CHAPTER 13

# Investigation of the Death Effects of Four Different Chemotherapeutic Agents on Breast Cancer Cell Lines Under the Microscope

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**What is a cell?**

Cells are the smallest building blocks of living things that can be seen with a microscope (1). All cells in our body have specific functions and these cells divide regularly (except muscle and nerve cells) (2). When cells get old or damaged, they die and are replaced by new cells that can renew themselves. When these cells begin to multiply uncontrollably, cancer cells, also called tumoral structures, are formed (3). As seen in Figure 1, cancer cells continue to grow and form new cells, forming an abnormal structure and leaving no room for normal cells.

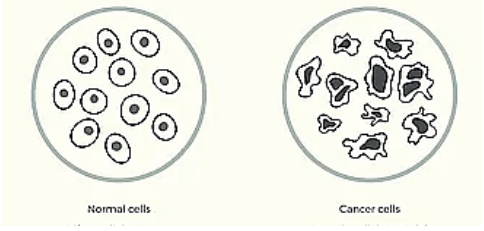


***Figure 1. Normal and cancer cells***

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**What is cancer cell?**

Because this abnormal growth affects normal cells, it creates a complex that threatens human health (4). Cancer, a complex condition that millions of people are diagnosed with every year, is still a serious health problem from past to present (5). Despite advances in the detection of cancer cases worldwide, studies to prevent detected cancer, and modern methods of treatment, these cases still affect millions of people. This disease lowers the living standards of patients and often causes people to lose their lives. According to the records of the World Health Organization, 11 million people are diagnosed with cancer annually and it is determined that there are still around 25 million cancer patients in the world (6). When we look at the causes of death in the world, cancer is the second cause of death after coronary heart diseases (7) with a rate of 20.06%. In our country, cancer causes the most deaths after heart diseases (Table 1).

***Table 1. % ratio of cancer diseases among the most common fatal diseases***

| **Diseases** | **% ratio** |
|---|---|
| Coronary heart ailments | %25,9 |
| Cancer | %20,6 |
| Cerebrovascular diseases | %13,7 |
| Pneumonia | %8,0 |
| Chronic bronchitis | %4,1 |
| Accidents | %3,8 |

While the death rate due to heart diseases is 42% in Turkey, the rate due to cancer is 12.9%. In the research conducted in 2018; While 18.1 million people were diagnosed with cancer, it was determined that 9.9 million people died due to these cases (8).

**Factors that cause cancer**

According to researches; Many factors such as radiation, genetics, DNA mutations, stress, environment, biochemical and synthetic chemicals cause cancer formation (9). Moreover; Environmental causes such as X-rays, UV rays, pollution and chemicals, lifestyles including alcohol and smoking habits, and diets containing vitamins, antioxidant factors, fatty or fibrous foods affect the frequency of tumor formation (Figure 2) (10).

