

Research Article

Effects of Fenugreek Extract on Total Antioxidant/Oxidant Status at Ehrlich Ascites Tumor Bearing Mice

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Abstract

Objectives: In this study, we aimed to effect of extract that derived from fenugreek on total antioxidant/oxidant status at Ehrlich ascites tumor bearing Balb/C mice.

Methods: The phenolic of Fenugreek extract was calculated as gallic acid equivalent using Folin-Ciocalteu method. Fenugreek extract concentration determined 200–400 mg/kg. In the end of in vivo studies, while the weight of experimental animals was estimated.

Results: It was found that fenugreek extract delaying the weight gain. Administered fenugreek extract group had lower serum TOS and OSI index and had higher serum TAS compared to the control group. These effects were higher in the group receiving fenugreek 400 mg/kg intraperitoneally.

Conclusion: As a result, the fenugreek extract shows antioxidant effect on EAT cells. We believe that our studies will be guiding for new studies about fenugreek and fenugreek could be advised as a food because of its antioxidant effect. Thus, it may have anticancer effect.

Keywords: Ehrlich ascites tumor, fenugreek, total antioxidant status, total oxidant status, oxidative stress index

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Free radicals play a role in the pathogenesis of various clinical conditions due to their structure, physical and chemical properties, cellular sources, reactions they take part in and the effect. The shift in the delicate balance between free radicals and the antioxidant defense system in favor of pro-oxidant and oxidant substances lead to the development of oxidative stress. Oxidative stress has been shown to cause tissue damage and is effective in development of various pathological conditions such as cancer, diabetes and atherosclerosis.^[1–5] The role of oxidative stress in cancer pathogenesis is highly debated. It is thought that

the chronic presence of Reactive Oxygen Species (ROS) facilitates the integration of viral oncogenes with cellular DNA and leads to the formation of tumor cells as a result of overexpression of some oncogenes.^[6] Total Oxidant Status (TOS), Total Antioxidant Status (TAS) and Oxidative Stress Index (OSI) are oxidative stress parameters used to evaluate the general oxidative stress in the body.^[7]

Cancer is the uncontrolled or abnormal growth and proliferation of cells as a result of DNA damage. Cancer forms a tissue mass that overshadows the development of normal tissues and does not conform to normal tissues.^[8] Surgi-

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cal treatment, chemotherapy, radiotherapy and hormonal treatment are primarily used in cancer treatment. Because of the side effects of these treatments and the long-term treatment itself, patients may sometimes look for other solutions. Alternative treatment modalities could be counted as other solutions. Thousands of cancer patients in the world use complementary and alternative treatment (CAT) methods in addition to medication.^[9] In a study conducted in 14 European countries including Turkey, it was found that rate of CAT usage among cancer patients was 36% and it showed a high variability between 15% and 73%.^[10] Phytochemicals obtained from spices and herbs have important anti-cancer properties.^[11] Fenugreek is made from fenugreek herb,^[12, 13] red pepper,^[14] garlic,^[15] and cumin^[16] and is reported to have an anticarcinogenic effect. It is commonly consumed in Central Anatolia. Possible anticarcinogenic effects of Fenugreek have not been studied so far.

Fenugreek is a mixture used in pastrami making in Kayseri and it is a widely consumed food among the public. Fenugreek is a member of the legume family and the plant has a wide therapeutic diversity. Pharmacological studies have shown that the extract obtained from this plant exhibits antidiabetic, antihypertensive and cholesterol-lowering effects as well as immunomodulatory effects.^[17-19] Traditionally used in diabetes, high cholesterol, wound healing and digestive system disorders, fenugreek has been reported to show apoptosis-enhancing effect and anticarcinogenic effect on breast cancer cells in a recent study.^[12, 13] Rosin et al.^[20] stated that fenugreek is capable of inducing genotoxic effects of inflammatory cells and therefore may have antitumor activity. The chemical components of fenugreek extract include polysaccharides, saponins, flavonoids, fiber, trigonellin, and choline. Polysaccharides have been described in many studies as immunomodulators that stimulate the function of macrophages. They have anticancer effects and exhibit immunoregulatory activity.^[17, 18]

Considering the therapeutic effects of these substances in the composition of fenugreek, this study was designed to investigate effect of fenugreek extract on the total antioxidant/oxidant status in Balb/C mice bearing Ehrlich ascites tumor using in vivo methods.

