x10^6$ cells (1 mL)	3.98 mL (medium)

Evaluation of EAT cells *in vitro*

Cells cultured for 3 and 24 hours were transferred to 100-µl Eppendorf tubes and then pipetted with 100 µL of Trypan blue. The culture was transferred to 100 µl Eppendorf tubes and then treated with 100 µL of Trypan blue. From the resulting cell and Trypan blue solution, a 50 µL aliquot was taken and placed into the wells of a Thoma slide. The counting area was identified using 40X objective lenses, and transparent living cells were distinguished from non-living cells containing blue dye and counted separately.

Statistical evaluation

The data were analyzed using IBM SPSS Statistics 22.0 (IBM Corp., Armonk, New York, USA). Descriptive statistics were given as the number of units (n), percentage (%), mean ± standard deviation ($\bar{x} \pm ss$), and median (25^{th} and 75^{th} percentiles). The normal distribution of numerical variables was assessed using the Shapiro–Wilk normality test and Q-Q graphs. Comparisons of the groups according to time were made by two-way analysis of variance for variables with normal distribution and multiple comparisons by performing Tukey's honest significant difference test. Intergroup comparisons were made by performing Kruskal–Wallis analysis for variables with non-normal distribution. If there was a difference due to the Kruskal–Wallis analysis, the Dunn test was used as the multiple comparison test. Wilcoxon analysis was used for intragroup comparisons for variables that did not show normal distribution. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Analysis results of fenugreek

The fenugreek paste extract was analyzed for its phenolic substance content by the Department of Pharmacognosy at Erciyes University's Faculty of Pharmacy. The results showed a content of 51.832±1.632 mg GAE/g.

***In vivo* findings**

Weight tracking in experimental groups

When comparing weight gain between the experimental groups, no significant difference was observed in the negative control group. However, significant differences were observed in the positive control and experimental groups. In the positive control group, the difference between the weights on day 1 and day 4 was significant (p=0.006), and the difference increased on days 5, 6, and 7. In the groups receiving fenugreek in doses of 200 and 400 mg/kg, there was a significant difference between days 1 and 5 (p=0.001), and the difference increased on the following days. It should be noted that the experimental groups experienced significant weight gain on day 5, indicating that fenugreek delayed weight gain for one day (Table 2 and Figure 1).

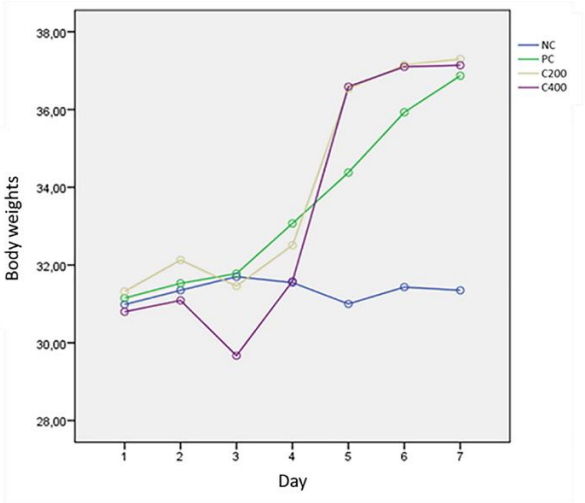

Figure 1. The daily weight tracking graph for the body weights of the experimental groups

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Table 2. The daily weight tracking results for the body weights of the experimental groups.

Groups	Day 1 M±SD	Day 2 M±SD	Day 3 M±SD	Day 4 M±SD	Day 5 M±SD	Day 6 M±SD	Day 7 M±SD
Negative control	30.98±2.3	31.35±2.3	31.70±23	31.55±2.2	31.00±2.0	31.43±2.2	31.35±2.0
Positive control	31.15±1.7	31.53±2.3	31.78±1.9	33.07±2.2	34.38±2.5	35.93±2.3	36.87±2.3
200 mg/kg	31.32±1.0	32.13±1.5	31.46±1.6	32.51±1.7	36.53±1.3	37.15±1.5	37.30±1.9
400 mg/kg	30.80±1.3	31.09±1.0	29.67±2.3	31.58±2.1	36.59±2.0	37.10±2.3	37.14±2.6

