Objectives: Cancer is the second most common cause of death in the world. Several chemotherapeutic drugs have been studied for their anticancer features. Paclitaxel is one of these chemotherapeutic drugs with high medicinal interest. This study was conducted in order to investigate effects of paclitaxel on lipid peroxidation and antioxidant enzymes in tissues of mice bearing solid-form Ehrlich tumors.

Methods: In this study, 36 Balb/C male mice aged 8-10 weeks and weighing 25-30 g were used. Six mice were kept as cancer stock to produce Ehrlich Ascites Tumor (EAT) cells. Thirty mice were distributed into three groups as healthy control, tumor control and paclitaxel treatment. 0.1 ml physiologic saline solution was administered into mice in healthy control subcutaneously (s.c.) for 15 days. The animals in tumor control and Paclitaxel treatment groups received 1x10^6 EAT cells s.c. through nape skin on the first day of the experiment. After the application of EAT cells, 10 mg/kg Paclitaxel was injected intraperitoneally on days 4, 9 and 14. At the end of the study (on day 15), animals were sacrificed and liver, kidney, brain and testis tissues were excised and analyzed for Malondialdehyde (MDA), by using TBARS method, superoxide dismutase (SOD) and catalase (CAT) activities spectrophotometrically.

Results: Compared to the results of healthy control group, tumor increased kidney and liver MDA levels development slightly but not significantly. Paclitaxel treatment significantly reduced the increased MDA levels in kidney and liver (p<0.001). Paclitaxel had no effect on testis MDA but brain MDA level reduced with the help of EAT cell injection and Paclitaxel returned the brain MDA level close to the level of healthy control (p<0.001). EAT cell injection reduced catalase activity in kidney and liver (p<0.001) and Paclitaxel had no effect on catalase activities in these tissues. In EAT cell injected mice; testis and brain catalase activities were higher than that of healthy control group that were returned to control levels by Paclitaxel treatment. Paclitaxel had no significant effect on decreased kidney and liver SOD activities whereas it significantly reduced the increased SOD activities in testis (p<0.05) and in brain (p<0.01).

Conclusion: Paclitaxel alleviated the lipid peroxidation in kidney and liver but had no effect on antioxidant status in these tissues of solid-Ehrlich tumor-bearing mice.

Keywords: Antioxidant enzymes, lipid peroxidation, paclitaxel, solid-form Ehrlich tumor

Cancer, a genomic disease, is a worldwide public health problem. Cancer, which is accepted among chronic diseases, is frequent and has the second leading mortality score following cardiovascular diseases. Cancer is one of the most important health problems in Turkey as it is in the whole world. In Turkey, the standardized cancer rate for age in 2013 is 186.5 per hundred thousand for women and 267.9 for men. Total cancer incidence is 227.2 per hundred thousand. In 2002, deaths caused by cancer in our country constituted 12% of all deaths whereas this rate increased up to 21% in 2009. When the 2012 data is evaluated, over 175,000 new cancer cases have emerged in our country within a year. If a similar course continues, it is expected to be 22 million new cases annually in 2030.

A number of experimental cancer models have been developed for use in cancer-related studies and Ehrlich solid carcinoma is a commonly used tumor model among them. Ehrlich ascites carcinoma is a spontaneous murine mammary adenocarcinoma adapted to ascites form and carried in outbred mice by serial intraperitoneal (i.p.) passages. Ehrlich solid carcinoma is an undifferentiated tumor. It was reported that Ehrlich ascites tumor (EAT) cells undergo rapid proliferation in almost any mouse host because they lack H-2 histocompatibility antigens. Morphological and metabolic changes occur following implantation of EAT cells. It was shown that subcutaneous implantation of EAT cells into mice causes changes in oxidant and antioxidant status in the tissues.

Several chemotherapeutic drugs have been studied for their anticancer activities. Paclitaxel, aka Taxol, is a chemotherapeutic drug with high medicinal interest. Paclitaxel is one of the most commonly used chemotherapeutics in clinical studies. It is a broad-spectrum anticancer drug effective in various solid tumors such as ovarian and breast cancer, lung cancer, melanoma, head and neck cancer and bladder cancer. Paclitaxel has potent anti-proliferative action against tumor cells and this agent shows its functions by stabilizing microtubules, blocking mitosis and inducing apoptosis.