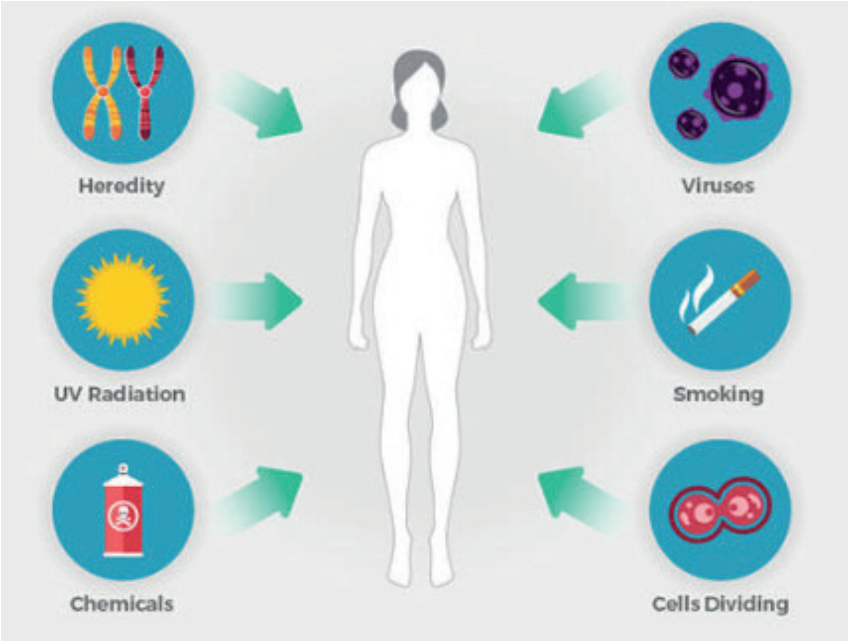


***Figure 2. Factors that cause cancer***

Although the incidence and mortality of cancer have increased rapidly in recent years, it threatens humanity more and more as time goes on (11). Cancer, which initially starts locally, tends to spread rapidly to other parts of the body, making the disease incurable.

**Breast cancer**

Breast cancer ranks first among the most common types of cancer among women (12). In the cancer rankings detected in Turkey, breast cancer is the most common type of cancer in women, and it is also identified as the leading cause of death from cancer cases in many countries. Breast cancer, which is a malignant tumor that starts in breast cells, is tumoral structures that occur with uncontrolled proliferation of cells in the mammary glands (lobular) or milk ducts (13). Breast cancer is frequently seen in women, although this type of cancer is rarely seen in men. Breast cancer, which occurs in two forms, is called ductal carcinoma (cancer arising from the milk ducts) and lobular carcinoma (cancer arising from the lobules) (12). Breast cancer, which starts mostly in duct cells, can sometimes start in lobules.

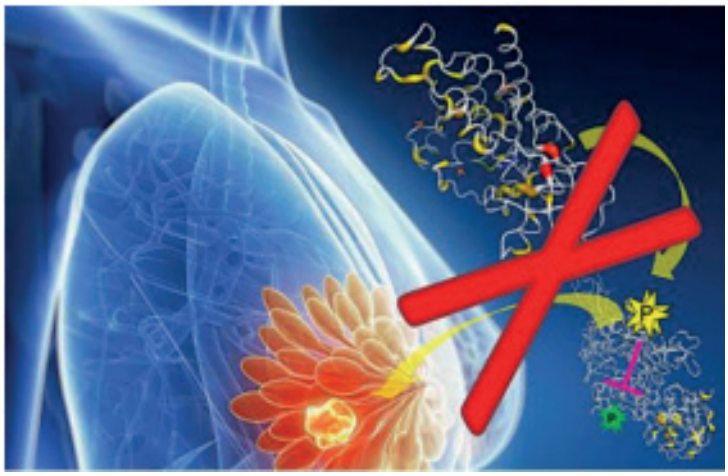

*Figure 3. Breast cancer resulting from excessive FGFR4 (Fibroblast growth factor receptor 4) growth (12)*

**Cancer treatment methods**

In cancer treatments, being aware of the methods of prevention from cancer cases, early diagnosis and obtaining positive results from the treatment lead to a decrease in the fear of cancer cases in people over time (14). Today, the treatment possibilities of many types of cancer have increased, and the life expectancy of patients is increasing compared to other years. However, cancer has not escaped being talked about as synonymous with fear and even death (15). Cancer and cancer treatment are among the most important health problems of the people of many countries. Many treatments are used to reduce the death rate and increase survival in cancer treatment (16). Among them, there are many options such as surgery, chemotherapy-hormone therapy, radiotherapy, immunotherapy, new drug types and treatment methods. Treatments such as surgery, radiotherapy, chemotherapy are limited by the accessibility of the tumor. Among these important problems that need to be solved are the early diagnosis delayed in cancer diagnosis and treatment, lack of drug compliance and use of systemic drugs that are not specific to the tumor, incomplete drug concentrations reaching the tumor site, and inability to display post-treatment responses (14-15). Cancer biotechnology focuses on the techniques and materials necessary to overcome these problems, and cancer biotechnology research activities are divided into seven categories as a result of the researches (15).

- First; early detection of cancers in the early stages and development of imaging agents to be used.

