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COMPARISON OF MEBENDAZOLE ANTICANCER EFFICACY WITH PACLITAXEL AND VINCRISTINE

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ABSTRACT

Mebendazole is a commonly used antiparasitic drug. In addition, it is mentioned in the literature that mebendazole may have anti-cancer effects. The present study was conducted to compare the anti-cancer effects of mebendazole when combined with vincristine and paclitaxel used in cancer treatment. In the study, following the induction of Solid Ehrlich Carcinoma (SEC) in Balb-C mice, vincristine (0.5 mg/kg, i.p.), paclitaxel (20 mg/kg i.p.), mebendazole (50 mg/kg p.o), vincristine+mebendazole and paclitaxel+Mebendazole application was made for 21 days. During the application process and at the end of the experiment, SEC mass volumes, tissue hemoglobin levels and apoptotic DNA-levels were examined. At the end of the study, while the SEC mass volume decreased in all treated groups, the most significant decrease was observed in the group in which mebendazole was administered alone ($P<0.05$). While SEC tissue hemoglobin level decreased in all treatment groups, an increase was observed in apoptotic DNA levels ($P<0.05$). We suggest that mebendazole may have apoptosis-inducing effects and anti-cancer effects on SEC tissues in single and combined applications.

Keywords: Apoptosis, Anti-cancer**,** Mebendazole, Paclitaxel, Vincristine.

INTRODUCTION

Mebendazole (Mbz), a benzimidazole derivative, is mostly used for antiparasitic purposes in medicine (Dayan 2003). Mebendazole shows its antiparasitic activity by disrupting energy metabolism in parasites and tubulin protein structure in cells (Laclette et al. 1980). It is known that the application of mebendazole does not cause any serious side effects in humans and animals, and even in overdose, no serious toxicity occurs (Braithwaite et al. 1982; Sanchez et al. 2000). Tubulin is a protein that plays an important role in cell proliferation (Katsetos et al. 2003; Parker et al. 2017). Any change in this protein structure causes disruption of the cytoskeleton, disruption of the cell cycle and death of the cell by producing apoptotic signal (Kavallaris 2010).

It is reported that Paclitaxel (Pac) and Vincristine (Vin), which are classical antineoplastic drugs, show their effects by disrupting the microtubule structure in which tubulin protein participates and disrupting the cell cycle (Fong et al. 2019; Himes et al. 1976).

Studies have suggested that mebendazole may have an anti-cancer effect resulting in the induction of apoptosis and that this effect may be based on the inhibition of tubulin protein, which is an important component of the cytoskeleton (Laclette et al. 1980; Tapas et al. 2002). Paclitaxel and Vincristine are also known to act by disrupting the microtubule structure on the basis of their antineoplastic activity (Mary Ann Jordan and Wilson 1998). However, it has been reported that paclitaxel and vincristine have serious side effects such as nephrotoxicity, hepatotoxicity, neurotoxicity, and immune deficiency (Argyriou et al. 2007; Rosenthal and Kaufman 1974). A number of combinations have been tried to minimize the undesirable effects of these antineoplastic agents (Pantziarka et al. 2014). However, in the literature review, no combination study was found with mebendazole, paclitaxel and vincristine.

In this study, it was aimed to compare the effects of Mebendazole alone and in combination with paclitaxel and vincristine, which are conventional anti-cancer drugs, in mice with Solid Erlich Carcinoma (SEC). We think that combinations of mebendazole with paclitaxel and vincristine can create synergistic effects with conventional anti-cancer drugs due to possible anti-neoplastic effects on cancerous cells and serious side effects of these drugs can be minimized.

Material and Methods

Mebendazole, Paclitaxel and Vincristine were obtained from abcam (Cambridge, UK) in analytical purity. Ehrlich Carcinoma cell was obtained free of charge from Erciyes University.

Experimental Animals and Groups

36 Balb-C mice in 6 groups were used in the study. The study was carried out in Cumhuriyet University Experimental Animals Unit with the permission of Sivas Cumhuriyet University Animal Experiments Local Ethics Committee dated 26/01/2017 and numbered 09. During the study, attention was paid to animal welfare. Animals were kept in 12 hours of darkness and 12 hours of light, and feed and water were given ad libutum.

After the SEC was established in all of the experimental animals within 10 days, the applications were made as follows. Drug administrations were continued for 21 days.

