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ORIGINAL PAPER

Investigation of the effect of rhamnetin on mice injected with solid and ehrlich ascites tumor

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Abstract

Rhamnetin is a flavonoid which contained in especially clove, such as apple, tea, and onion plant. Rhamnetin has been used in cancer research due to its antitumor and antioxidant properties. In this study, effects of rhamnetin administration at different doses on ascites and solid tumors were investigated in Balb/C mice bearing EAT model that originating from rat breast adenocarcinoma. Experimental procedure: Overall, 92 Balb-c mice were used in this study. EAT cells (1×10^6 cells) that harvested from stock animals were injected to all rats via intraperitoneal and subcutaneous route. Rhamnetin (100 µg/kg–200 µg/kg) were given intraperitoneally and subcutaneously during 10 and 15 days to the animals bearing ascites tumor and solid tumor, respectively. Throughout experiments, weight changes were recorded in all groups. The maximum weight increase was observed in the control group among all groups (ascites and solid tumor groups). In the treatment groups, the least weight increase were determined in 200-µg/kg rhamnetin applied. The lowest increase in tumor volume was observed in the group that received 200-µg/kg rhamnetin (2.84) when compared to tumor control group (3.67). Result and conclusion: We determined that the number of live and dead cells in the treatment groups administered with the mean rhamnetin dose (2.5 µg/ml) was found in the count made in the EAT cell line after the incubation periods. We observed that rhamnetin plays an important role against cancer formation. We have obtained important results in our study, but detailed studies on the relationship between rhamnetin and cancer are needed.

Keywords Ehrlich ascites tumor · Rhamnetin · Apoptosis · Factor VIII

Abbreviations

µg	Microgram
µl	Microliter
cm	Centimeter
kg	Kilogram
g	Gram
mg	Milligram
h	Hour
DAB	Diaminobenzidine
DAPI	4,6-Diamidino-2-phenylindole
TdT	Terminal deoxynucleotidyl transferase
dUTP	Nonisotopic labeled nucleotides
TUNEL	*Terminal deoxynucleotidyl transferase-mediated dUTP Nick-end Labeling*
EAT	Ehrlich Ascites Tumor
PS	Physiological saline
FITCH	Fluorescein isothiocyanate
FVIII	Factor VIII
ml	Milliliter
mm^3	Cubic millimeter
NSCLC	Non-small cell lung cancer cells
PBS	Phosphate buffer saline
DNA	Deoxyribonucleic acid
W	Watt
ip	Intraperitoneal
sc	Subcutaneous

Introduction

Cancer is a disease of the genome. The main problem in the process that begins with a change in the structure of DNA is uncontrolled reproduction [1]. Cancer, which is considered one of the chronic diseases, is one of the leading health problems today due to its frequent occurrence and high Dec of deaths. In the 1970s, it was ranked fourth among the diseases

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Dec cause is known in the world and in our country, while it is currently ranked second after heart diseases [2–4]. While the diagnostic rate of cancer has been increasing continuously with the scientific studies conducted, the death rate from cancer has remained almost constant. This situation is promising that cancer is a winnable battle with scientific studies. The development of cancer long term being diagnosed at an early stage makes it easy to treat the disease. The more effective and urgent of these diagnoses will undoubtedly be possible with the increase of scientific studies [5, 6].

Recently, crucial studies have been conducted on the treatment of cancer. Among the treatments in the clinic Dec surgical removal of the tumor, chemotherapy and radiotherapy.

Chemotherapy can be used either alone or in combination [7]. But because chemotherapy has many side effects and treatments are long term, patients can go on other quests. These searches are usually carried out by herbal treatment methods, respectively. Some medicinal herbal products can be used against cancer due to the absence of serious side effects [8]. The plant that we will use for this purpose is rhamnetin, a flavonoid. Rhamnetin is also found in some plants, such as buckthorn and akdiken, which grow in the Mediterranean region, especially in the clove plant. It is known that rhamnetin has antitumoral and antioxidant properties [9]. We planned to determine the rhamnetin's antitumoral effect on Ehrlich's ascites and solid tumor for this study.