Antioxidants are naturally present in many fruits and vegetables and can be synthesized in laboratories. They delays or improves cellular oxidative damage, so there are various health benefits in the prevention and treatment of diseases. They can be used alone or in combination with other drugs as adjuvant therapies. The study of free radicals and antioxidants includes promising new solutions in disease and health management. Many original studies aim to eliminate this toxicity by the antioxidants of the drug’s healing effect and the toxicities associated with chemicals. Reactive oxygen species (ROS) are produced continuously in the body because of aerobic metabolism as well as external factors and these are balanced by antioxidant defense systems. When ROS are produced in excess, they cause tissue damage. Cellular damage resulting from oxidative stress is involved in the initiation and progression of cancer. Cancer cells increase production of ROS compared to normal cells and it is speculated that tumorigenic signaling also increases expression of antioxidant proteins to balance the high ROS production to maintain redox homeostasis. Studies indicated that the levels of oxidative stress markers increase in cancer cases. Several adverse effects of chemotherapy treatments have been reported and most of these effects are associated with oxidative metabolism. Anti-cancer drugs can also cause oxidative stress as a side effect. Previous studies investigating the effects of Taxol on lipid peroxidation and antioxidant status in different cancer types in animal models and human have revealed distinct results. Therefore, this study was performed to investigate the effects of Paclitaxel on tissue MDA levels and antioxidant enzyme activities in mice bearing Ehrlich solid carcinoma.

Methods

Animals, Management and Experimental Design

In this study, 42 Balb/C male mice aged 8-10 weeks and weighing 25-30 g were obtained from Erciyes University Experimental and Clinical Research Center (DEKAM). The study was held at DEKAM with the permission of Erciyes University Experimental Animals Local Ethics Committee, Approval No. 15/03 and dated 14.01.2015.

Animals were maintained in polycarbonate cages sized 42x26x15 cm (five mice in each) at this center that provides appropriate standard conditions (21±2 °C room temperature, 50±5% humidity, environmental ventilation systems providing air flow rotation of 12 per hour and 12 hours light/dark light cycle) for highest health status throughout the study. A commercially available pellet diet containing 24% crude protein, 3.85% crude cellulose, 5% fat, 6.98% ash as well as amino acids and vitamin-mineral mix that met the daily nutritional requirement of the mice (routinely used in DEKAM) was provided throughout the experiment. Water and feed were supplied ad libitum during the study.

In the beginning of the study, all animals were weighed. Before initializing the experiment, 12 mice were kept as cancer stocks to obtain sufficient EAT cells. The remaining 30 animals were assigned into three experimental groups consisting of 10 mice in each. Five individually labeled mice were maintained in one cage.

Group I was kept as healthy control and a-0.1 ml injection
of physiologic saline solution was done subcutaneously (s.c.). On the first day of the experiment, a single dose of 1x10^6 EAT cells in 0.1 ml of phosphate buffer saline (PBS) was injected s.c. through nape skin to each animal in Group II and Group III for solid tumor development. Mice in Group II were kept as solid tumor control following the EAT injection. Mice in Group III received 10 mg/kg Paclitaxel i.p. on days 4, 9 and 14. Mice in Control Group also received physiologic saline solution i.p. on the same days (Fig. 1).

**Sample Collection and Preparations**

At the end of the experiment (on day 15), all of the animals were sacrificed with ketamine-xylazine under general anesthesia, and the liver, kidney, brain and testis tissues from each animal were collected into sterile plastic bags for determination of MDA levels, SOD and CAT activities. The samples were transferred to the laboratory under cold chain and stored at -80 °C until biochemical analyses immediately.

**Homogenization of Tissues**

Tissue samples (500 mg) were thawed and homogenized in a glass homogenizer with physiological saline solution (pH=7.4) (1/10, w/v). The homogenates were centrifuged at 12,000 rpm for 20 minutes under 4 °C. Some parts of the supernatants were kept for MDA and CAT analyses. The remaining supernatants were mixed with ethanol/chloroform mixture [5/3 (v/v)] at 1/1 ratio and they were centrifuged again at 12,000 rpm for 20 minutes in a refrigerated centrifuge. The supernatants were separated for SOD enzyme activity.

