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Evaluation of the Anticarcinogenic Effect of White Radish Extract (*Raphanus sativus var. Longipinnatus)* on *In Vitro* Ehrlich Ascites Tumor Cells

Beyaz Turp Ekstraktının (*Raphanus sativus var. Longipinnatus*) *In Vitro* Ehrlich Asit Tümör Hücreleri Üzerinde Antikanserojenik Etkisinin Değerlendirilmesi

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ABSTRACT

In this research, it was aimed to research the time and different dose effects of white radish extract (*Raphanus sativus var. Longipinnatus*) on Ehrlich Ascites Tumor (EAT) cell lines. EAT cell line was used in the study. EAT cells were treated white radish extract (*Raphanus sativus var. Longipinnatus*) at 37°C and 5% CO_2 for differing periods (24 and 48 hours) and doses (100-200 and 300 µg/ml white radish extract). At the end of the incubation duration, Argyrophilic nucleolar organizing region (AgNOR) protein condition of EAT cells were investigated.It was determined among the control and 300 µg/ml Raphanus sativus extract group the significant differences for mean AgNOR number and TAA/NA (Total AgNOR area/Total nuclear area) in 48 hours period. It was detected also between the 100 and 300 µg/ml Raphanus sativus extract groups for AgNOR number and TAA/NA in 48 hours incubation ($p<0,05$). This study demonstrated that Raphanus sativus had a important role against cancer cells. Also, both AgNOR values mit be used as biomarkers for identification of the most true therapeutic dose option for cancer and it has been shown that suitable ingestion of Raphanus sativus can be effective in avoid cancer development and slowing its spreading.

Keywords: AgNOR, Cancer cell line, EAT, *Raphanus sativus L.*

ÖZ

Bu çalışmada beyaz turp (*Raphanus sativus var. Longipinnatus*) ekstresinin Ehrlich Asit Tümör (EAT) hücre hattı üzerinde zaman ve doza bağlı etkilerinin incelenmesi amaçlanmıştır. Çalışmada EAT hücre hattı kullanıldı. EAT hücreleri, değişen sürelerde (24 ve 48 saat) ve dozlarda (100-200 ve 300 µg/ml beyaz turp ekstresi) 37°C ve %5 CO^2'de beyaz turp ekstresine (*Raphanus sativus var. Longipinnatus*) maruz bırakıldı. İnkübasyon süresinin sonunda EAT hücrelerinin argyrophilic nükleolar organize bölge (AgNOR) protein durumu incelendi. 48 saatlik inkübasyonda ortalama AgNOR sayısı ve TAA/NA (Total AgNOR alanı/Total çekirdek alanı) açısından kontrol ve 300 µg/ml *Raphanus sativus* ekstresi grubu arasında anlamlı fark bulundu. Ayrıca 48 saatlik inkübasyonda AgNOR sayısı ve TAA/NA için 100 ve 300 µg/ml *Raphanus sativus* ekstre gruplarında istatistiksel olarak fark görüldü ($p<0,05$). Mevcut çalışmamızda, *Raphanus sativus*'un kanser gelişimine karşı çok önemli bir işlevi olduğunu göstermiştir. Ayrıca, AgNOR kanser için en güvenilir terapötik doz seçiminin saptanmasında biyobelirteç olarak kullanılmaktadır. *Raphanus sativus*'un doğru tüketiminin kanser oluşumunu önlemede ve ilerlemesini yavaşlatmada etkili olabileceği gösterilmiştir.

Anahtar kelimeler: AgNOR, Kanser hücre hattı, EAT, *Raphanus sativus L.*

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INTRODUCTION

Surgery, chemotherapy, radiotherapy and hormone therapys are mainly utilized the cure of cancer, which is one of the most important threats to human health. Due to some of the side effects of these treatments and the long duration of the treatments, sometimes patients are they can enter into quests. Sometimes, the practice and use of alternative treatments has traditional value and history. The Chinese herb has been used medicinally for therapeutic purposes since 770 BC.[1]

Proliferation of cancer cells can be assessed by argyrophilic nucleolar organized region staining (AgNORs). (NOR) regions can be stained with silver (Ag) and therefore these regions, called AgNOR, are used to evaluate the increase in cancer cells.[2] AgNOR staining, which stands for silver-staining nucleolar organizer regions, is a technique used to detect and quantify the number and size of nucleoli in a cell. Nucleoli are regions within the nucleus of a cell where ribosomal RNA synthesis and assembly occur. AgNOR staining can provide information about cell proliferation and can be particularly useful in cancer research.[3]