Materials and methods

This study with experimental animals was conducted in accordance with the decision No. 14/30 taken on February 12, 2014 from the Erciyes University Local Ethics Committee of the Department of Animal Experiments. Male BALB/c mice weighing 25–30 g and 8–10 weeks were used throughout the study. Eight groups were identified in the study. It was designed to have 10 mice in each group. Unlike these groups, 4 animals were used to create a stock animal group. During the study period, the mice used in the experiment were maintained at a stable temperature of 21 °C in specially set and air-conditioned place with a day cycle of 12 h and a night cycle of 12 h. The created stock mice were arranged at the initial stage in such a way to get enough EAT cells before the formation of experimental groups. Stock mice's EAT cells were used both solid and liquid tumor formation in vivo for in vitro cell culture.

Sterilization and dissolution of the rhamnetin extract

Rhamnetin (Sigma-Aldrich) was purchased as a powder and solution was prepared freshly before the experiment. For each experimental group rhamnetin extract was dissolved in methanol to provide the desired concentrations.

Stock mice formation

When creating stock animals, EAT cells were stored at − 80 °C. After the cells were thawed at standard laboratory conditions (25 °C), 0.1 ml was injected intraperitoneally (IP) into the stock animals from the joint of the abdomen and left hind leg. An acid tumor in an animal was expected to form within about 1 week. Cells in ascitic fluid from 1 × 10 °C stock animal were injected intraperitoneally into mice in PBS buffer (0.1 ml) to form a liquid tumor. To form a solid tumor, it was again applied subcutaneously (SC) to the neck areas with PBS (0.1 ml).

Formation of the experimental groups

Experimental groups *Groups of ascites tumors*: In this group, the subjects were divided into 4 groups. Cancer was not established in the negative control group. Animals were included in the study by providing 0.1 ml of physiological saline (PS) instead of acid liquid. Mice in the positive control group were given 0.1-ml acid liquid with 1×10^6 EAT cells intraperitoneally. The liquid tumor + 100 µg/kg treatment study was started by intraperitoneally administering 0.1-ml acid liquid with 1×10^6 EAT cells to mice in the rhamnetin group. 24 h after the start of the experiment, the animals were injected intraperitoneally with rhamnetin 100 µg/kg/day in 0.1 ml for 10 days. The liquid tumor + 200 µg/kg treatment study was started by intraperitoneally administering 0.1-ml acid liquid with 1×10^6 EAT cells to mice in the rhamnetin group. 24 h after the start of the experiment, animals were intraperitoneally injected with 0.1 ml of 200 µg/kg/day rhamnetin intraperitoneally for 10 days. On the 11th day in the treatment groups, intraperitoneal acid fluid was withdrawn in that day and a cell count was performed. For subjects in all groups, 11th-day animals were sedated with ketamine-xylacine (50 mg/kg, 15 mg/kg). After this procedure, intra-abdominal organs were removed and tissues were prepared for histological examinations.

Groups of solid tumors: Cancer was not created in the negative control group. Instead of acid liquid, 0.1 ml of physiological saline (PS) was administered subcutaneously from the back of the neck to the animals and 0.1 ml intraperitoneally to animals for 15 days; 24 h after the start of the experiment, saline was continued to be given physiologically. The study was started by giving 0.1 ml of acid liquid subcutaneously from the back of the neck region to the mice in the positive control group with 1×10^6 EAT cells. For solid tumor + 100 µg/kg of 0.1-ml treatment groups, acid liquid with 1×10^6 EAT cells was injected subcutaneously from the back of the neck to mice in the rhamnetin group and

the study was started. 24 h after the start of the experiment, animals were intraperitoneally injected with 0.1 ml of 100-µg/kg/day rhamnetin intraperitoneally for 15 days. For solid tumor + 200 µg/kg of 0.1-ml treatment groups, acid liquid with 1×10^6 EAT cells was administered subcutaneously from the back of the neck to mice in the rhamnetin group and the study was initiated. 24 h after the start of the experiment, animals were intraperitoneally injected with 0.1 ml of 200-µg/kg/day rhamnetin intraperitoneally for 15 days. For subjects in all experimental groups, 16th-day animals were sedated with ketamine-xylacine (50 mg/kg, 15 mg/kg). The tumor tissue formed in the back of the neck region was removed and prepared for histopathological examination. Solid tumor volumes developed in animals over 15 days were measured with an electronic compass (Tumor Volume (mm^3) = Width.^{2}XLengthX0, 52).

