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The Antitumor Effect of Rhamnetin on the Heart

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Abstract: Rhamnetin has a wide usageduetoits anti-inflammatory, anti-carcinogenicand anti-oxidantproperties. In this study, the effects of different doses of Rhamnetin on ascitetumor were investigated in the Ehrlich Assit Tumor (EAT) model originating from mouse breast adenocarcinoma and developed in Balb / c mice.

Inthisstudy 10Balb / c mice were used each group, six of them were in stock animals and the others were in ascitestumor groups. EAT cells ($1x10^6$ EAT cells) taken from all stock mice were injected intraperitoneally. 200 µg /kg rhamnetin was administered intraperitoneally to the treatment groups of animals with ascites tumor for 10 days. At theend of the experiment, the animals were decapitated and the hearts of the animals were removed and determined orhistological evaluation.

The histological results of hearttissues of ascitestumor-induced animals were observed to be less in the 200 µg / kg rhamnetin treated group compared to the tumor control group than EAT cells ($p < 0.05$).

It has beenshown in our study that rhamnetin has antitumoral effect on ascitetumor development with EAT cells.

Keywords: EAT, rhamnetin, cell, anti-inflammatory.

1. Introduction

Worldwide, more than 6 million people die each year from cancer, one of the most common deadly diseases today. Chemotherapy, radiotherapy and complementary medicine are used in cancer treatment (1).

It is very important to diagnose cancer in the early stages and its treatment is facilitated. Cancer development can be prevented with early intervention. Cancer is diagnosed by taking biopsy in suspicious cases with laboratory tests and Computed Tomography (CT) imaging used in diagnosis (2).

Various treatments are applied to the type of cancer diagnosed. Surgery, chemotherapy, and radiotherapy are common methods used in treatment. In surgical methods, cancerous tissues surgically removed, and in radiotherapy treatment, they are burned with specific radiation to cancer cells. In chemotherapy, it is aimed to kill cancer cells with toxic drugs and to eliminate the mechanisms that allow these cells to divide. Besides these treatments, it is used in alternative therapy. This alternative treatment is widely used as herbal extracts (3).

The antitumor effects of herbal extracts have been tested on many cancer models, one of which is the EAT model. After EAT first emerged as spontaneous mammary adenocarcinoma in female mouse, tumor fragments were transformed into experimental tumors by subcutaneous transplantation from mouse to mouse (4).

After ward the growing form of the liquid tumor in the abdomen of mice was obtained, and the tumor was named EAT, since acidic fluid was formed in addition to cells in the peritoneum. Flavonoids are a class of exclusive metabolites from plants, characterized by the flavan nucleus. These compounds are commonly found in the leaves, seeds, bark and flowers of plants.

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Human intake is estimated to range from 26 mg to 1 g per day (5). This substance is especially obtained from the clove plant. Clove is from the Myrtaceae family and includes blueberries, clove, eucalyptus in the family (6).

The rhamnetin we used in our study is in the group of flavonoids. This substance is especially obtained from the clove plant. Rhamnetin, a powerful antioxidant, appears in the literature as an anticancer.

In our study, the anticancer effects of clove-derived rhamnetin substance on mice were investigated in terms of histopathology.

2. Material and Methods

Our study was supported by the Scientific Research Projects Unit of Erciyes University. During the study period, the male mice of BALB/c type, which were 8-10 week-old and weighed 25-30 gr, were used. While 2 groups were formed in a way that there would be 10 mice in each group, 6 animals were used to form a group of stock animals apart from the groups in question. During the experiment, the mice were kept in a specially prepared room with automatic conditioning at 21 0C, 12 hours of daylight and 12 hours of night cycle. While creating groups for our study, stock mice were first arranged to obtain a sufficient amount of EAT cells.