Methods

The compliance of animal practices performed during this study with animal rights and ethics of animal experiments was approved by Erciyes University, Local Ethics Committee of Experimental Animals dated 12.03.2014 and numbered 14/053.

Animals

Forty 8-week-old male Balb-c mice weighing between 25-

30 g were used in the study. Mice were maintained in automatically climatized rooms with 12 hours of light/dark cycles at a constant temperature of 21°C and fed with normal pellet feed. The animals were divided into 4 groups with 10 mice per group. Thirty mice were used in the experimental groups and 10 were used to generate stocks. In this study, Ehrlich Ascites Tumor (EAT) cells were used to create liquid tumors in Balb/C mice while EAT cells were grown in culture medium and the effects of fenugreek on EAT cells were investigated in vivo and in vitro. Cells to be used in the liquid tumor model and cell culture experiments were obtained by creating stock mice.

Fenugreek Extract

Fenugreek powder (500 g) was extracted three times for 24 hours at 37 °C in a shaking water bath with 70% methanol. The obtained extracts were combined and concentrated in a rotavapor (37-38 °C) under vacuum. The extract was lyophilized and stored at -20 °C until analysis. Fenugreek extract was dissolved in 5% ethanol solution before use. Extract obtained from plant materials was examined spectrophotometrically for phenolic compounds. In accordance with the doses used in other studies in the literature, 200 mg/kg and 400 mg/kg fenugreek extract was intraperitoneally administered to rats with liquid tumor and investigated for its in vivo effect.^[21, 22]

Total Phenolic Content of The Substance

The total content of phenol contained in the extract was calculated using the Folin-Ciocalteu method as gallic acid equivalent (GAE).^[23] 100 µL of sample solution and 500 µL of Folin-Ciocalteu reagent were added to a 10-ml flask containing 6 mL of distilled water. After 1 minute, 1.5 mL of 20% aqueous Na₂CO₃ was added to 10 mL of water. As a control, a non-extracting reagent mixture was used. After 2 h of incubation at 25 °C, absorbance at 760 nm was measured and compared with the GAE calibration curve. Total amount of phenolic content was calculated as GAE.

In Vivo Experiments

Cancer was not induced in the negative control (- control) group and the animals were fed with a normal diet for 7 days. Normal saline (NS) was administered intraperitoneally for 7 days (n=10). On the other hand, 0.1 ml of ascitic fluid containing 1x10⁶ EAT cells was administered intraperitoneally to the abdominal region of other mice on day 0. The mice were then divided into 3 groups and one group received 0.5 ml of NS (positive control), the other group received 200 mg/kg/day fenugreek extract and the last group received 400 mg/kg/day fenugreek extract intraperitoneally.

Serum TAS-TOS and OSI

Serum TAS was measured using an automated colorimetric measurement method developed by Erel et al.^[24] This technique measured the antioxidant effect of the sample against a potent free radical reaction. The results were expressed as $\mu\text{mol Trolox Eq/L}$. Serum TOS was measured using the automated colorimetric measurement method developed by Erel et al.,^[25] in which the color intensity, as determined by spectrophotometry, was correlated with the total amount of oxidant molecules in the sample. This assay was calibrated against hydrogen peroxide (H_2O_2) and the results were expressed in micromolar H_2O_2 equivalents per liter ($\mu\text{mol H}_2\text{O}_2 \text{ Eq/L}$).

Statistical Evaluation

Data were analyzed by IBM SPSS Statistics 22.0 package software (IBM Corp., Armonk, New York, USA). Descriptive statistics were expressed as number of units (n), percentage (%), mean \pm standard deviation ($x \pm \text{SD}$), and median (25.-75th percentile) values. The distribution of numerical variables was evaluated by the Shapiro Wilk normality test and Q-Q graphs. Time comparisons of variables with normal distribution were performed by two-way analysis of variance in repeated measures and Tukey HSD test was used for multiple comparisons. Kruskal-Wallis analysis was used for inter-group comparisons of non-normally distributed variables. Dunn test was used as multiple comparison test in case of a significant difference in Kruskal-Wallis analysis results. Wilcoxon analysis was used for intra-group comparisons of non-normally distributed variables. $P < 0.05$ was considered statistically significant in all analyses.