Immunohistochemical practices

TUNEL labeling ("Terminal deoxynucleotidyl transferase-mediated dUTP Nick-end Labeling") technique, which is the most sensitive and fastest method of apoptosis tunnel staining, was used in tumor tissue sections of the experimental and control groups (each group includes 10 mice). It is used for DNA strand breaks in apoptotic cells in situ detection. Briefly, 5-µm pieces placed on Poly-L-Lysine-coated laminates were deparaffinized in xylene. In the next step, it was added to alcohol in decreasing concentrations and incubated in PBS buffer for 5 min at standard laboratory temperature. Fragments incubated with proteinase K (15 min) were washed with pure water and then treated with hydrogen peroxide (30%) for the endogenous peroxidase activity suppression. In the next step, immunofluorescence staining was performed according to the staining technique included in the kit as given by the manufacturer ("Millipore, S7110, ApopTag In Situ Cell Death Detection Kit"). In these experimental steps, the pieces washed with PBS buffer were incubated with TdT enzyme for 60 min at 37 °C in a humid and dark environment. Fractions washed with PBS began to react again with the anti-digoxigenin conjugate at standard laboratory temperature. All incubation steps were performed in a humid environment. An immunofluorescent microscope (Olympus BX 51) was used to get images from the slides. Apoptotic cells were detected in the images obtained after 4′,6-diamidino-2-phenylindole (DAPI) and fluorescence isothiocyanate (FITCH).

Factor VIII staining

Factor VIII staining is a method used to detect increased angiogenesis in tumor tissue. Therefore, we used this technique in this study. The biotin-avidin-peroxidase technique was applied to solid tumor tissues obtained from mice for the determination expression of the factor VIII. In this study, tumor tissue sections placed on laminate with polylysine from all mice in groups were placed in trays/cassettes and incubated overnight (60 °C). It was then treated with a series of xylenes and then with decreasing degrees of alcohol, which was then rehydrated. It was washed 3 times with pure water for 2 min. Next, for antigen retrieval, 10% citrate buffer was heat exposed in a microwave oven at 600 W (5 min) and then cooled to room temperature (10 min). Sections washed with PBS buffer were treated with hydrogen peroxide (3%) for 12 min and washed once more with PBS to inhibit endogenous peroxidase activity. A staining kit (Thermo Scientific) was used in the next steps.

After the repeated washing steps, the peroxidase substrate with diaminobenzidine (DAB) (Thermo Scientific) in the kit was treated for 1.5 min to make the immunoreactivity visible. Contrast-stained sections were washed several times with gill hematoxylin and pure water. Lastly, the parts that were dehydrated with alcohol and xylene were sealed with a sealing solution (Entellan®, Merck). Samples were investigated under an Olympus BX51 microscope (Tokyo, Japan, Olympus BX51).

In vitro experimental group

The doses of rhamnetin effects of 1, 2.5, and 5 µg/ml on EAT cells were investigated on the cell culture. Rhamnetin was dissolved with methanol (1%). The cell culture medium was prepared along with 80% Dulbecco's Middle Eagle Medium, 20% fetal bovine serum, and 1-ml penicillin/streptomycin solution: 10-mg/ml streptomycin and 10,000 unit/ml penicillin. The plates with 96 sections were divided into 4 groups as follows: the tumor control group, 1-, 2.5-, and 5-µg/ml rhamnetin treatment groups in the way that there would be 24 wells in each group. In each well 104.000 EAT cells were cultured. After these steps, the different doses of rhamnetin effects were examined by performing dead and vital cell counts 3 and 24 h later.

The cell count

In the form of suspension, the MEAL cells in the medium were tube laced with indoor swimming pools and 100-ml trypan was added. 50 µl of cell solution was pipetted on the Thomas slide. After keeping the Thomas slide on the microscope for 15–20 s, the cells were counted at 40 × zoom.