Total volume and packed volume calculations of intraperitoneal ascites fluid

At the end of the experiment, ascites fluid was extracted from the abdomen of all animals using an injector, and the total volume was calculated. The liquid was then centrifuged at 1000 rpm for 5 minutes, the supernatant fraction was discarded, and the remaining volume was calculated as the packet volume. The total volume results for the positive control and treatment groups (200 mg/kg and 400 mg/kg) were 6.4±1.9 cc, 6.7±1.7 cc, and 6.9±2.2 cc, respectively. The treatment groups had a higher total volume than the positive control group, but a lower packed volume (4.3±1.0, 4.0±1.1, and 4.2±1.7 cc, respectively). The supernatant volume was measured as 2.1±1.4, 2.7±0.9, and 3.0±1.3 cc in the positive control and treatment groups (200 and 400 mg/kg), respectively (Table 3).

Table 3. Total, packed, and supernatant volume results of ascites fluid obtained from the abdomen

Group	Total volume	Packed volume	Supernatant
Negative control	---	---	---
Positive control (n=10)	6.4±1.9 cc	4.3±1.0 cc	2.1±1.4 cc
EAT cell + 200 mg/kg fenugreek (n=10)	6.7±1.7 cc	4.0±1.1 cc	2.7±0.9 cc
EAT cell + 400 mg/kg fenugreek (n=10)	6.9±2.2 cc	4.2±1.7 cc	3.0±1.3 cc

The cells in the ascites fluid collected from the animals were counted by staining with trypan blue. The mean numbers of live cells in 1 mL were 67.60×10^6, 55.08×10^6, and 47.28×10^6, respectively, as shown in Table 4. The treatment groups showed a statistically significant decrease in live cells per 1 mL compared to the positive control group. The 400 mg/kg group had the most significant decrease ($p=0.041$).

Table 4. Mean number of cells in intraperitoneal ascites fluid in experimental groups

Group	Mean number of cells (1 mL)
Negative control	---
Positive control (n=10)	67.60×10^6
EAT cell + 200 mg/kg fenugreek (n=10)	55.08×10^6
EAT cell + 400 mg/kg fenugreek (n=10)	47.28×10^6

The reduction in ascites fluid volume in treatment groups compared to the positive control group suggests that the fenugreek paste extract may have inhibited the proliferation of EAT cells, rather than reducing the volume of the cells themselves. This is supported by the mean cell numbers presented in Table 3.

Histopathological results

On day 8, all mice in the experimental groups were subjected to intramuscular general anesthesia, and their kidney, liver organs were extracted. The intra-abdominal organs of the animals that were intraperitoneally injected with EAT cells did not exhibit any morphological differences compared to the healthy control group. Routine histopathological follow-up was performed on the extracted tissues. 5-µm-thick sections were taken and stained using the hematoxylin and eosin method. The examinations revealed normal histological features in the healthy control group tissues. In contrast, the EAT cell groups showed scattered EAT cell populations in the tissue capsule in the form of connective tissue. Upon histopathological examination of the EAT cells, it was observed that they varied in size and shape, each containing a large hyperchromatic nucleus and eosinophilic cytoplasm (Figure 2).

It was found that the EAT cells invaded the connective tissue capsule in organs of the healthy, tumor control, and treatment groups. The cells were characterized by a large hyperchromatic nucleus, eosinophilic cytoplasm, and distinct morphological features. Although the EAT cells exhibited intense

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aggregation in the tumor control group, they were individually observed surrounding the connective tissue capsule in the treatment groups (Figures 3 and 4).

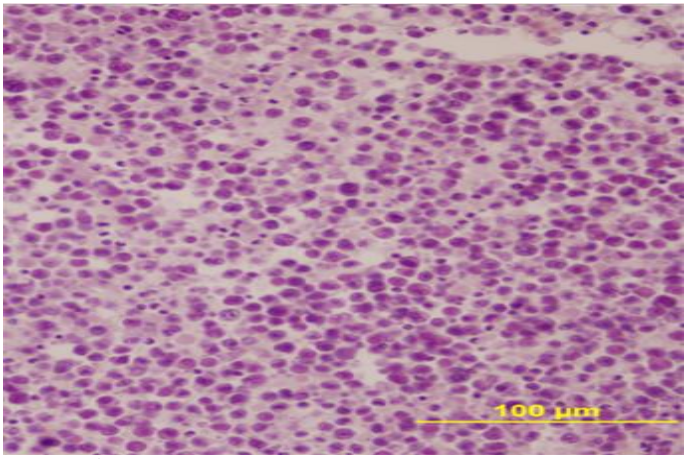

Figure 2. Histopathological view of EAT cells (H&E 40X).