- Latter; deriving methods that can enable assessments of the effects of treatments in the region.
- Third; will transfer the therapeutic agents to the cancer area without being damaged by biological agents; develop targeted materials, devices and carriers.
- Fourth; development of agents to distinguish malignant from benign cancer cells and to observe predictive molecular changes.
- Fifth; making various observations to detect genetic conditions predisposing to cancer and developing mutations that trigger cancer.
- Sixth; development of new methods for monitoring cancer symptoms that reduce quality of life.
- Seventh; development of techniques to rapidly identify target sites and predict drug resistance in clinical therapy to support researchers.

In cancer technology research; In addition to the imaging of cancerous cells recently, it is also important to monitor the effect of drug materials applied to the tumor area (14). If drug materials can be used effectively in cancer treatment in accordance with the desired target, it is predicted that it will be one of the biggest developments in this field (16). Another important situation is; It is aimed to apply many drugs to the tumor area at the same time, and thus to eliminate or minimize the problem of drug resistance, which is one of the most undesirable problems in cancer treatment (17-18). In recent years, many chemotherapy drugs and new treatment methods have been developed for cancer treatment. There are many cancer drugs and cancer drug combinations. They have individual side effects.

**Cisplatin**

Cisplatin is indicated for the palliative treatment of the following diseases, either alone or in combination with other approved chemotherapeutic agents (19): Metastasized testicular tumors: In established combination therapy with other approved chemotherapeutic agents in patients with metastasized testicular tumors who have undergone appropriate surgery and/or radiotherapy (20). Established combination therapy includes cisplatin and cyclophosphamide. Cisplatin as a single agent is indicated as a secondary therapy in patients with metastatic ovarian tumors refractory to standard chemotherapy who have not previously received cisplatin therapy.

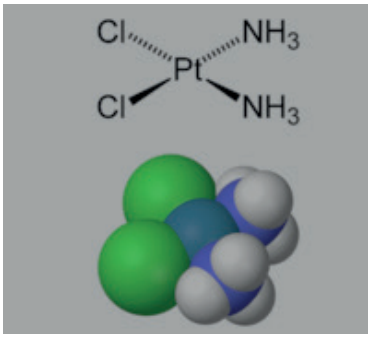


***Figure 4. Cisplatin structure***

**Docetaxel**

Docetaxel, sold under the brand name Taxotere, is a chemotherapy drug used to treat a number of cancer types. This includes breast cancer, head and neck cancer, stomach cancer, prostate cancer, and non-small cell lung cancer. It can be used alone or in combination with other chemotherapy drugs (21).

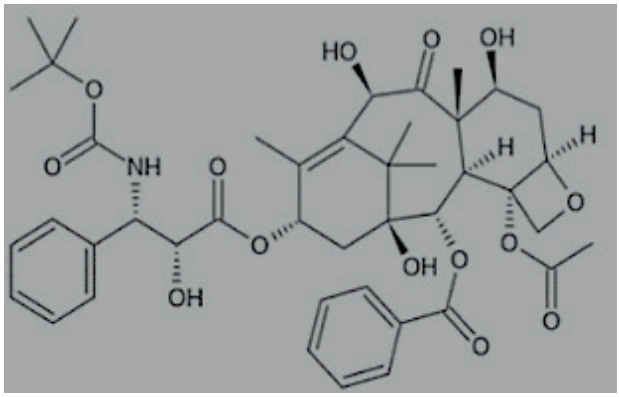


***Figure 5. Docetaxel structure***

**Gemcitabine**

Gemcitabine is a chemotherapy drug. It treats cancers such as testicular cancer, breast cancer, ovarian cancer, non-small cell lung cancer, pancreatic cancer, and bladder cancer. It is administered by intravenous infusion (22).

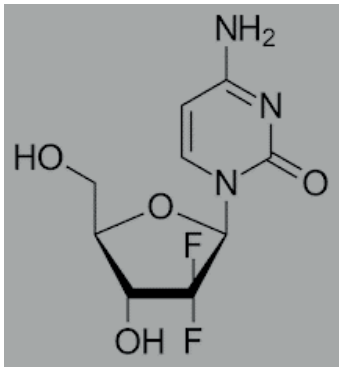


*Figure 6. Gemcitabine structure*

**Taxol**

Paclitaxel (Taxol) is a chemotherapy drug used to treat various types of cancer. This includes ovarian cancer, esophageal cancer, breast cancer, lung cancer, Kaposi's sarcoma, cervical cancer, and pancreatic cancer. It is administered by intravenous injection. There is also an albumin-dependent formulation (23).