1. Group: Control (Cont.) - Daily 50 mg/kg saline was given orally.
2. Group: Vincristine (Vin.) - 0.5 mg/kg vincristine given weekly via intraperitonally (i.p.).
3. Group: Paclitaxel (Pac.) - 20 mg/kg Paclitaxel given once a week via intraperitonally (i.p.).
4. Group: Mebendazole (Mbz.) - 50 mg/kg mebendazole was given orally daily.
5. Group: Vincristine+Mebendazole (Vin+Mbz) - 0.5 mg/kg vincristine (once a week) +50 mg/kg mebendazole (daily).
6. Group: Paclitaxel+Mebendazole (Pac+ Mbz) - 20 mg/kg Paclitaxel (once a week) +50 mg/kg mebendazole (daily).

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Solid Ehrlich Carcinoma (SEC) Tumor Formation

Ehrlich ascites fluid ($2x10^5$ Ehrlich tumor cells in 200 µl) was injected subcutaneously into the left dorso-lateral lumbar region of each mouse to create a solid mass. It was observed that solid carcinoma developed between the following 8-10 days. SEC xenograft mice were treated with drugs for 3 weeks. The volume of the cancerous mass was measured with the help of the wernier caliper. With these measurements, the relative volume of SEC cancerous tissue was calculated based on the equation mm^3= (the smallest measurable diameter a^2 X the biggest measurable diameter b)/2. As a result of the applications, Solid Ehrlich Carcinoma masses in mice sacrificed under Ketamine + Xilazine anesthesia were surgically removed, and their volumes were calculated with the formula $mm^3 = (a^2xb)/2$ (Doudican et al. 2013).

Determination of Tissue Hemoglobin Level in Cancerous Tissues

Tissue hemoglobin level was measured using the EPOCH ELISA reader device (BioTek, Vermont, USA) with the help of kits produced by EIAaB company (cat no: M1402e).

Apoptotik DNA Laddern Experiment

Commercial kit of Biovision (Catalog no: K120-50, Massachusetts, USA) was used for apoptotic DNA assay. At the end of the experiment, DNA fragments were run on 1.2% agarose gel containing 0.5 µg/ml ethidium bromide and read in a UV transilluminator (Maestrogene, Hsinchu, Taiwan). The pixel density of the DNA bands of the samples in the photographed gel image was expressed by digitizing with the help of the image J (National Institutes of Health, USA) program.

Statistical analysis

The analysis of the data obtained from the study was made using the IBM SPSS vers.26 (USA inc) program. Apoptotic DNA, antemortem and postmortem cancer tissue mass volume analyzes were tested with one-way analysis of variance (one way ANOVA), and Tukey test was used to determine the difference between groups. Data obtained from tissue hemoglobin experiments were determined by one-sample t-test. $P<0,05$ was considered significant in all analyses.

Results

Apoptotik DNA Laddern Results

In our study, apoptotic DNA levels were tested in samples taken from cancerous tissues in order to examine whether the decrease in mass volume with SEC is through apoptosis. A significant increase in apoptotic DNA levels was observed in all groups compared to the control ($P<0,05$). However, while no significant difference was observed in the Pac+Meb group compared to the Pac group ($P<0,05$), an increase was observed in the Vin+Meb group compared to the Vin group. (Figure 1.)

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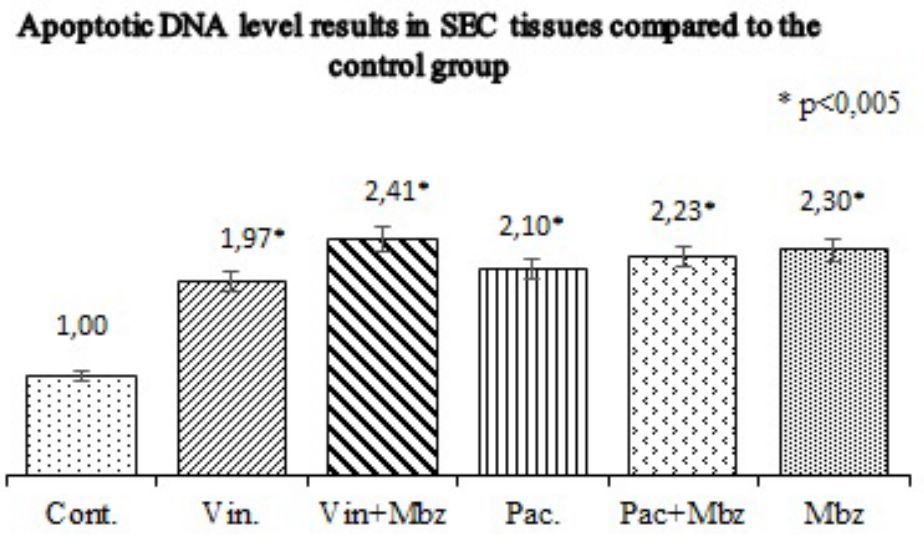