**Determination of MDA Level, CAT and SOD Activities**

Malondialdehyde, a secondary product of lipid peroxidation, is an important indicator of lipid peroxidation. Malondialdehyde forms a pink complex with thiobarbituric acid (TBA) under aerobic conditions at pH=3.4 following the incubation at 95 °C. The absorbance of this complex was measured at 532 nm by using a UV-Visible spectrophotometer (Shimadzu, UV 1601, United states) through freshly prepared 10, 20, 40, 60, 80, 100 nMol/ml 1,1,3,3-tetramethoxypropane (density: 0.99 g/ml) solutions according to the method described by Ohkawa et al.[25] Briefly, 100 μl tissue homogenate was mixed with 8.1% of sodium dodecyl sulfate (SDS), 20% of acetic acid (HAc) (pH=3.5) and 0.8% of TBA (pH=3.5) and incubated at 95 °C for 30 minutes. Then they were cooled and n-Butanol-pyridine (nBu-Pri) solution and distilled water were added in the vortex-mixed solution. The supernatant was separated following the centrifugation at 4,000 rpm for 10 minutes and the absorbance was monitored. The result was recorded in nMol/mg protein.

The activity of SOD was measured spectrophotometrically according to the method described by Sun et al.[26] This method is based on reduction of nitroblue tetrazolium (NBT) by superoxide radicals which are formed by the enzymatic reaction of xanthine oxidase (XO). The colorless NBT ion is transformed into a blue formazan giving maximum absorbance at 560 nm when reduced with the superoxide radical. The tissue was homogenized with the help of 1/10 of distilled water. The sample was mixed with the chloroform/ethanol mixture 1/1 (v/v) and centrifuged at 12,000 rpm for 2 hours at +4 °C. The supernatant was separated to determine SOD activity. 50 μl tissue supernatant and 50 μl XO in 2 M ammonium sulfate solution (1/100, v/v) were added 2.9 ml of the reagent mixture consisting of xanthine solution + NBT + Na2CO3 + BSA. After incubation at 25 °C for 20 minutes, 1 ml of 0.8 mM CuCl2 was added to the tube and optical density of the sample was recorded at 560 nm. The SOD activity was displayed in Unit/mg protein (1 unit=50% inhibition of NBT reduction) and % inhibition was calculated with the following formula: % inhibition = [(blank abs-tissue abs)/blank abs] x 100.

Catalase enzyme catalyzes the conversion of H2O2 to H2O. This conversion can be monitored by a decrease in absorbance at 240 nm. The decrease in absorbance at 30th sec-

**Figure 1.** (a) Solid tumor on the back of the rat, (b) Solid tumor removed from the back of the rat (c) Solid tumor.
ond is related to catalase activity. The CAT activity was determined as described previously by Aebi. The CAT assay was performed briefly as follows: Tissue homogenate was mixed with $\text{H}_2\text{O}_2$ solution (30 mM) + freshly prepared PBS (50 mM, pH=7.0) then the absorbance was measured spectrophotometrically at 240 nm after 30 seconds against blank. The extinction coefficient was 0.004 (0.0039) mM-1mm-1. The CAT activity was expressed in U/mg protein/min for tissue.

**Histopathological Examination**

After the experiment, kidney and liver tissue samples were taken from animals and fixed with formalin solution for histopathological examination. Following the two-day fixation of the tissues; Histological tissue monitoring including tap water, graded alcohols (50, 70, 80, 90, 100%), xylol (xylol 1 and xylol 2) and paraffin (paraffin 1 and paraffin 2) were performed. The prepared paraffin blocks were cut into 5 μm sections in the microtome. Tissues were stained with Hema-toxylin-Eosin (HE) and evaluated under light microscope.

**Results**

**Biochemical Findings**

Compared to that of the healthy control group, tumor development increased kidney and liver MDA levels slightly but not significantly. Paclitaxel treatment significantly reduced the increased MDA levels in kidney and liver (p<0.001). Paclitaxel had no effect on testis MDA but brain MDA level was reduced by EAT-cell injection and Paclitaxel returned the brain MDA level to the level of the healthy control group (p<0.001). EAT-cell injection reduced catalase activity in kidney and liver (p<0.001) and Paclitaxel had no effect on catalase activities in these tissues. In EAT-cell-injected mice, testis and brain catalase activities were higher than that of the healthy control group that were returned to control levels by Paclitaxel treatment. Paclitaxel had no significant effect on decreased kidney and liver SOD activities whereas it significantly reduced the increased SOD activities in testis (p<0.05) and brain (p<0.01).