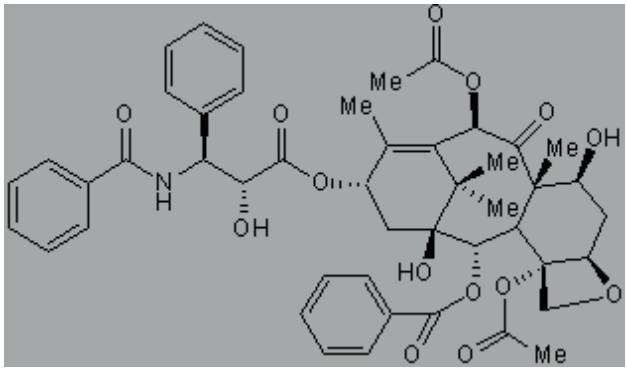


*Figure 7. Taxol structure*

**Application Of Four Different Drugs To Breast Cancer Cell Lines**

Cancer is usually called continuous and fast growing tumor cells. It is a disease characterized by uncontrolled growth and metastasis by activation of oncogenes, epigenetic modifications, and inactivation of tumor suppressors (24). Treatments are based on researched methods with the aim of minimizing tumor progression, recurrence and mortality. Generally, many treatment methods are applied, especially chemotherapy, radiotherapy and surgical

methods (25). Cytotoxic chemotherapy is most commonly used. Since these chemotherapies target all body cells, they can damage some healthy cells as well as cancer cells (26). Existing chemotherapy drugs (Docetaxol, taxol, cisplatin and gemstabin) used during the treatment are examined under the microscope for their harmful effects on cells.

In many studies, the anticancer activities of such drugs and their derivatives have been determined by examining them with a microscope. In this study, the death effects of four different drugs on breast cancer cells were examined under a microscope and compared.

Although cancer treatment, which is still a major problem in the world, is treated with three methods (surgery, radiotherapy and chemotherapy), chemotherapy, also known as drug therapy, is known as the only treatment method that can affect all parts of the body systemically (14-15). Cancer is a systemic disease that affects many organs of the body. The common feature of chemotherapy drugs used in drug treatment applications is that they damage cells with a high growth and division rate and that the tumor cell growth rate is too uncontrolled, so that these chemotherapeutic drugs are damaged by these chemotherapeutic drugs (3, 12). Because these chemotherapy drugs affect the normal cells of the body with a high rate of division as well as cancer cells, nausea, decrease in blood counts, hair loss, etc., which are common during treatment. side effects may occur (9, 23). One of the most important distinctions between these cells is that while normal cells can repair themselves over time, cancerous cells cannot fully repair themselves. Therefore, in about two to three weeks, while normal cells can recover from the effects of chemotherapy drugs, cancer cells cannot tolerate this and fail. In this case, the cells die over time (20).

All these treatment methods may become insufficient over time. While the cell becomes resistant to the drug and the death rate is expected to increase, the cell may grow further and metastasize. All these treatment methods may become insufficient over time (16). While the cell becomes resistant to the drug and the death rate is expected to increase, the cell may grow further and metastasize. Anticancer drugs used in cancer treatments show their effects mainly in five ways: apoptosis, necrosis, autophagy, mitotic catastrophe and senescence (27). In addition, two more death pathways known as necroptosis and proptosis can be activated with anticancer drugs.

Apoptosis and autophagy pathways are genetically tightly controlled mechanisms of programmed cell death. Necrosis and mitotic catastrophe are types of unscheduled and uncontrolled cell death that occur in the face of severe cellular attacks. Senescence, which is characterized by telomere

damage and activation of tumor suppressor factors, literally means cellular senescence (27).

It is expected that the development of drug active substances that are synthesized or derivatized for cancer treatments, cause the death of tumor cells by causing the above-mentioned cellular functions or mechanisms in cell lines. For this reason, macrocyclic molecules and their derivatives, especially calixarene molecules, are functionalized for many uses such as drug transport, release, solubility and preservation in cancer treatments, in vitro and in vivo studies are carried out and still being investigated.