Statistical analysis

Q–Q graphs and histograms were examined than Shapiro–Wilk test was applied to evaluate the normality of the data. ANOVA test was used for the comparison between the groups. Tukey test was used for multicomponent

comparisons. One-way analysis of variance (ANOVA) for continuous variables or Kruskal–Wallis test was performed to compare the differences between groups. Levene's test was used to test the homogeneity of variance. Kruskal–Wallis analysis was used for comparisons between groups. Tukey and Dunn–Bonferroni tests were used for multiple comparisons. Dunn–Bonferroni test was used for multiple comparisons. Kruskal–Wallis and ANOVA were used for apoptosis and factor VIII between groups. In the in vitro experimental groups, Kruskal–Wallis test was used for dose comparison. Analysis was performed using the IBM SPSS Statistics 22 programs. A *P*-value of less than 5% was considered statistically significant.

Results

Ascites tumor groups' body weight changes

When the animals information on the daily body weights in the groups were examined during the 10-day experimental period, it was found that there was an increase in the body weights of the rhamnetin treatment groups and tumor control group. It was observed that there were higher differences in the treatment groups compared to the tumor control group. While the animal weight on the last day in the tumor control group was 50.3 g, whereas the weight in the 100-µg/kg rhamnetin group of the treatment groups was 43.8 g, the weight in the 200-µg/kg rhamnetin group was found as 41.2 g (Table 1) ($P < 0.05$).

Ascites tumor groups histopathological results

While kidney EAT cells, spleen, and liver tissue belonging to treatment and tumor control groups are invasive toward the connective tissue capsule and the cells in question were found to be invasive toward the talked layer, with an eosinophilic cytoplasm, bulky hyperchromatic nucleus and various morphological features were detected, compaction of the stomach, small intestine, and large intestinal tissues. While EAT cells were found individually in the connective tissue capsule in the treatment groups, it was found that the tumor showed dense clustering in the control group as shown in Figs. 1 and 2, 3.

Solid tumor groups' body weight changes

The mean of the animal body weights in the treatment and control groups was measured during the entire experimental period, and on the experiment last day, the tumor control weight was 37.17 g in the group. The highest increase rate was found in the tumor control group (Table 2) ($P < 0.05$).

Table 1 Body weight results of mice in the liquid tumor group

Days	Experimental and control groups			
	Negative control	Positive control	100 µg/kg	200 µg/kg
	mean±SD (n=10)	mean±SD (n=10)	mean±SD (n=10)	mean±SD (n=10)
1	30,98 ± 2,29	31,19 ± 1,45	30,47 ± 1,83	33,42 ± 2,60
2	31,35 ± 2,26	31,77 ± 1,53	31,19 ± 2,40	33,94 ± 2,13
3	31,70 ± 2,32	32,74 ± 1,57	31,70 ± 2,60	33,33 ± 1,62
4	30,98 ± 2,29	35,24 ± 1,74	33,78 ± 2,36	34,77 ± 1,59
5	31,35 ± 2,26	37,89 ± 2,07	36,88 ± 3,81	36,26 ± 2,60
6	31,70 ± 2,32	41,11 ± 2,76	39,14 ± 5,38	38,22 ± 4,30
7	31,55 ± 2,15	43,39 ± 2,07	42,31 ± 6,81	39,50 ± 5,43
8	31,00 ± 2,00	45,43 ± 2,71	43,29 ± 6,91	39,69 ± 5,54
9	31,43 ± 2,22	47,53 ± 2,11	43,50 ± 6,83	40,06 ± 5,93
10	31,35 ± 1,90	50,30 ± 2,38	43,88 ± 6,93	41,25 ± 7,19

Solid tumor groups' tumor volume changes

Tumor size was measured from the day on which tumor volume was allowed to be measured from the skin surface in the treatment and control groups. While measurements were made from the 7th day in the tumor control group, these measurements could be started from the eighth day in the treatment groups. The tumor volumes were determined as 3.67 cm in the tumor control group, 2.93 cm^3 in 100-µg/kg rhamnetin group, and as 2.85 cm3 in 200-µg/kg rhamnetin group on the last day of the experiment. Total tumor sizes in all animals were dimensionally compared animals. When the tumor control group and treatment groups were compared, the increase in tumor size was found to be statistically significant. ($P < 0.05$).

Results of angiogenesis

A positive staining was found in the vascular endothelial cells of the treatment and control groups by immunohistochemical staining for factor VIII. (Fig. 2). The tumor tissue's necrotic regions were excluded from the evaluation. The mean vascular density in the control group was 6.033, when the vascular density between the rhamnetin treatment groups and tumor control group was examined. It was found as 1.866 in the group to which 100-µg/kg rhamnetin was applied and as 1.7000 in the group to which 200-µg/kg rhamnetin was applied in the treatment groups. The treatment groups and tumor control group were compared in terms of factor VIII expression intensity and a statistically significant difference was found between the groups. ($P < 0.05$).