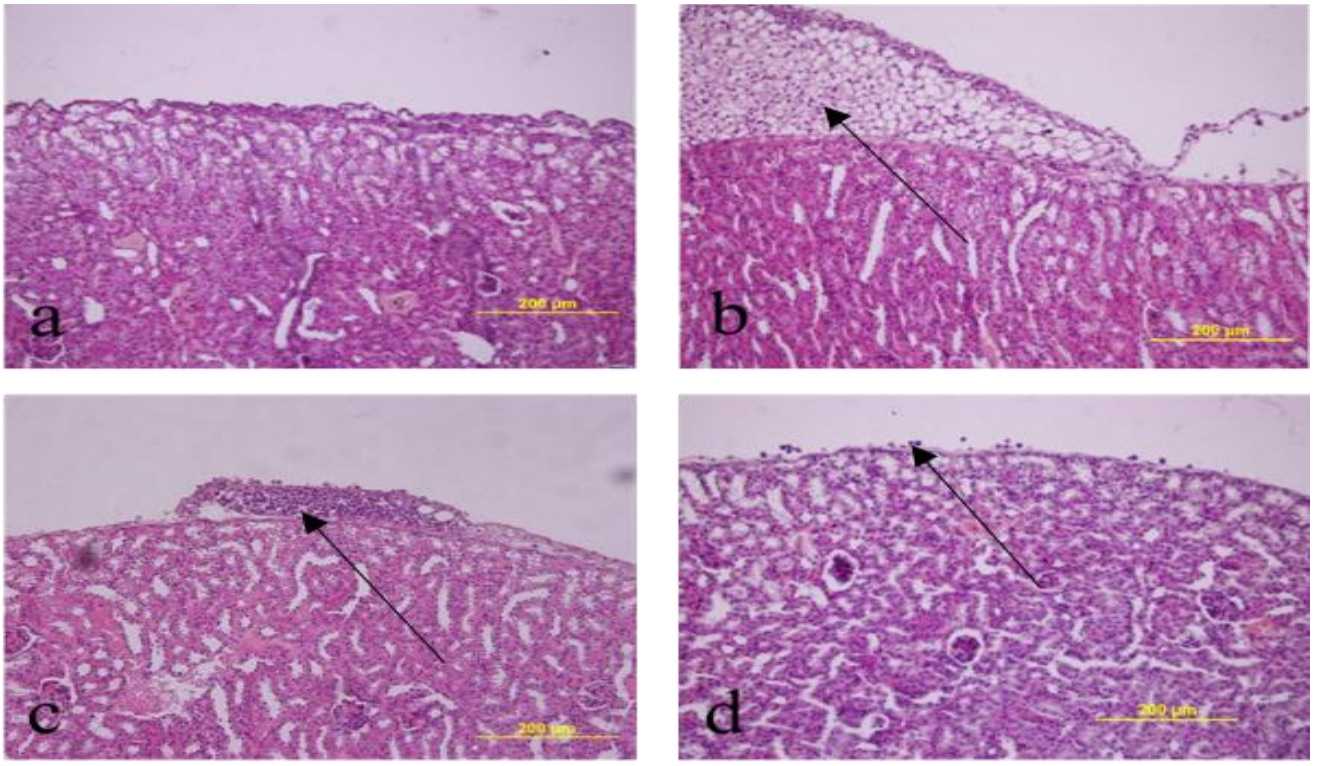

Figure 3. The histological and histopathological findings of kidney tissue in groups are presented in Figures a-d. a) The healthy control group (H&E, 20X). b) Tumor control group (H&E, 20X). c) The group that received 200 mg/kg of fenugreek (H&E, 20X). d) The group that received 400 mg/kg of fenugreek (H&E, 20X). The EAT cell, which invades the kidney capsule, is represented by the black arrow.