The four different cancer drugs used have been investigated by many research groups for their anticancer activities. These drugs have multiple mechanisms of action for their anticancer properties, including enzyme inhibition, antagonism of angiogenesis, suppression of oncogenes, upregulation of tumor suppressor genes, and DNA binding. In other literature studies, it has been observed that platinum derivatives cause growth inhibition, while drug molecules in the taxol structure inhibit phosphatases such as alkaline phosphatase and tyrosine phosphatase. In addition, many studies have suggested that new cancer drugs may interact with the platelet-derived growth factor (PDGF) receptor, preventing it from interacting with PDGF and phosphorylation, resulting in an anti-angiogenic effect (28). It has been shown in many studies that cancer drugs and their equivalents can increase the activity of tumor suppressor genes by stabilizing the relevant protein.

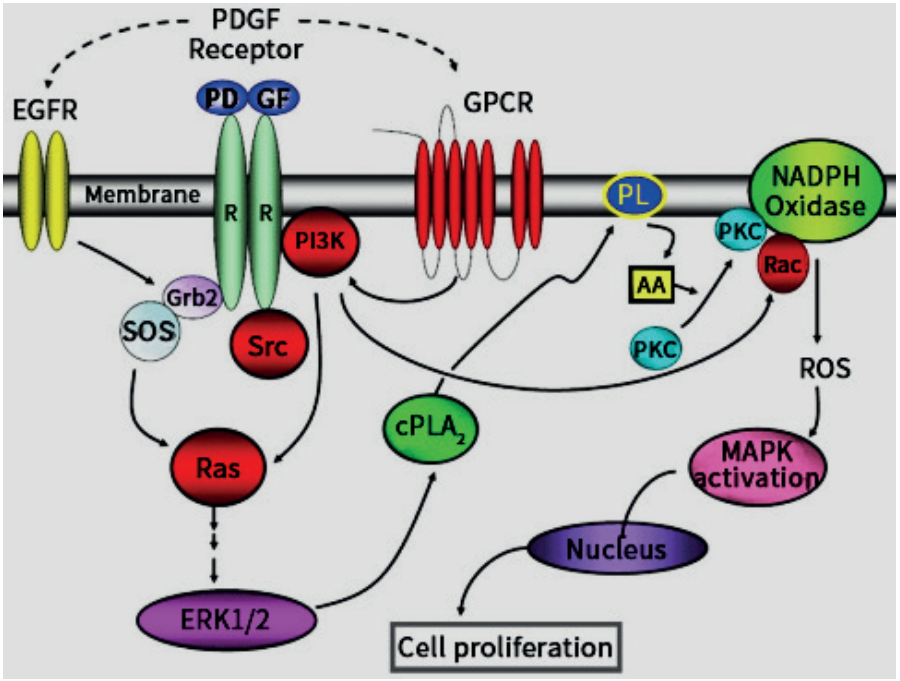


*Figure 8. Growth factor (PDGF) receptor (29)*

Again, as a result of research; it has been observed that gemstabin inhibits glutathione S-transferase and prevents multidrug resistance of cancer cells. It has also been proven to regulate the tumor suppressor gene and the p53 gene (30).

Drug molecules containing sulfo and/or sulfur in their structure initiate the apoptotic caspase system with the help of photodynamic therapy, thereby destroying the membrane and mitochondria. While apoptosis was expected to be preferred over the above-mentioned death pathways by metal-based anticancer drugs, other death pathways showed greater activation. It has been reported that Cisplatin, which has been used in clinical applications for years, causes cell death by apoptosis, but rarely causes necrosis (31).

Figure 9 shows images of cisplatin-treated breast cancer cells (MCF-7). When we look at the appearance of breast cancer cell before and after drug administration, it is clearly observed that cisplatin is a very effective drug.