Results

1×10^6 EAT cells administered to the experimental groups at the beginning of the experiment caused swelling in the abdomen from day 4-5. From day 7 onwards, this swelling in

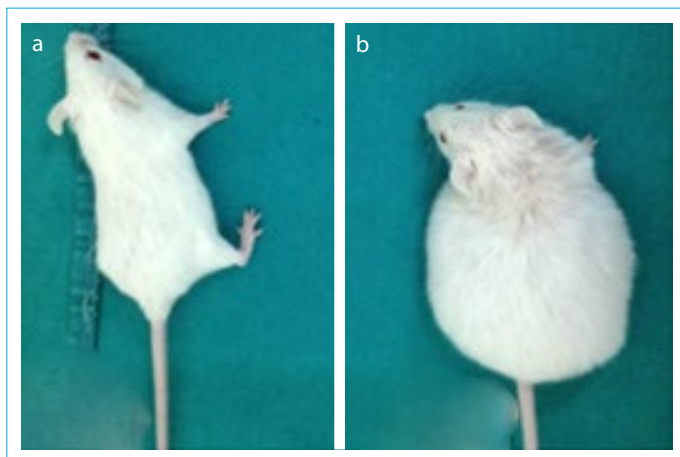


Figure 1. Morphological image of the negative control (a) and the positive control (b) animals on the seventh day.

the abdominal region had reached a level that prevented the animal from walking (Fig. 1).

When the daily weight gain of the animals in the experimental groups was examined, there was no difference in the mean weight gain of the animals in the negative control group, whereas there was an increase in the weight of the other groups.

Fenugreek Analysis Results

Phenolic content of fenugreek extract was found to be $51.832 \pm 1.632 \text{ mg GAE/g}$.

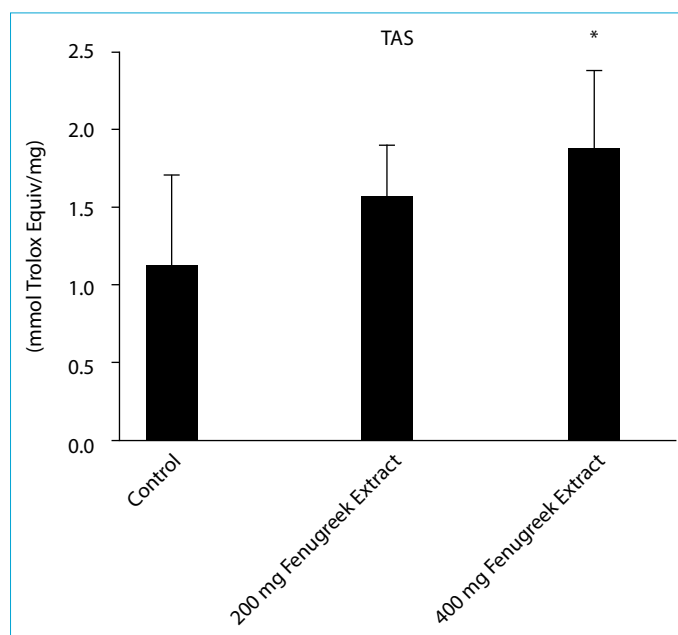


Figure 2. Serum TAS of EAT cell experiment groups.

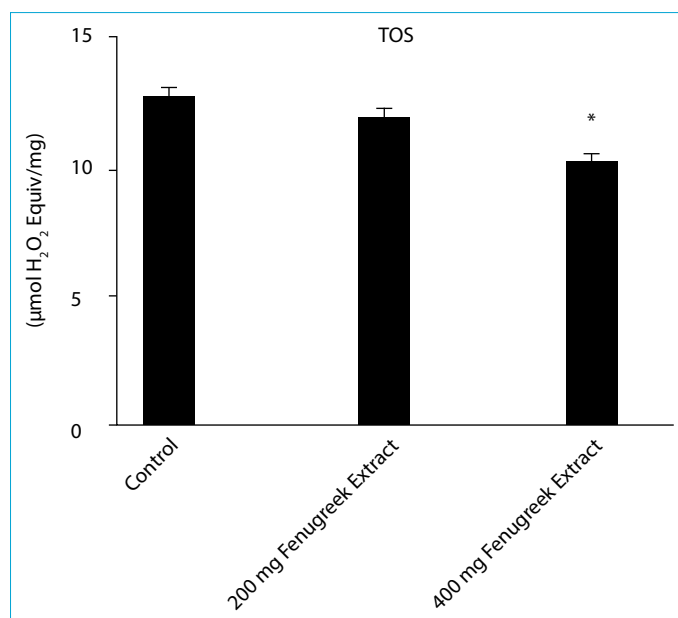


Figure 3. Serum TOS of EAT cell experiment groups.