In cell culture, AgNOR staining can be performed on cultured cells to examine their nucleolar characteristics. The procedure involves treating the cells with a silver solution that specifically stains the nucleolar proteins associated with ribosomal RNA synthesis. This staining results in the appearance of dark, discrete dots or patches within the nucleoli.[4] Not eating enough fruits and vegetables in general has been connect an increased danger of stomach cancer for years., worldwide association focusing on epidemiological research of gastric cancer, reports that people who consume a lot of fruit have a much lower risk of developing gastric cancer.[5-7]

The consumption of leaves and radish sprouts along with the root of the White Radish (Raphanus sativus L.), which is one of the most eated vegetables of the Brassicaeae family, is increasing. While radishes typically have a peppery flavor, white radishes tend to be milder and slightly sweet. White radishes are most commonly found in Asian cuisines, particularly in Japanese, Chinese, and Korean dishes. From a nutritional standpoint, white radishes are low in calories and high in dietary fiber. They contain vitamin C, potassium, folate, flavonoids and other beneficial compounds. Like other radishes, they are known for their potential digestive and detoxifying properties, although scientific evidence in these areas is limited. Sulfur and nitrogen-containing glycosides are organic compounds derived from glucose and amino acid. In previous studies on glucosinolates, which have benefits for human health, it has been reported that dietary consumption of these substances has positive effects against the danger of carcinogenesis, antioxidant, antitumor and oxidative stress in the cardiovascular system.[8-13]

The aim of this study is to evaluate the antitumoral effect of Raphanus Sativus L. on EAT cells in vitro by the AgNOR method.

MATERIAL AND METHODS

After EAT cells were removed from -80, they were thawed and washed with medium. Cell culture application was performed as in the previous study by Ateş et al. [14]

As a result of the experiment for AgNOR staining, EAT cells cultured with 100,200 and 300 µg/ml Raphanus Sativus L. extract were spread on a slide and dried at room temperature for approximately 30 minutes. The silver staining solution obtained from 50% $AgNO_3$ and gelatinous formic acidmixture was dripped 3-4 drops on the preparations with a staining pipette and covered with a coverslip. Then the lid of the petri dish was quickly closed, wrapped with aluminum foil in such a way that it would not get any light,and left in an oven at 37ºC for 15 minutes. At the end of the15th minute, the preparations that were removed from the oven were washed with distilled water until the coverslips fell off. Photographs of the preparations covered with Entellan were taken

under a light microscope (Leica DM3000) at a magnification of 100 (Imaging Color 12 BIT,Made in Canada).

Analyzes were performed in the ImageJ program (ImageJ version 1.47t, National Institutesof Health, Bethesda, Maryland, USA). By evaluating cell nuclei, both the total AgNOR area (TAA/NA) and theaverage AgNOR number per nuclear area were calculated using the "freehand selections" tools. Ethics committee permission is not required for this study.

Statistical analyses

Graphpad Prism version 9.0 program (for Mac, GraphPad Software, La Jolla, California, USA) was used for all statistical analysis. With the Shapiro-Wilk test, it was observed that the data belonging to the groups were normally distributed. Since there is a normal distribution in the data, one-way ANOVA test in multiple comparisons between groups; Post-hoc Tukey test was used for paired group comparison. Statistically, a value of $p < 0.05$ was considered significant.

Ethics Committee approval

The article does not require ethics committee permission.

Funding sources

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Conflict of Interest

The authors declare no conflict of interest to disclose

RESULTS AND DISCUSSION

24 hours incubation, mean AgNOR number value was statistically significant ($p < 0.05$) in 300 µg/ml Raphanus Sativus L. extract groups compared to the control and other extract groups (Fig. 1).