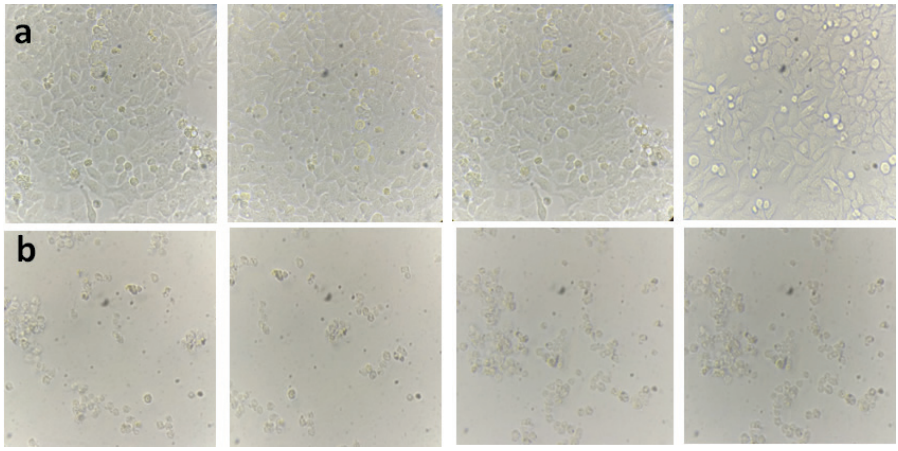


***Figure 9. (a) Non-medicated and (b) Drug-administered (Cisplatin) MCF-7 cell line***

As a result of researches, with the use of platinum-based Cisplatin against cancer, the search for other metal-based drug candidates that are less toxic on healthy tissues and more effective against cancer cells has accelerated.

In the light of the literature information; Gemstabin has a water-soluble structure thanks to the active groups it contains. It has been proven that this structure acts like cisplatin and inhibits DNA (genetic material) synthesis and acts against tumor cell DNA. Although not as effective as cisplatin, it has been proven to inhibit DNA synthesis of tumor cells (Figure 10). It is clearly seen that the drug molecule Gemstabin produces less toxic effects than Cisplatin (29-31).

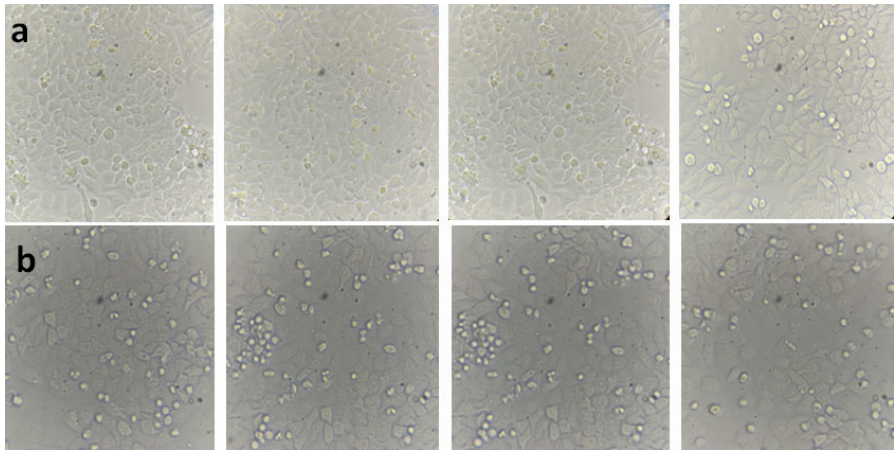


***Figure 10. (a) Non-medicated and (b) Drug-administered (Gemstabin) MCF-7 cell line***

These studies, thanks to the groups and structures of drug molecules and derivatives of different structures against cancer cell structures; DNA, mitochondria, growth factors and their effects on cell cycles illuminate the mechanisms in cell death.

The more cytotoxic effects of drug molecules on the cancer cell line, the effects on cell cycles, DNA structure, growth inhibition or many cell functions such as protein synthesis were better observed with microscopic images. These observed cell lines were evaluated by comparing drug treated and untreated control cells. When we look at Taxol and Docetaxel drug molecules, it is clearly observed in Figure 11 and Figure 12 that they cause similar deaths.