Serum TAS-TOS-OSI Values

Serum TAS value (Fig. 2) was significantly higher whereas serum TOS value (Fig. 3) was significantly lower in the group administered with 400 mg/kg fenugreek extract compared to the group administered with 200 mg/kg fenugreek extract. OSI showing oxidative stress status was also significantly lower in the group administered with 400 mg/kg fenugreek extract compared to the positive control and the group administered with 200 mg/kg fenugreek extract (Fig. 4). Based on these results, it can be said that fenugreek extract reduces oxidative stress.

Discussion

Natural dietary compounds are used in combination with chemotherapy in cancer treatment. These diets commonly contain flavonoids and phenolic compounds. Phenolic compounds, which constitute a very important part of human diet, attract attention due to their antioxidant, anti-inflammatory and anticarcinogenic properties. There are studies showing that many plant species have anticarcinogenic effects. Therefore, it is thought that there may be a connection between the inclusion of such plants in the diet and a decrease in cancer incidence.^[26] Fenugreek is an herbal mixture containing mostly fenugreek herb in addition to lesser amounts of red pepper, garlic and cumin, and very small amounts of black pepper, clove, coriander, cinnamon, ginger and allspice that is widely used as a food item among the people in Kayseri and the surrounding region. Fenugreek mixture used in our study consisted of fenugreek herb (35%), red pepper (5%), garlic (5%), cumin

(2%), black pepper (0.5%), clove (0.5%), coriander (0.5%), cinnamon (0.5%), ginger (0.5%), and allspice (0.5%). Phenolic compounds, which are defined as secondary metabolism products of plants, constitute the most common group of substances found in plants. Studies have shown that the phenol contents of the spices that make up the fenugreek mixture are variable (13.98-33.96 mg GAE/g).^[27-34] In our study, total phenolic content of fenugreek mixture was found to be 51.832 ± 1.632 mg GAE/g and it was higher than the phenolic content of plants constituting the mixture. The high phenolic content of the fenugreek mixture may also indicate a greater likelihood of antioxidative and anti-carcinogenic effects.

Fenugreek has a broad therapeutic activity and pharmacological studies have shown that the extract obtained from this herb has antioxidant, antihypertensive, cholesterol-lowering and anti-inflammatory effects^[17-19] as well as the antioxidant effects of its components.^[35, 36] Prema et al.^[37] showed that fenugreek herb extract given to rats with Alzheimer's disease significantly reduced oxidative stress thanks to its antioxidant activity. In another study, fenugreek extract was shown to increase free radical scavenging activity against CCl₄-induced liver and kidney damage in rats and thus prevent oxidative damage.^[38] Jagadeesan et al.^[39] examined the anticancer activity of diosgenin, which is dominant in the fenugreek, in breast cancer-induced rats. They found that this saponin attenuates lipid peroxidation via enhancing antioxidant defense system in rats and thus shows anticancer activity.^[39] In a study investigating the effect of the fenugreek powder on hepatic lipid peroxidation, antioxidant status and tumor incidence in colon cancer-induced Wistar male rats, it was found that fenugreek powder given 2 g/kg body weight in addition to the diet decrease tumor incidence to 16.6%, increase the antioxidant enzyme levels and decrease the lipid peroxidation.^[40] In the present study, added fenugreek extract showed antioxidant properties by increasing serum TAS value, decreasing serum TOS value, and decreasing OSI value and it was thought to be protective against oxidative damage.

Anticarcinogenic properties of various spices in fenugreek have been investigated in various studies. In vivo and in vitro studies on various cancer types have shown that red pepper,^[41, 42] carnation^[43, 44] and allspice^[45-47] in fenugreek composition increased apoptosis in tumor cells and showed cytotoxic effect, red pepper^[48-52] and cinnamon^[53, 54] reduced metastasis and suppressed tumor growth, while coriander^[55-58] and ginger^[59, 60] showed antiproliferative and antioxidant effects. Thus, literature data shows that each of the plant extracts used in fenugreek mixture has anticarcinogenic effects, both in vivo and in vitro.