Figure 1. Apoptotic DNA levels change in SEC tissue samples as percentages compared to the control group and (*p<0.05).

Antemortem SEC Tumor Volume Level Results

The diameters of the cancerous mass were determined externally by the wernier caliper every 3 days following SEC injection in mice. Tumor growth rate was similar in all groups until the 10th day when the SEC mass was formed, and after the 10th day, there were differences in the tumor mass according to the groups (P<0,05). At the end of the study, it was found that the SEC volumes of the treatment groups were significantly reduced compared to the control (Figure 2).

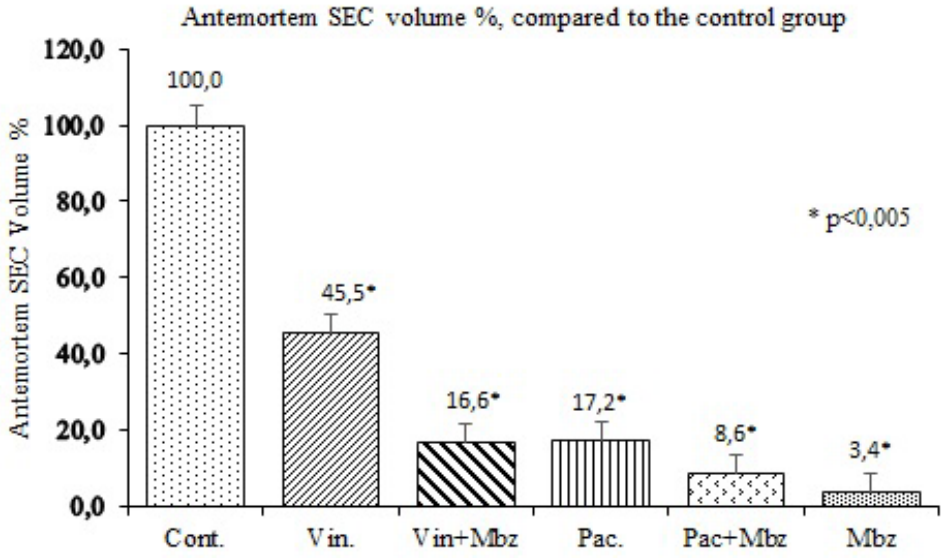

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Figure 2. The change of antemortem SEC mass volume in percentages compared to the control group (*p<0.05).

Among the drugs were administered alone, the most regression was measured in the Mbz group in the groups. While a significant decrease was observed in Vin+Mbz and Pac+Mbz combinations compared to Vin and Pac groups, it was measured smaller than the tumor volume before drug administration in Meb and Pac+Mbz groups, (Figure 3).