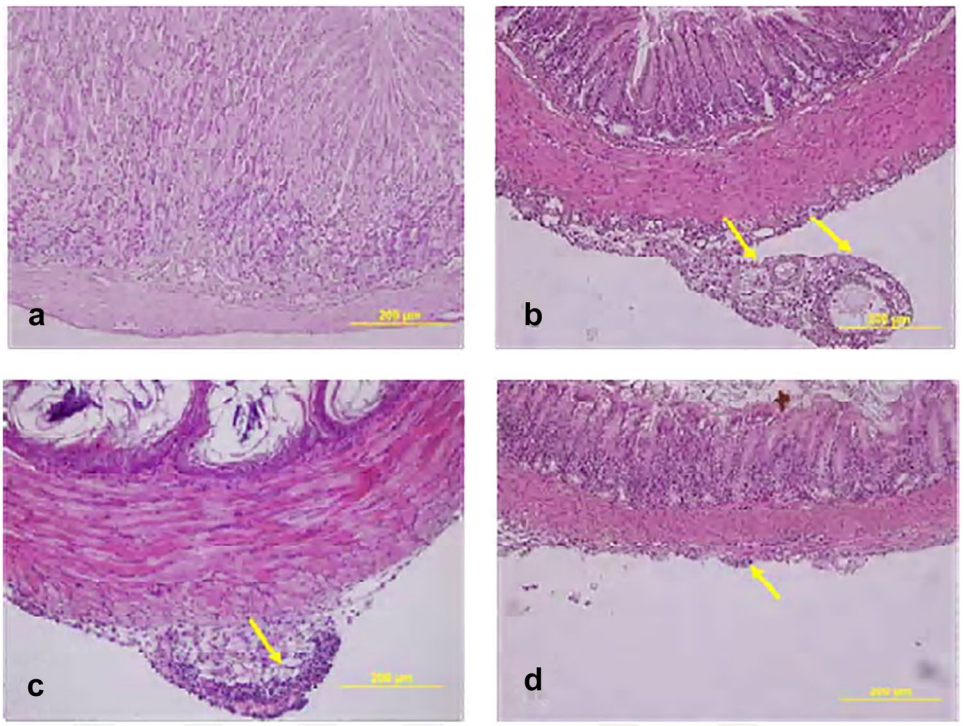

Fig. 1 Negative control of gastric tissue and histopathological findings of EAT cell-treated groups. **a** Negative control group (H&E, 20X). **b** Positive control group (H&E, 20X). **c** Tumor and group to which 100-µg/kg rhamnetin was administered (H&E, 20X). **d** Tumor and group to which 200-µg/kg rhamnetin was administered (H&E, 20X)