2.1 Dissolition and Sterilization of the Rhamnetin

5mg of rhamnetine obtained from Sigma Aldrich was dissolved with 1% methanol and diluted with distilled water to become a solution. A total of 0.5ml of methanol was used in the dissolution process. Then the solution was completed to 10 ml with distilled water. After dissolution, the solution was sterilized through 0.45 micrometer filters. The desired doses of rhamnetine (200µg / kg) were drawn from the sterilized solution and injected into the experimental groups.

2.2 Formation of the Stock Mice

EAT cells kept at -80 °C were used to create stock animals. Stock cells were thawed at room temperature and administered intraperitoneally to the stock animals in 0.1 ml. Ascites tumor was expect to ocur within one week in the stock animal. $1x10^6$ EAT cells in ascitic fluid drawn from the stock animal with an injector were administered to mice 0.1 ml Phosphate Buffered Saline (PBS) as intraperiton.

2.3 Forming the Experimental Groups

Group 1. Positive Control Group (n=10) Themiceincluded in thisgroupwereadministered $1x10^6$ EAT cellthroughthe intraperitoneally(IP) way on the 1st day. Startingfromthe 1st dayonwards, 0.1 ml of PBS wasinjectedtothemicethroughthe(IP) wayfor 10 days.

Group 2. Treatment Group (200 µg /kg Rhamnetin) (n=10) Themiceincluded in this group were administered $1x10^6$ EAT cells IP on the 1st day. Starting from the 1st day onwards, 200 µg /kg/day rhamnetin was injected to the mice through the IP wayfor 10 days.

On the 10th day, all mice were sacrificed under anesthesia (ketamine-xylazine). General anesthesia was administered with ketamine and xylazine.

2.4 Histologic Analysis

Heart tissues removed from experimental groups were fixed in 10% formalin for 24 hours for routine paraffin embedding. Then samples were dehydrated through a graded series of ethanol, cleared with xylene and finally embedded in paraffin. After then, paraffin sections at 5µm thickness were stained with Harris Hematoxylin and Eosin (H&E) examined under light microscopy (Olympus BX53) for histopathologic evaluation (7).

2.5 Immunohistochemical Analysis

The Avidin-Biotin-peroxidase method was used to detect immunoreactivity of factor VIII (8). Briefly, after deparaffinization of 5µm sections, citrate buffer (pH: 6.0) was used for antigen retrieval. Then the slides were placed in %3 hydrogen peroxide in

methanol to block endogenous peroxidase. Ultra V block was applied to block non-specific staining. After removing blocking solution, the slides were incubated with primary antibodies factor VIII (BiossInc; BS10048R) overnight at 4°C. Biotinylated secondary streptavidin-HRP(horseradish peroxidase) and DAB (3,3'-diaminobenzidine)chromogens were applied respectively and then the slides were counterstained with Gill Hematoxylin, dehydrated, mounted in entellan (9, 10). The sections were examined by Olympus BX53 light microscopy. The evaluation of the immunoreactivity levels was performed by ImageJ program. For each slide, 10 different areas were evaluated from each group.

2.6Statistical Analysis

The Levene test was used to test the variance homogeneity. To compare the difference among groups, either one-way analysis of variance (ANOVA) or Kruskal Wallis test were applied for continuous variables. The Tukey test was used in multicomponent comparisons. ANOVA was used in the comparison between groups. The Kruskal Wallis analysis was used in the intergroup comparisons. For factor VIII, Kruskal Wallis and ANOVA was used. The analyses were conducted using IBM SPSS Statistics (Statistical Package for the Social Sciences) 23 program. P- value less than 5% was considered as statistically significant.

3. Results

As shown in Figure 1, in the light microscopic examination of the heart samples of the positive control group showed more mild hemorrhage was observed between muscle fibers compared to the group of rhamnetin 200 µg. In the heart sections of both groups, eosinophilically stained apoptotic cells were detected. (Fig. 1).