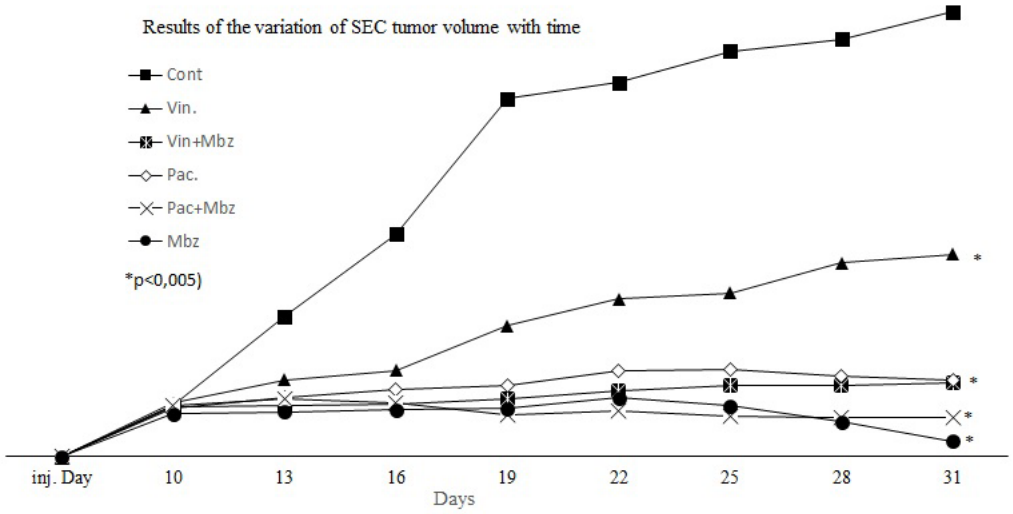

Figure 3. Variation of antemortem cancer mass volumes of mice with SEC over time (*p<0.05)

Postmortem SEC Tumor Volume Level Results

At the end of the study, the SEC mass volume calculated using the averages of the measurements obtained from the groups is expressed in Figure 4 and the SEC tumor volume change calculated from the postmortem measurements is expressed in Figure 5 as percentages. When compared to the control group, tumor volume decreased in all groups and this decrease was statistically significant (p<0,05). Tumor volume regression was found at the highest level in the group given mebendazole, while it was observed at the lowest level in the group given vincristine.

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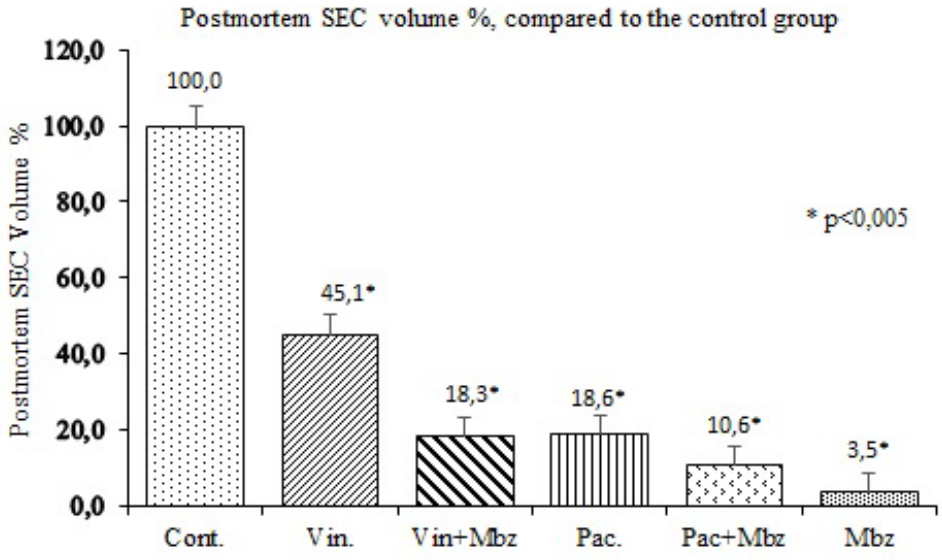

Figure 4. Volumes of SEC masses extracted from experimental mice postmortem at the end of the study (*p<0.05)