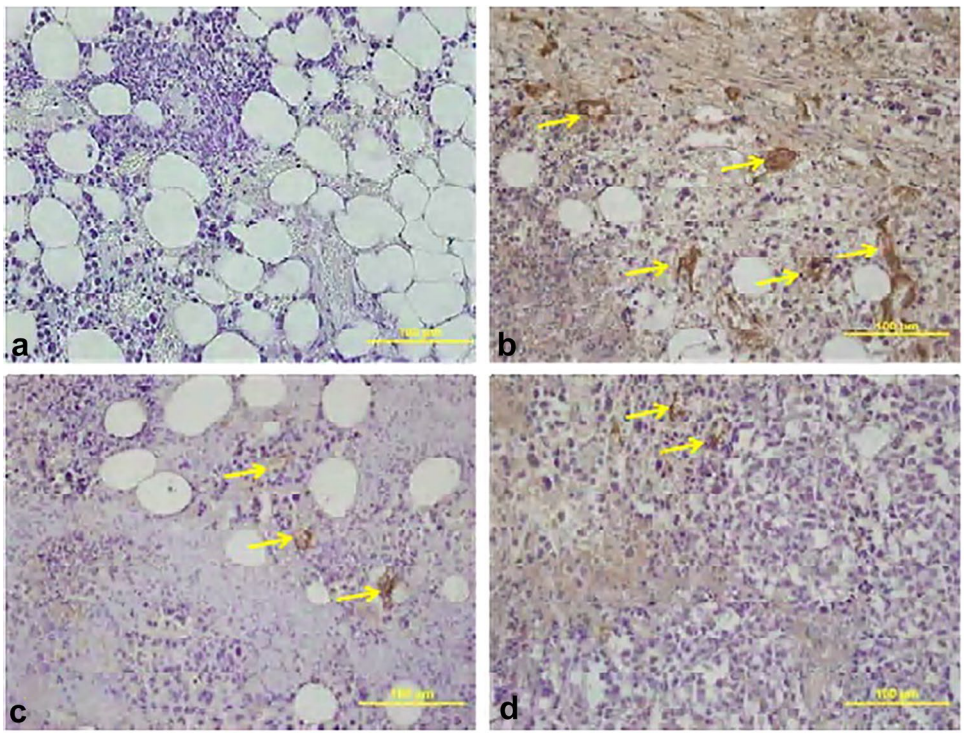

Fig. 2 **a** Negative control group, **b** Positive control group, **c** Treatment group 100-µg/kg rhamnetin, and **d** Treatment group 200-µg/kg rhamnetin expression of Factor VIII in the vascular endothelium (arrow = Factor VIII positive capillaries). 40X. Scale bar =100µm

Apoptotic results

It was shown that apoptotic cells had spread to the tumor tissue in the experimental groups in the solid tumor tissue sections (Fig. 3). In accordance with these results, a significant increase in the number of apoptotic cells was found in both treatment groups. Compared to the treatment groups and the tumor control group, numerous apoptotic cells were found in the group in which we applied higher-dose rhamnetin. When the control group was compared with the treatment

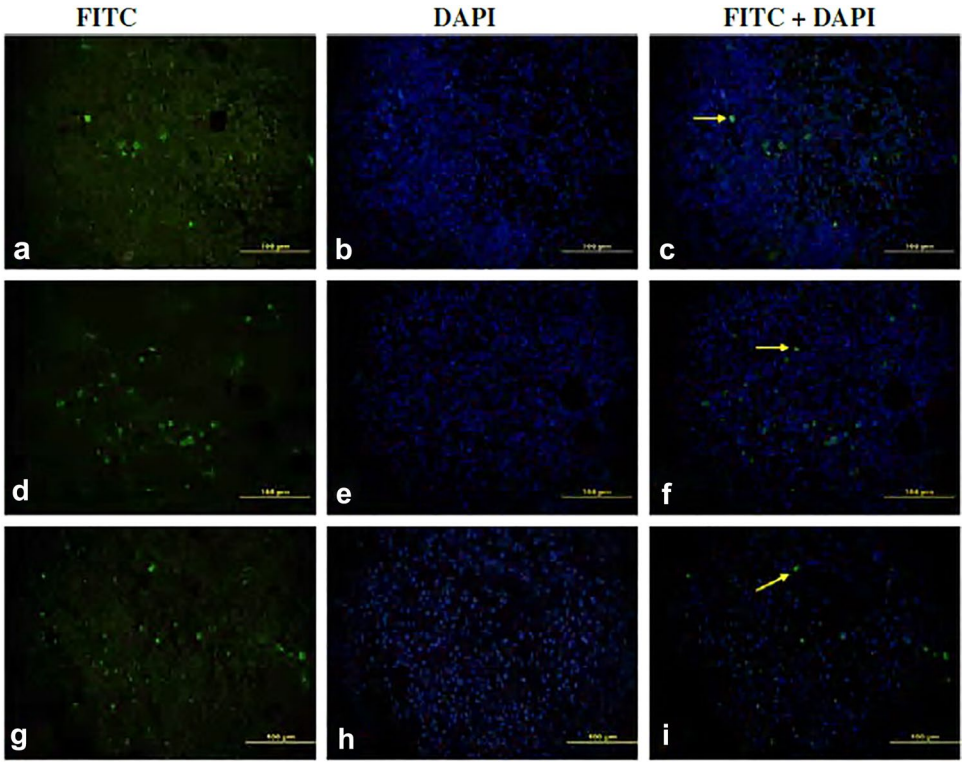

Fig. 3 **a**–**c** Apoptotic cells belonging to the control group. **d**–**f** Apoptotic cells belonging to the treatment group (100-µg/kg rhamnetin). **g**–**i** Apoptotic cells belonging to the treatment group (200-µg/kg rhamnetin). (Shape showing with arrow). Scale bar=100µm