In vitro findings

Effect of fenugreek paste extract on cell-cultured EAT cells

The effect of fenugreek paste extract on EAT cells was evaluated *in vitro* using 3- and 24-hour culture results. A literature review of cell culture studies revealed that the extract was used at 250, 500, and 1000 µg/mL concentrations. The cell count of EAT cells was measured after incubation periods of 3 and 24 hours. Following 3 and 24 hours of cell culture in

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the positive control group, the number of living and dead cells were recorded as 5.3 ± 0.2/0.7±0.1 and 5.9±0.2/5.3±0.2, respectively. In the 250 µg/mL group, the numbers were 5.4±0.2/0.7±0.1 and 5.7±0.2/5.5±0.1, respectively. In the 500 µg/mL group, the numbers were 5.3±0.2/0.7±0.1 and 5.7±0.2/5.5±0.1, respectively. In the 1000 µg/mL group, the numbers were 5.2±0.2/0.7±0.1 and 5.6±0.2/5.7±0.1, respectively (Table 5).

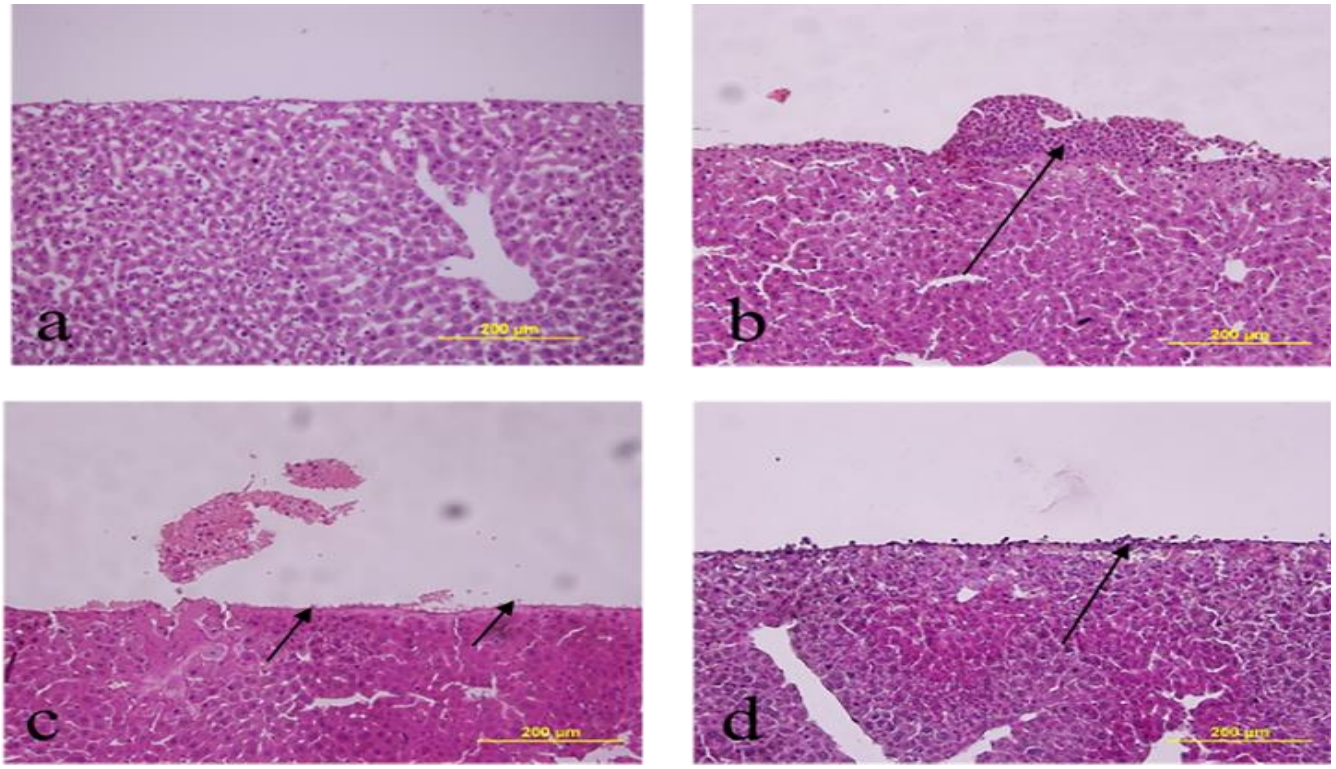

Figure 4. The histological and histopathological findings of liver tissue in in groups are presented in Figures a-d. a) The healthy control group (H&E, 20X). b) Tumor control group (H&E, 20X). c) The group that received 200 mg/kg of fenugreek (H&E, 20X). d) The group that received 400 mg/kg of fenugreek (H&E, 20X). The EAT cell, which invades the peritoneum surrounding the liver tissue by the black arrow.