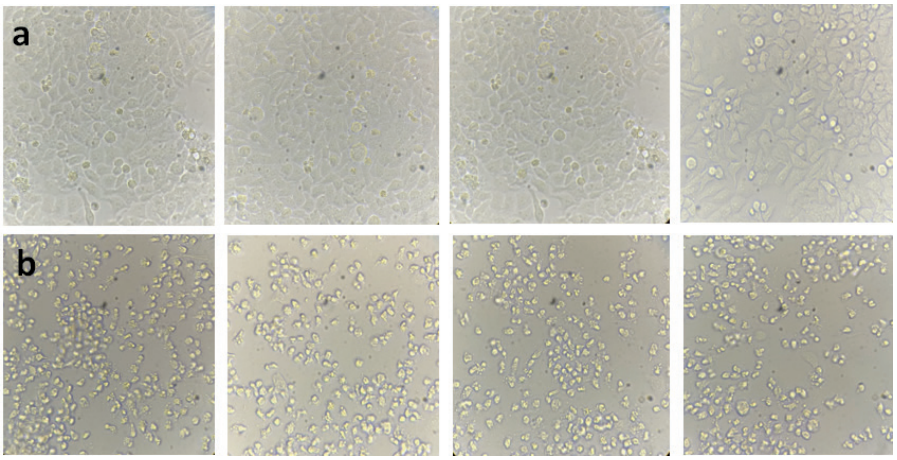


***Figure 11. (a) Non-medicated and (b) Drug-administered (Taxol) MCF-7 cell line***

In general, membrane integrity and cell structure were evaluated by examining the drug-administered cells. Cells with impaired membrane structure are generally observed as the cause of death due to external influence (26). Although the integrity of the membrane is preserved, the deaths observed as a result of intracellular interactions are generally considered as controlled cell death. This explains the occurrence of necrosis (self-death of the cell). It is observed that nuclear fragmentation, which also evokes an apoptic (programmed death of the cell) appearance, creates deep folds and cellular disorders in the cells (28-29). Necrosis and apoptosis processes occur more in cancer cell lines (32). As a result of microscopic images, necrosis and apoptosis processes in cancer cell lines were clearly demonstrated.

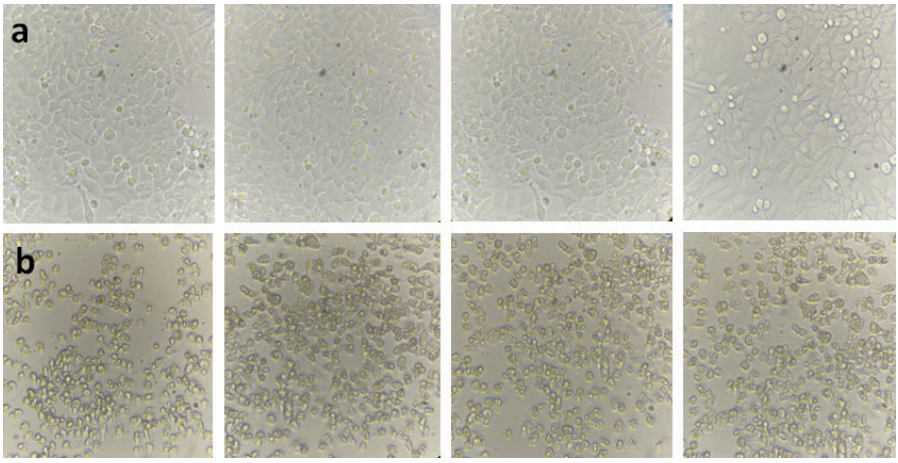


***Figure 12. (a) Non-medicated and (b) Drug-administered (Docetaxel) MCF-7 cell line***

In all studies, the mechanism of drug molecules against cancer cells is explained (33). Considering these mechanisms, the effects of drug molecules on cell growth and division have been studied directly and indirectly. In addition to the cytotoxic effects of drug molecules against cancer cells, it is also important that they do not harm healthy cells. In this study, the toxic effect and mechanism of 4 different drug molecules on breast cancer cell line were investigated. Binding of amine side chains of different drug molecules to single-stranded DNA can inhibit cell proliferation by causing DNA replication to be inhibited (32-33).

The drug molecules have the ability to induce apoptosis in endothelial cells and cardiomyocytes through the activation of cardiomyopathy, p53 protein, and reactive oxygen species.

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