Table 2 Body weight results of mice in the solid tumor group

Days	Negative control Mean±SD	Positive control Mean±SD	100-µg/kg rhamnetin Mean±SD	200-µg/kg rhamnetin Mean±SD
1	28,69 ± 3,25	28,84 ± 1,55	33,18 ± 1,55	31,78 ± 1,48
2	29,67 ± 2,37	28,97 ± 1,42	31,99 ± 1,49	30,23 ± 1,51
3	29,63 ± 2,47	29,43 ± 1,34	33,18 ± 1,42	31,20 ± 1,35
4	29,59 ± 1,80	31,37 ± 1,27	33,65 ± 1,23	31,68 ± 1,40
5	30,03 ± 1,58	33,70 ± 1,07	34,53 ± 2,09	32,05 ± 1,62
6	30,32 ± 1,59	34,58 ± 1,16	34,82 ± 1,58	31,14 ± 1,29
7	31,26 ± 1,84	35,31 ± 0,97	34,89 ± 1,54	32,62 ± 0,92
8	31,14 ± 1,71	35,59 ± 0,91	35,82 ± 1,98	35,87 ± 2,38
9	31,57 ± 1,47	35,68 ± 0,74	35,87 ± 2,38	32,88 ± 1,75
10	31,62 ± 1,82	36,31 ± 0,89	37,35 ± 2,48	33,61 ± 1,31
11	32,46 ± 1,68	36,38 ± 0,65	38,24 ± 3,18	34,40 ± 1,58
12	32,89 ± 1,81	36,65 ± 0,76	39,44 ± 3,41	35,73 ± 2,11
13	32,14 ± 1,78	36,15 ± 2,05	40,49 ± 2,98	36,46 ± 1,72
14	32,41 ± 1,54	37,08 ± 0,86	40,57 ± 2,68	36,78 ± 1,74
15	32,52 ± 1,48	37,17 ± 0,90	41,61 ± 2,35	37,39 ± 2,17

groups, the difference between the groups was statistically significant. ($P < 0.05$).

Rhamnetin extract applied to EAT cells in vitro effects

The effects of various concentrations of rhamnetin (1, 2.5, and 5 µg/ml) added onto the EAT cells on the number of dead and live cells in the wake of 3- and 24-h incubation periods were examined. Counting was carried out after two separate incubation periods (3–24 h), the highest number of dead cells was found in the group administered with 5-µg/ml rhamnetin, and the data recorded are given as standard deviation and the mean cell number (Table 3).

Discussion

Cancer is a major disease throughout the world [10–13]. Methods such as surgical removal, radiotherapy, immunotherapy, hormone therapy, and chemotherapy are often used for treatment [14]. It has been seen that some flavonoids are used in cancer treatment and successful results are obtained [15]. Srivastava et al. [2] found that the quercetin, which is a flavonoid, interacts directly with DNA and may be a mechanism to inducing apoptosis in both tumor tissue lines and cancer cells by activating the intrinsic pathway. Young et al. [8] investigated the preventive effect of flavonoids and they distinctly demonstrated that fisetin (a flavonoid) inhibited pancreatic, hepatic, and colorectal cancer cellular growth and proliferation by affecting multiple important signaling pathways involved in tumor cell differentiation and growth.

Tsai et al. [16] investigated the preventive effect of dietary flavonoids of quercetin and luteolin and they reported that they suppressed the highly invasive malignancy, anchorage-independent spheroid expression and formation, and Du145-III cells of some CSC markers.

A flavonoid used to cancer treat is the rhamnetin, which is most found in especially clove, such as tea, onion, and apple. For years, Rhamnetin has been used in cancer research because of its antitumoral and antioxidant properties. You jung kim [17] results suggest that rhamnetin has potent anti-melanogenic properties owing to its anti-inflammatory and antioxidant effects. Kang et al. [18] investigated the preventive effect of rhamnetin or cirsiliol, and they showed that it reduced the proliferation of non-small cell lung cancer cells (NSCLC) through the suppression of radiation-induced Notch-1 expression.