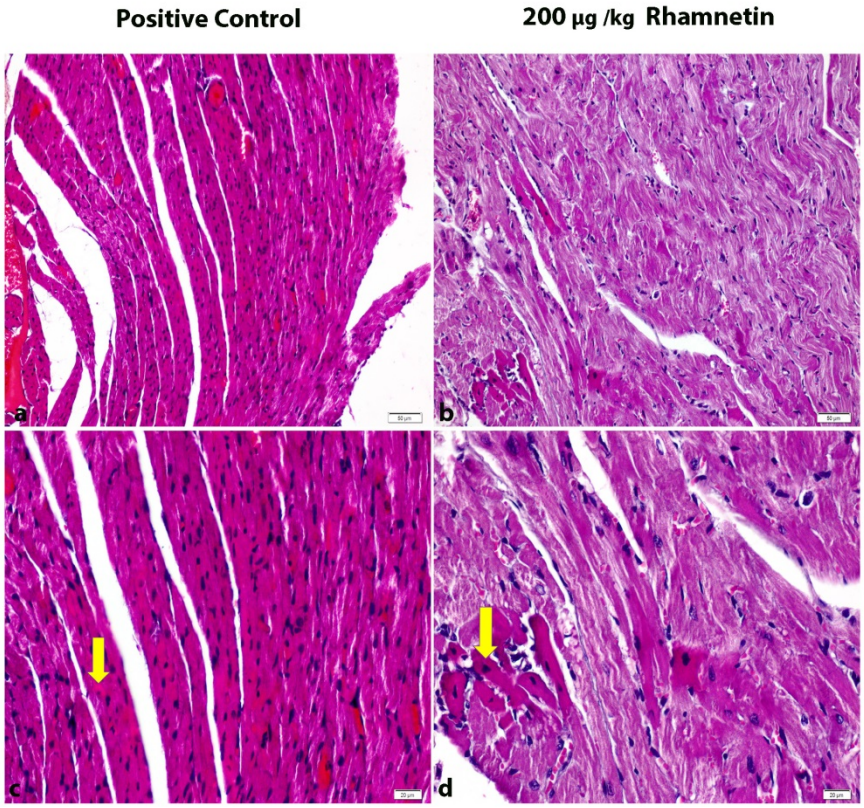

Fig. 1 H&E images (arrow: apoptotic cell) of heart tissues belonging to control (a, c) and 200 µg Rhamnetin (b, d) groups. magnification a and b 20X, bar = 50 µm; magnification c and d is 40X, bar = 20 µm.

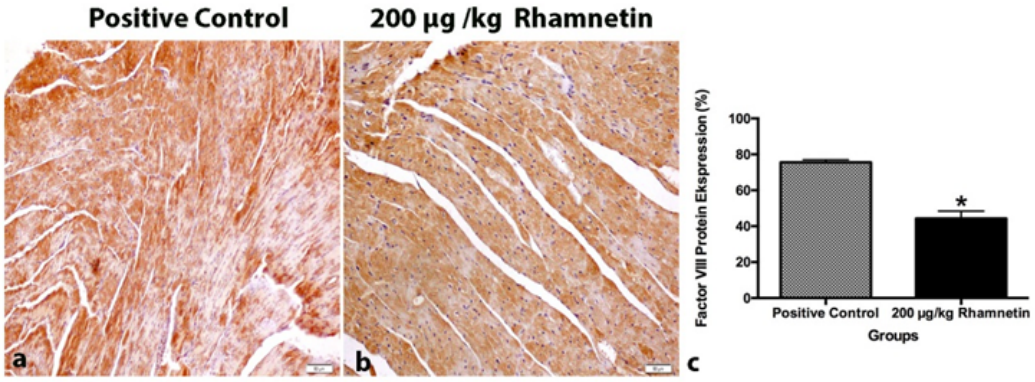

Fig. 2 Immunestaining of Factor VIII in hearttissues of control (a), 200 µg Rhamnetin (b) experimentalgroups. B. Histopathologicalscoredatashown in thehistogramgrapharemean. Expressed as ±SD. Independentsample t test wasapplied (* P <0.05 controlgroup; P <0.05 200 µg Rhamnetingroup).