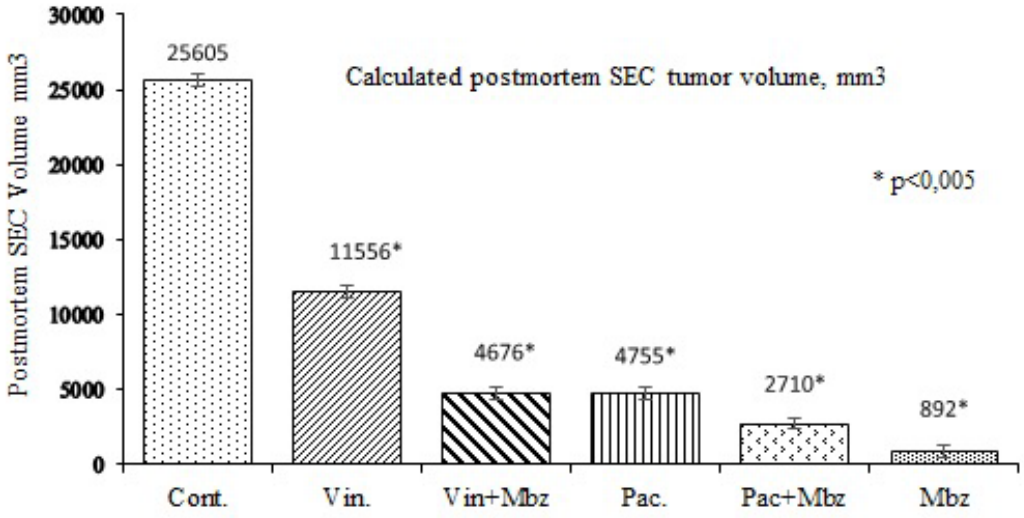

Figure 5. Change of postmortem SEC cancerous tissue volume in percentages compared to the control group (*p<0.05), the smallest measurable diameter a, the bigest measurable diameter b, tumor volume= [(a^2 X b)/2]

SEC Tissue Hemoglobin Level Results

Hemoglobin levels in cancerous tissues are given in Figure 6. When Figure 6 is examined, it is seen that the hemoglobin level decreased significantly in all groups compared to the control (p<0,05).

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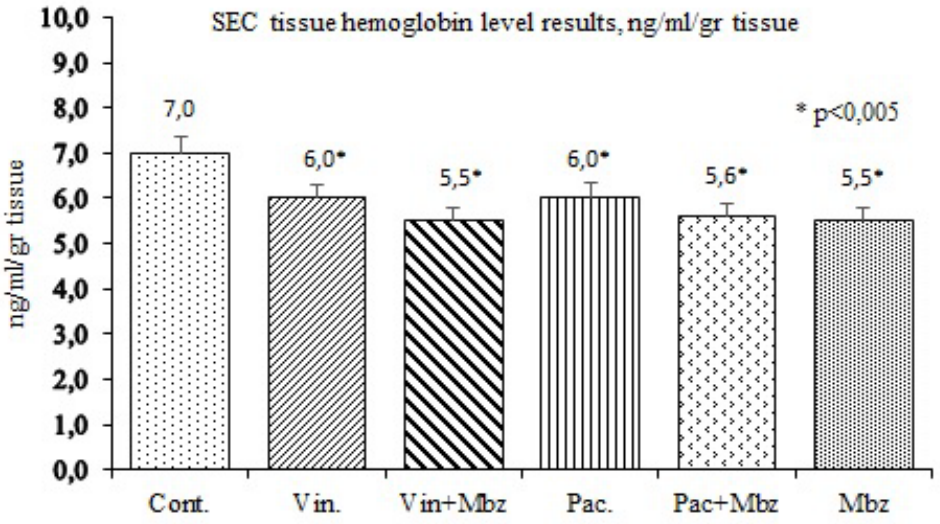

Figure 6. Cancerous tissue hemoglobin levels according to drugs (ng/ml/gr tissue *p<0.05)

DISCUSSION

Mebendazole shows anthelmintic activity by inhibiting tubulin in parasites (Laclette et al. 1980; Lai et al. 2017). It has also been reported that clinical trials are underway as an antineoplastic agent in some tumor types (Guerini et al. 2019). Paclitaxel and Vincristine used in cancer chemotherapy have similar mechanisms of action. However, these agents have very serious toxicities (Marupudi et al. 2007; Mora et al. 2016). Some combinations are tried in order to reduce these side effects and increase the therapeutic efficacy (Smorenburg et al. 2001; Zhang et al. 2017). Comparison of the efficacy of Mebendazole, Paclitaxel and Vincristine with similar mechanism of action in SEC mass is the subject of this research.

Apoptotic pathways are important in oncogenesis and cancer chemotherapy. In cancer cells, disruption of microtubulin structure disrupts the cell cycle and causes the death of the cell by producing apoptotic signals (Mary A Jordan and Kamath 2007).

In previous studies, it has been reported that apoptosis is formed by inhibiting mitosis as a result of mebendazole disrupting tubulin protein polymerization on cancerous cells (Sasaki et al. 2002; Tapas et al. 2002). In these studies, when the in-vivo activity of mebendazole was compared with paclitaxel, it was stated that it had an anti-cancer effect without any side effects.