In our study, when the weights of the mice in the groups injected with EAT tumor cells as IP were examined, it was found that the weight of the treatment groups decreased significantly compared with the tumor control group. In the histopathological evaluation, invasive EAT cells with different forms of eosinophilic cytoplasm and hyperchromatic nuclei were found around the stomach, kidney, large intestine liver, small intestine and spleen tissue from the treatment and tumor-positive groups. Additionally, when high-dose rhamnetin was given to EAT-treated mice, EAT cells in the tumor control group were denser than EAT cells in the surrounding tissue. In the evaluation, it was found that the tumor size reached the dimensions that could be measured in the control group from the 7th day onward, and it increased measurably from 8th day in the treatment groups. We detected a decrease in the volume of tumor in the high-dose Rhamnetin group.

Lan et al. [19] showed that rhamnetin suppressed the viability of MCF-7 cancer cells from human breast carcinoma. When treated with various concentrations of rhamnetin for 72 or 48 h, proliferation of MCF-7 cells was determined to be significantly less than untreated cells. These findings indicated that rhamnetin treatment could suppress the proliferation of human breast cancer MCF-7 cells. The tunnel staining method for solid tumors was used in our study. The number of cells undergoing apoptosis in the tumor control group is 5455, 9925 in the 100-µg/kg rhamnetin group, and 10,416 in the 200-µg/kg rhamnetin group. Whether the agents examined in experimental studies have any anti-angiogenic effects on tumor vascularization plays an effective role in assignation the agent effect [20–22].

In our study, the FVIII expression was evaluated in solid tumor kits. The density of the vascular was 6.033 in the tumor control group, 1.866 in the treatment group administered with 100-µg/kg rhamnetin, and 1.700 in the group given 200-µg/kg rhamnetin. The decrease in expression FVIII in the treatment groups was statistically significant ($P < 0.05$).

Several phenolic compounds have received a lot of attention recently because of the possible beneficial roles they play in cancer therapy [23–28]. One of them is Rhamnetin. In this study, we conducted both in vitro and in vivo investigations on the antitumor effect of rhamnetin on EAT cells, and it was found that rhamnetin was effective in the groups that received high-dose treatment. We found that there were a lot of EAT cells in the connective tissue capsule compared to the 100-µg/kg rhamnetin group and the 200-µg/kg rhamnetin group. When the tumor volumes were examined, it was measured as 2.2 mm^3 in the 100-µg/kg rhamnetin treatment group, 2.1 mm^3 in the other group of treatment treated with 200-µg/kg rhamnetin, and 2.9 mm^3 in the tumor control group. The rhamnetin treatment groups were compared with the tumor control group. There was a statistically significant difference between the groups using the Tunnel Staining method and the group using 200-µg/kg rhamnetin ($P < 0.05$).

Table 3 Average live and dead cell values calculated in in vitro experimental groups

Group	Vital cell (3 h) Mean±SD	Dead cell (3 h) Mean±SD	Vital cell (24 h) Mean±SD	Dead cell (24 h) Mean±SD
Tumor control group	5.53 ± 0.7	2.86 ± 2.7	5.79 ± 0.2	5.63 ± 0.2
EAT cell + 1-µg/ml rhamnetin	5.61 ± 0.2	2.82 ± 2.2	5.81 ± 0.2	5.79 ± 0.2
EAT cell + 2.5-µg/ml rhamnetin	5.45 ± 0.2	2.19 ± 2.5	5.70 ± 0.1	5.62 ± 0.2
EAT cell + 5-µg/ml rhamnetin	5.45 ± 0.2	2.19 ± 2.5	5.73 ± 0.2	5.69 ± 0.2

Conclusion

As a result of this study, we determined that the number of live and dead cells in the treatment groups administered with the mean rhamnetin dose (2.5 µg/ml) was found in the count made in the EAT cell line after the incubation periods. These differences were found to be statistically significant ($P < 0.05$). We observed that rhamnetin plays an important role against cancer formation. We have obtained important results in our study, but detailed studies on the relationship between rhamnetin and cancer are needed. Because of these studies, good therapeutic approaches can be developed to make cancer and its management more accurate.

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Data availability Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

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