**Histopathologic Findings**

The kidney tissues of Group I, the connective tissue capsule appeared normal. Also kidney parenchyma, renal corpuscles, proximal and distal tubules, peritubular capillaries showed normal. In Group II, EAT cells were observed attached to the connective tissue capsule. When the kidney sections of this group were examined, invasive tumor cells were observed at different densities in the tissue capsule. The presence of tumor cells in tumor group was also detected in the kidney capsule of Group III, but the density was lower (Fig. 2).

![Figure 2](image-url)
In the liver tissues of Group I, normal parenchymal structure was observed. Liver cells progressing radially around the vena centralis in the lobules were normal. Liver sinusoids were observed to extend normally between the hepatocyte cords. In the portal areas, bile duct, hepatic artery and venous structures were visible in the connective tissue. In Group II, EAT cells were observed attached to the connective tissue forming the glisson capsule whereas in Group III EAT cells were observed attached to the capsule of liver tissue (Fig. 3). Paclitaxel had no significant effect on decreased kidney and liver SOD activities whereas it significantly reduced the increased SOD activities in testis (p<0.05) and brain (p<0.01) (Tablo 1).

Discussion
Cancer cells demonstrate alterations in oxidative metab-

![Figure 3. Histopathological findings of liver tissue (H&E, 20X) (a) Healthy control group (b) Tumor control group (c) Paclitaxel group.](image)

<table>
<thead>
<tr>
<th>Organs</th>
<th>n</th>
<th>Healthy Control</th>
<th>n</th>
<th>Cancer Control</th>
<th>n</th>
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Oxidative stress is defined as an imbalance between ROS and the anti-oxidant capacity of the cell. Patmavathi et al. have shown the increases in the level of lipid peroxidation in the breast and liver of breast cancer bearing rats. Didziapetriene et al. detected elevated MDA level in ovarian cancer. In the present study, the MDA levels in the kidney and liver were increased by the tumor development in the EAT-cell-injected mice. The elevated kidney and liver MDA levels were reversed with Paclitaxel treatment and the result was lower than that of the healthy control group. However, Paclitaxel had no significant effect on testis MDA level. On the other hand, brain MDA level was lower than that of the healthy control group mice but it returned to the level of controls after paclitaxel treatment.

In cancer-bearing animals, significant decreases were reported in the SOD and CAT activities in the breast and liver of breast cancer bearing rats. Didziapetriene et al. reported lower CAT activity in ovarian cancer patients. Similarly, in the present study, kidney and liver SOD and CAT activities were decreased by the cancer development but in contrast to the findings of Patmavathi et al., paclitaxel had no effect on the activities of these antioxidants. In tests and brain, the activities of SOD and CAT were higher in cancer group than both control and paclitaxel treated cancer-bearing animals. Catalase is a tetrameric protein that consists of four similar subunits containing heme group. It is excessively expressed in some tissues to protect cells against excess ROS formation. It has an oxidase activity as well as it is involved in ROS generation. Thus, higher CAT levels in cancer group may be attributed to the modification of CAT levels in cancer cells resistant to some chemotherapy or hydrogen peroxide.

In the study of Salem et al., EAT cells were inoculated into CD-1 mice subcutaneously to form solid tumor and then treated with intraperitoneal injection with venom (0.22 mg/kg) every other day. A. amoreuxi venom treatment decreased MDA level while increased GSH in liver kidney and serum. In our study Paclitaxel decreases the lipid peroxidation in kidney and liver. In addition, histopathological evaluations of liver and kidney tissues also support these results. In the study of Cosan et al., Paclitaxel treatment returned the altered MDA level and SOD and CAT activities to control levels and paclitaxel restored the damaged kidney and liver structure. Campos et al. determined decreases in catalase activity and metahemoglobin levels after paclitaxel infusion in rats. Panis et al. detected high oxidative stress status characterized by elevated lipid peroxidation and reduced CAT activity in advanced breast cancer and these authors found that paclitaxel enhanced lipid peroxidation due to systemic oxidative stress and red blood cell oxidative injury with anemia development.

Conclusion

The results of this study have shown that Paclitaxel alleviates the lipid peroxidation in kidney and liver but has no effects on antioxidant status in these tissues whereas it significantly reduced the increased SOD activities in testis and brain of Ehrlich solid tumor-bearing mice.

Disclosures

Ethics Committee Approval: The study was held at DEKAM with the permission of Erciyes University Experimental Animals Local Ethics Committee, Approval No. 15/03 and dated 14.01.2015.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.


References