Doudican et al. (Doudican et al. 2013) reported that when 0.32 µM mebendazole was administered on chemoresistance metastatic melanoma cancer, it was effective in terms of tubulin inhibition, increased apoptosis in cancerous cells, but did not make any changes in healthy cells, and these effects occurred as a result of microtubulin depolymerization.

According to the results of apoptotic DNA levels, while no significant difference was observed in the Pac+Meb group compared to the Pac group (P<0,05), an increase was observed in the Vin+Meb group compared to the Vin group. This result indicates that mebendazole acts as a tubulin stabilizer like vincristine (Figure 1). This situation is similar to the literature sources (Sasaki et al. 2002).

When we look at the findings of our study, we can state that mebendazole's and vincristine's inhibitory effects on microtubulin polymerization are the reason why combinations of mebendazole with vincristine are more effective. It is understood that these results are in parallel with the relevant literature (Guerini et al. 2019).

Mukhopadhyay et al. by using lung cancer cell xenografts, the metastasis abilities of cancerous cells were tested in-vivo, and it was observed that the metastasis in mice administered oral mebendazole was 5 times lower than the control group. In this study, the level of neovascularization was also tested in-vivo and it was found that angiogenesis was suppressed due to apoptosis of endothelial cells in the subjects in the mebendazole group (Tapas et al. 2002).

Nicole et al. (Doudican et al. 2013) conducted an animal modeling study with human melanoma xenografts using athymic mice to examine the in-vivo efficacy of mebendazole on anti-apoptotic factors and to investigate this efficacy. Following administration of melanoma xenografts, 1 and 2 mg mebendazole (oral) daily and 100 mg/kg temozolomide (i.p.) for 5 days were given after 3-5 mm of tumor development. As a result of the study, they reported that there was an 83% reduction in tumor mass in those administered 1 mg of mebendazole, and 77% in those administered 2 mg of mebendazole.

When Figure 2 is examined, it is seen that the SEC volume decreased in all experimental groups. The growth rate of the cancerous mass was similar in all groups until the 10th day, and after the 10th day, there were differences in the tumor mass according to the groups (Figure 3). As expressed in Figure 4, it is understood that the changes in postmortem cancerous mass volumes are also parallel and compatible with antemortem measurements.

The mebendazole's reduction of 96.6% in cancerous tissue volume in the present study is in line with the study of Nicole et al. (Doudican et al. 2013).

In another study (Martarelli et al. 2008), it was stated that the tumor volume was reset in the subjects who were administered mebendazole at 1 μM concentration in an in-vivo trial created with two different adenocortical cancer cells. In this study, the researchers reported that mebendazole induced apoptosis in cancerous cells in-vitro and in-vivo conditions, inhibited the invasion and migration abilities of cancerous cells in-vitro, and prevented metastasis in in-vivo conditions. In the same study, findings indicated that all cancerous cells were completely killed in 20 days. The decrease in the volume of cancerous tissue in the group administered mebendazole on the termination of the experiment is similar to our study.

In the present study, indirect information about angiogenesis was obtained by examining SEC tissue hemoglobin levels. Although tissue hemoglobin level decreased in all drug-administered groups, the highest decrease was measured in the mebendazole group with a decrease of 21.43% (Figure 6). In the groups in which the drugs were administered in combination, close results were obtained between the hemoglobin levels, and it was understood that these similarities within the group were statistically insignificant.

In the light of the findings obtained from our study and the information obtained from the literature sources, we are of the opinion that mebendazole may have apoptosis-inducing effects on cancerous cells and tissues. Cell cycle and mitotic division by mebendazole is supported by the death of cells damaged by apoptosis by the demonstration of apoptotic DNA fragments in the terminated cells.

CONCLUSION

In conclusion, it appears to have antineoplastic activity in experimental SEC tumors when mebendazole is administered alone or in combination with paclitaxel and vincristine. We are of the opinion that mebendazole can be used as an anti-neoplastic drug, and if it is used in combination with vincristine or paclitaxel, it can reduce the side effects of vincristine and paclitaxel, while increasing their pharmacological efficacy in treatment. However, we suggest that it would be appropriate and beneficial to conduct more detailed studies on this subject, including different types of cancer.

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Conflict of Interest
The authors declared that there is no conflict of interest.

Ethical Statement
This study was approved by the Sivas Cumhuriyet University Animal Experiments Local Ethics Committee (approval date/no: 26.01.2017 / no:09).

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