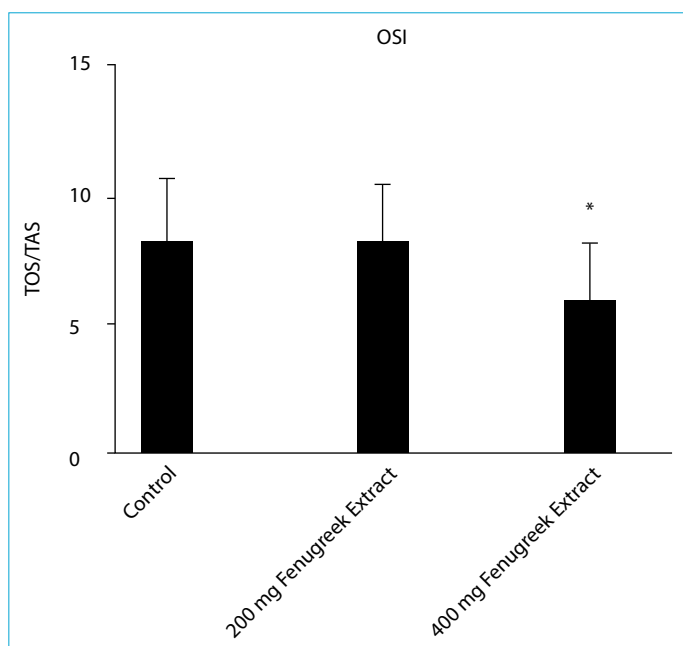


Figure 4. OSI of EAT cell experiment groups.

The anticarcinogenic effects of fenugreek have also been investigated in various studies. The effects of fenugreek extract on cancer cells have been tested in vitro in doses ranging from 10 µg/ml to 1000 µg/ml and a reduction in the number of viable cells has been reported in extracts above 250 µg/ml.^[22] Alshatwi et al.^[12] reported that fenugreek extract increased apoptosis in a dose and time dependent manner in MCF-7 cells. In another study, Amin et al.^[35] reported that 200 mg/kg fenugreek extract inhibited 7,12-dimethylbenz(a)anthracene induced breast cancer, and fenugreek oil obtained from fenugreek seeds was reported in another study to reduce cell viability in cancer cell lines (HEp-2, MCF-7, WISH).^[22] Studies have shown that fenugreek seed extract has cytotoxic effects on T-cell lymphoma, B-cell lymphoma, Thyroid Papillary carcinoma and human breast cancer (MCF7) cells and 300 µg/ml fenugreek seed extract causes significantly higher rates of apoptosis in cancer cells compared to normal cells.^[61, 62] Sur et al.^[21] tested the in vivo effects of 100 mg/kg and 200 mg/kg fenugreek seed extract on EAT cells and reported that 70% of tumor cells were inhibited compared to the control group.

In the present study, the effects of fenugreek extract on EAT cells were investigated. In vivo results showed that EAT cells administered intraperitoneally caused a rapid weight gain in the control group animals, whereas there was a delay in weight gain in the treatment groups receiving 200 mg/kg and 400 mg/kg fenugreek extract daily. Also, fenugreek extract showed antioxidant properties by increasing serum TAS value, decreasing serum TOS value and OSI value and it was thought to be protective against oxidative damage. Considering the uncontrolled proliferation of cancer cells as a result of oxidative damage, it is possible to say that fenugreek may show anticarcinogenic effects due to its antioxidant effect.

Conclusion

In conclusion, the results obtained in this study showed that fenugreek extract at the administered concentrations caused a decrease in oxidative status and has an antioxidant effect. Thus, it can be shown antitumor activity. These results are consistent with the literature and will contribute to future studies on this subject. On the other hand, we believe that the dissemination of the use of fenugreek, which is a regionally consumed food, may be useful in slowing the formation and development of cancer, one of the most important health problems of our time.

Disclosures

Ethics Committee Approval: The study was held at DEKAM with the permission of Erciyes University Experimental Animals Local Ethics Committee, Approval No. 14/053 and dated 12.03.2014.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – M.N., Ş.A.; Design – M.N., Ş.A., S.Y.; Supervision – M.N., Ş.A., S.Y.; Materials – M.N., Ş.A., S.Y.; Data collection &/or processing – M.N., Ş.A., S.Y., N.İ.; Analysis and/or interpretation – M.N., Ş.A., S.Y., N.İ.; Literature search – M.N., Ş.A., S.Y., N.İ.; Writing – M.N., Ş.A., S.Y., N.İ.; Critical review – M.N., Ş.A., S.Y., N.İ.

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