Table 5. The statistical evaluation involved taking the logarithm of the data obtained from the 3- and 24-hours cell culture results.

Group	Mean number of living cells±SD (3-hour)	Mean number of dead cells±SD (3-hour)	Mean number of living cells±SD (24-hour)	Mean number of dead cells±SD (24-hour)
Positive control	5.3 ± 0.2	0.7 ± 0.1	5.9 ± 0.2	5.3 ± 0.2
EAT cell + 250 µg/mL	5.4 ± 0.2	0.7 ± 0.1	5.7 ± 0.2	5.5 ± 0.1
EAT cell + 500 µg/mL	5.3 ± 0.2	0.7 ± 0.1	5.7 ± 0.2	5.7 ± 0.1
EAT cell + 1000 µg/mL	5.2 ± 0.2	0.7 ± 0.1	5.6 ± 0.1	5.7 ± 0.2

There were no significant statistical differences in dead and living cell numbers among the groups cultured for 3 hours. However, in the groups cultured for 24 hours, there were significant differences in the numbers of living cells between the positive control and 250 µg/mL (p=0.013), the positive control and 500 µg/mL (p=0.029), and the positive control and 1000 µg/mL (p=0.000). Significant differences were found in the number of dead cells between the positive control and the groups cultured with 250 µg/mL (p=0.024), 500 µg/mL (p=0.000), and 1000 µg/mL (p=0.000) for 24 hours. Additionally, significant differences were found between the 250 µg/mL and 500 µg/mL groups (p=0.033). An increase in the number of dead cells was observed with increasing

concentrations in the treatment groups. The EAT cells were imaged and photographed using a 20X objective lens in cell culture (Figure 5).