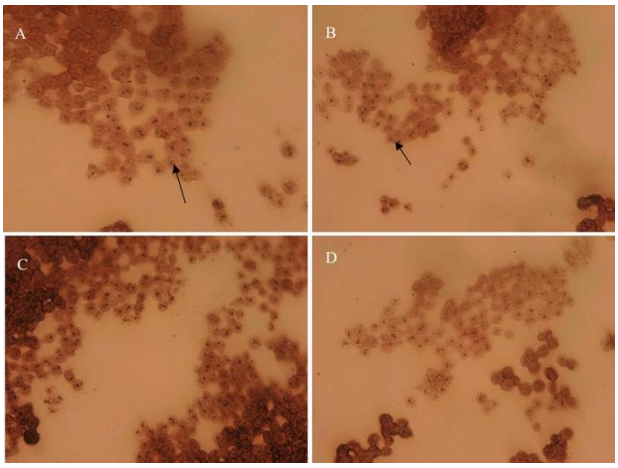

Fig.1 AgNOR Staining Images After 24 Hours A) Control group B)100 µg/ml *Raphanus Sativus L.* extract group C) 200 µg/ml *Raphanus Sativus L.* extract group D) 300 µg/ml *Raphanus Sativus L.* extract group.

The mean 48 hours incubation AgNOR number was significantly in the 300 µg/ml *Raphanus Sativus L. extract* groups compared to the control group ($p < 0.05$). Additionally 100 and 300 µg/ml *Raphanus Sativus L. extract* groups statistically significant (Fig. 2 and Table 1)

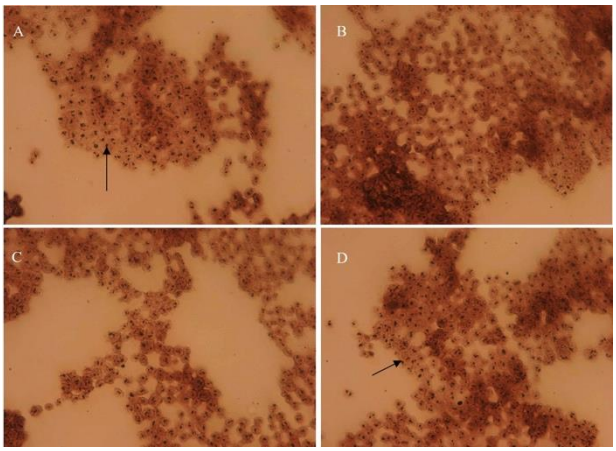

Fig.2 AgNOR Staining Images After 48 Hours A) Control group B)100 µg/ml *Raphanus Sativus L.* extract group C) 200 µg/ml *Raphanus Sativus L.* extract group D) 300 µg/ml *Raphanus Sativus L.* extract group.

After 24 hours incubation, TAA/NA ratio was statistically significant between control and 300 µg/ml *Raphanus Sativus L.* extract group. Additionally 100 -300 µg/ml and 200-300 µg/ml *Raphanus Sativus L.* extract groups statistically significant (($p < 0.05$).

At the end of 48 hours incubation, the TAA/NA ratio was statistically significant ($p < 0.05$) in 300 µg/ml *Raphanus Sativus L.* extract groups compared to the control group and between 100 -300 µg/ml extract groups (Fig. 3 and Table 2).

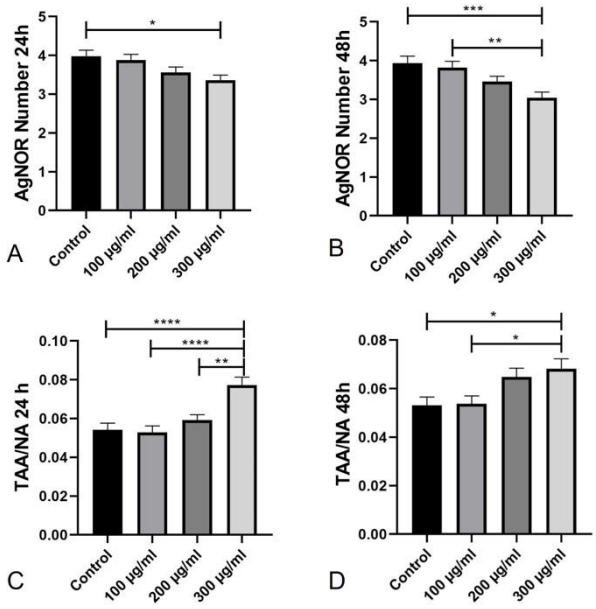

Fig. 3 Comparison of AgNOR Number (A And B) and TAA/NA Ratio (C And D) Between Groups After 24 And 48 Hours of Incubation. * statistically significant

Table1. Mean Agnor Number After 24 and 48 Hours of Incubation

H/G	Control	100 µg/ml	200 µg/ml	300 µg/ml	*p*
24 H	3,98±1,09[a]	3,88±1,04[a]	3,56±1,01[a]	3,36±0,94[b]	<0.001
48 H	3,94±1,23[a]	3,82±1,14[a]	3,46±0,97[ab]	3,04±1,07[b]	<0.001

p <0.05 was considered statistically significant. Data are expressed as mean ± SD (Standard deviation). There is no statistically significant difference between the groups containing the same letter (p> 0.05). 100, 200 and 300 µg / ml: ml *Raphanus Sativus L. extract* groups. **AgNOR:** Argyrophilic nucleolar organizer region.**H**: hour, **G**:group

Table2. TAA / NA Value at The End of 24 and 48 Hours of Incubation.