As shown in Figure 2 Factor VIII expressionwasobserved in thesarcoplasms of thecardiacmusclefibersbelongingtobothexperimentalgroups. Accordingtoimage j analysis, immunoreactivity of Factor VIII decreased in 200 µg Rhamnetincomparedtothepositivecontrolgroups(Fig. 2).

4. Discussion

Cancer is an important health problem worldwide. In the treatment, besides chemotherapy, radiotherapy and surgical intervention, alternative medicine was sought some herbs have been useful in cancer treatment shown in previous studies. One of these plants is the rhamnetin found in the clove plant. Rhamnetin is a flavonoid with antioxidant, anti-inflammatory and anti-carcinogenic effects (11).

In the fight against cancer, turmeric are four popular herbs commonly used by cancer patients: green tea, ginger, ashwagandha and reishi mushroom. Herbal products are considered "natural" and "safe" compared to invasive treatments (12).

Before the development of modern pharmacy, herbal therapies were widely used for diseases. Nowadays, it is often seen that patients undergoing cancer treatment also take supplements as herbal treatment (13, 14). In addition, one third of cancer survivors stated that they used herbal therapy a lot (15).

Turmeric, one of the herbs frequently used in the fight against cancer, originates in South Asia and is used in medicine to improve circulation and digestion. The turmeric plant, whose active ingredient is curcumin, is helpful in alleviating the negative effects of arthritis and cancer treatments. (16).

Asian green tea is of Asian origin and its leaves are used in tea making. It is used to prevent and treat hyperglycemia, hypertension, cancer. Green tea extract prevents the formation of the pre-cancerous polyp and induces apoptosis in breast cancer cells, thus showing a chemopreventive effect (17).

Ginger is often used in the common cold in Asian and Arab countries. Clinical studies appear to have a preventive effect on nausea and vomiting. Its effectiveness in preventing chemotherapy-induced nausea has been promising (18, 19).

The reason why EAT cells are preferred in cancer studies is the high rate of transplantation. The retention rate in Balb / C rats varies between 90-100%. In this mouse race, this rate is 90% in females, while it is 100% in male mice.(6).

Studies have shown that the number of EAT cells injected into mice is applied differently in the literature. Patel (20) ve Joseph (21) stated in their studies thet 1x106 cells were sufficient. Patra (22) stated thet it was $2x10^6$. Hanafy (23) stated it as 2,5x106.

Jia et al. first reported that ramnetin induced sensitivity to anti-tumor drugs in Hepatocellular carcinoma through in vitro or in vivo models. In this study, they noted that ramnetin could alleviate the multidrug resistance process and guide further development of new therapies to benefit hepatocellular carcinoma patients (24).

Oak et al. reported that ramnetin acts as a powerful antioxidant agent, reduces intracellular persistent oxidative stress levels, increases Klotho protein expression, induces apoptosis in prostate cancer cells, and may have an important role in the inhibition of prostate cancer progression (25).

Shukla et al. stated that ramnetin acts on cell cycle regulation and potential molecular targets of survival to elicit anticancer effects in prostate cancer cells (26).

The effect of plant extracts in cancer treatment is being investigated experimentally. Some plants/herbs have been beneficial in the treatment of cancer, which has been shown in previously conducted studies.

Commonly used in Chinese society, Arctiumlappa is used as a medicinal plant. This herb appears to be effective in acute inflammation and melanoma progression in mouse experimental models (27).

Yu et al. In experimentally induced acute pancreatitis in mice, it was observed that curcumin (50 mg / kg / day), which is used herbally, decreased while the damage to the pancreas increased, the serum amylase level was increased (28).

Researchers examining tissues in the abdominal cavity in mice injected with EAT cells abdominally reported that cancer cells usually accumulate in the capsules of organs (29).

5. Conclusion

The reduction of Factor VIII with rhamnetin administration, which proved to be a potent regulator of angiogenesis tumor growth and metastasis, suggests that rhamnetin could be used as a potential therapeutic drug. Further studies are planned on this subject.

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