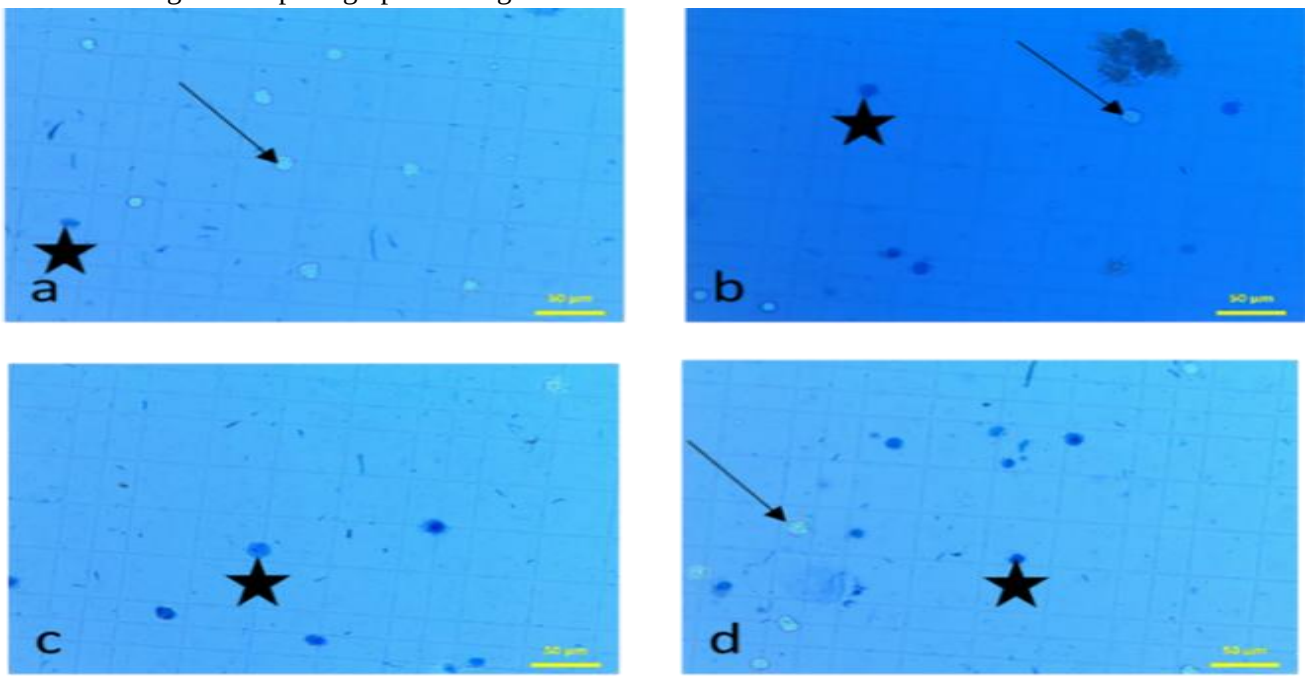

Figure 5. Images of living and dead EAT cells on the Thoma slide *in vitro* experimental groups. Arrow: Living EAT Cells, Star: Dead EAT Cells. a) Tumor control group b) 250 µg/mL fenugreek group. c) 500 µg/mL fenugreek group. d) 1000 µg/mL fenugreek group. (20X)

DISCUSSION

Flavonoids and phenolic compounds are used in cancer treatments, including chemotherapy. Phenolic compounds have been found to possess antioxidant, anti-inflammatory, and anticarcinogenic properties. Studies have demonstrated the anticarcinogenic effects of many plant species. It is believed that there may be a link between including such plants in the diet and reduced cancer rates[22]. Our study found that a fenugreek mixture, commonly consumed by locals, has antitumor effects. Fenugreek has a wide range of therapeutic applications. Studies show that the components of the extract have antioxidant effects[23, 24], as well as antidiabetic, antihypertensive, cholesterol-lowering, and anti-inflammatory effects[11, 25, 26]. Studies have reported that fenugreek extract increases apoptosis depending on dose and time[23, 27, 28]. Additionally, studies performed with the plants forming the mixture of fenugreek have reported anticarcinogenic effects. Sur et al. (2001) conducted an *in vivo* study on EAT cells, testing a fenugreek seed extract at 100- and 200-mg/kg treatment groups[17]. They reported a 70% inhibition of tumor cells compared to the control group. *In vitro* tests on cancer cells using fenugreek extract at concentrations ranging from 10-1000 µg/mL have shown a reduction in the number of living cells, particularly at concentrations ≥250 µg/mL[19]. In a separate *in vivo* study, it was found that administering 200 mg/kg of fenugreek extract intraperitoneally inhibited tumor cell proliferation by 70%[20]. Thoennissen et al. (2010) determined the dose of capsaicin, the active ingredient of red pepper, as 2 × 10-4 M in vitro. In an *in vivo* study, the amount of dose inhibiting the growth of solid tumor was 5 mg/kg/day[24]. Ban et al. (2007) investigated the effect of garlic extract on colon cancer in varying doses (30–150 µg/mL). The study found that the effective dose for SW620 colon cancer was 105 µg/mL, while for HCT116 colon cancer it was 130 µg/mL[25]. Pradeep et al. (2002) examined the effects of black pepper on melanoma cells and induced lung metastasis in mice. The study found that the active ingredient in black pepper, piperine, was present in the range of 10-100 µg/mL. A dose of 100 µg/mL was reported to be 100% effective[26]. Dwivedi et al. (2011) investigated the effect of clove extract on different cancer cell lines at 100, 200, and 300 µg/mL. They reported that the maximum cell death observed was 80% in groups administered 300 µg/mL of clove extract in a 24-hour cell culture[27]. Flores et al. (2010) reported

antitumor effects of coriander on lymphoma cells[28]. Kwon et al. (2009) investigated the effects of cinnamon extract on B16F10 and Clone M3 mouse melanoma cells through gavage and injection into the tumor. They reported that both treatments inhibited tumor growth[29] Karna et al. (2012) investigated the effect of ginger extract on prostate cancer cells. The study found that ginger extract reduced tumor volume *in vivo*. A high concentration of 1000 µg/mL was effective in shorter-term cell culture, while a low concentration of 50 µg/mL was effective in longer-term cell culture in vitro[30]. Jenny et al. (2005) evaluated the effect of an herbal mixture, including allspice extract, on lymphocytic leukemia cell line. The mixture was found to be effective at doses of 0.4 mg/mL and 1.0 mg/mL[31].