H/G	Control	100 µg/ml	200 µg/ml	300 µg/ml	*p*
24 H	0,05±0,02[a]	0,05±0,02[a]	0,05±0,02[a]	0,07±0,02[b]	<0.001
48 H	0,05±0,02[a]	0,05±0,02[a]	0,06±0,03[a]	0,06±0,03[b]	<0.001

p <0.05 was considered statistically significant. Data are expressed as mean ± SD (Standard deviation). There is no statistically significant difference between the groups containing the same letter (p> 0.05).; 100, 200 and 300 µg / ml: ml *Raphanus Sativus L. extract*. **TAA/NA:** Total AgNOR area (TAA)/Total nuclear area (NA) ratio.**H**:Hour, **G**:group

For assesment the clinical course and aggressiveness of tumors use AgNORs to cellular proliferation markers. This method was applied to various materials such as paraffin-embedded human pathological tissues. AgNOR staining is a representative method for the detection of NORs in tissue sections and provides convenience estimation of tumor activity.[15]

Jajoda et al. to measure the role of brush cytology in the screening of oral lesions with malignant suspicion and compare it with histopathology in north-eastern India used AgNOR staining method.[16] Furusawa et al.,studied to AgNOR staining method in the cytology of smears in dogs and cats.[17]

Srivastava et al. with the tumor marker potential of AgNOR pleomorphism counts had

assessed correlation HPV positivity.[18] Ferreira et al. evaluated the cytopathological changes in the epithelial cells of the oral mucosa of patients with oral lichen planus (OLP) by comparing them with patients without OLP using AgNOR staining methods.[19]

Jinza et al. performed AgNORs applied to cell imprint preparations in bladder cancer and stated that AgNORs of cell imprint preparations is an objective method in human bladder cancer.[20]

Rao et al. determined the diagnostic accuracy of rapid AgNOR in brush biopsies of potentially malignant lesions for early sensing of oral cancer.[21]Elangovan et al. evaluated the importance of various AgNOR parameters and their role in differentiating hyperplastic, premalignant and malignant oral lesions.[22]Tomazelli et al. in oral squamous cell carcinoma (OSCC) investigated the proliferative activity, using AgNORs quantification proteins, in low- and high-risk oral epithelial dysplasia, OSCC, and nondysplastic epithelium (inflammatory fibrous hyperplasia).[23] Studies have shown that Raphanus sativus extract reduces the viability of the breast cancer cell line MDA-MB-231 cells in the dose range of different concentrations (100, 200, or 300 µg/mL) and especilly in 200 and 300 µg/mL notably reduced cell proliferation after 48 hours incubation.[24]

Yılmaz et al. studied the effectiveness of curcumin (10 µg/ml, 20 µg/ml and 30 µg/ml) on EAT cells in 3 and 24 hour incubation periods. As a result, they found a significant difference at the dose of 10 µg/ml.[25]

Yılmaz et al. examined the effects of rutin, a flavonoid found in fruits and vegetables, on mice in which solid tumors were formed with EAT cells, using the AgNOR staining method and found a significant decrease in the rutin groups compared to the control group.[26]

In this study, the effects of radish extract on the proliferation of EAT cells were investigated and it was observed that it prevented proliferation especially at 300 µg/ml doses. There was also a decrease in the number of AgNORs with increasing dose.

AgNOR values can be used as biomarkers in determining the most reliable therapeutic dose selection for cancer and it has been shown that the correct consumption of Raphanus sativus can be effective in preventing cancer formation and slowing its progression.

In this study, it was seen that Raphanus sativus has a very important function against the development of cancer.

CONCLUSION AND RECOMMENDATION

Research has shown that regular nutrition with antioxidant nutrients prevents the formation and progression of cancer. In conclusion, we believe that adding radish with protective content against cancer to diets will be beneficial for human health. More studies are needed to further elucidate the mechanisms of radish's effect on cancer cells.

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