The literature review showed that the plant extracts used in fenugreek have anticarcinogenic effects. Although these plants are commonly used as spices, seasoning, aromatizers, and appetizers, they are also used as nutrients or preservatives in certain foods, such as pastrami, as in the case of the fenugreek paste used in Kayseri and Central Anatolia. These spices also have a significant place in traditional medicine. They are used in many diseases and their effects have been shown in *in vitro* and *in vivo* studies[32, 33, 34]. Phenolic compounds are secondary metabolic products commonly found in plants. Studies have shown that the phenolic content of fenugreek spices varies. Research has also been conducted on the total phenolic content of plants. In a study by Omezzine et al. (2013), the total phenolic content of fenugreek was 13.98 mg GAE/g[35]; in a study by Vega-Gálvez et al. (2009), the total phenolic content of red pepper was 43.2 mg GAE/g[36]; in a study by Chen et al. (2013), the total phenolic content of garlic was 21.27–33.96 mg GAE/g[37]; in a study by Alinian et al. (2016), the total phenolic content of cumin was 11.9–14.4 mg GAE/g[38]; in a study by Ahmad et al. (2014), the total phenolic content of cumin was 1.86–9.91 mg GAE/g [39]; in a study by Adefegha et al. (2012), the total phenolic content of clove was 0.88 mg GAE/g[40]; in a study by Tang et al. (2013), the total phenolic content of coriander was 1.73–1.38 mg GAE/g[41]; in a study by Krishnan et al. (2013), the total phenolic content of cinnamon was 11.11–12.4 mg GAE/g[42]; and in a study by Simon–Brown et al. (2016), the total phenolic content of ginger was 7.74 mg GAE/g[43]. In our study, the total phenolic content of fenugreek mixture was 51.832 ± 1.632 mg GAE/g and the phenolic content of the fenugreek mixture was higher than the phenolic content of each of the plants. The fenugreek mixture may have a higher phenolic content, which could result in greater antioxidative and anticarcinogenic effects.

This study investigates the effects of fenugreek paste extract on EAT cells *in vivo* and in vitro. The results of the *in vivo* study indicate that EAT cells administered intraperitoneally caused rapid weight gain in the control group animals, while there was a delay in weight gain in the 200- and 400-mg/kg per day fenugreek paste extract treatment groups. At the end of the experiment, a cell count was conducted by draining the ascites fluid in the peritoneum from the experimental animals using a liquid injector. The results showed a decrease in the number of living cells in the treatment groups. The weight gain and decrease in cell count observed suggest that the fenugreek paste extract inhibited the rapid growth of percutaneously injected EAT cells in the treatment groups. Histopathological examination of the tissues from the control and treatment groups showed that the EAT cells adhered more strongly to the tissues from the control group. Additionally, cancerous cell adhesion was observed in the experimental groups, particularly in the 400-mg/kg fenugreek extract treatment group. The *in vitro* findings supported the *in vivo* findings. The fenugreek paste extracts, which were increased in concentration (250, 500, and 1000 µg/mL) after 3 and 24 hours of culture, reduced the vitality rate of the EAT cells.

CONCLUSION

The study results indicate that the fenugreek paste extract, in the concentrations administered, did not completely inhibit the proliferation of EAT cells. However, it caused a decrease in the number of living cells. These findings are consistent with the literature cited above and could contribute to further research on this subject. The results suggest that the regional nutrient fenugreek may be useful in slowing down the formation and development of cancer.

Author contributions

Harun Ulger, Serife Alpa ve Tolga Ertekin designed the experiments; Serife Alpa, Ozlem Bozkurt, Seher Yilmaz, Mehtap Nisari, Gokce Seker Karatoprak ve Tolga Ertekin performed experiments and collected data; Şerife Alpa, Ozlem Bozkurt, Seher Yilmaz, Mehtap Nisari ve Tolga Ertekin perfomed analysis and Interpretation of Results; Harun Ulger, Serife Alpa ve Tolga Ertekin discussed the results and strategy; Serife Alpa, Ozlem Bozkurt, Seher Yilmaz, Mehtap Nisari, Gokce Seker Karatoprak, Tolga Ertekin, Harun Ülger final approved of the version to be published.

Conflict of Interest

There is no conflict of interest between the authors.

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