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Elimination of Reactive Oxygen Species Formed by Chemotherapeutic Agent in Imatinib Resistant K562r Cell Line by Sweetgum Oil

İmatinib Dirençli K562r Hücre Hattında Kemoterapötik Ajanların Oluşturduğu Reaktif Oksijen Türlerinin Sığla Yağı ile Ortadan Kaldırılması

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ABSTRACT

Objective: The antibacterial, antioxidant, antiseptic, and anti-inflammatory properties of Sweetgum oil (SO), a resinous exudate obtained from the injured trunk of the Liquidambar orientalis tree and named locally as "SO", have been reported in many studies.

Methods: In this study, cytotoxic doses of imatinib and ponatinib combined with SO were applied to determine differences in reactive oxygen species (ROS) formation in resistant K562R and susceptible K562S cell lines and to observe the effects of ROS on autophagy. Cytotoxicity, ROS formation, DNA damage due to ROS, autophagy, and the expression of Atg4A, Atg5, LC3α/β proteins in cell lines were investigated. In the cytotoxicity studies, the IC_{50} values of SO in K562R and K562S cells were determined as 250 µg/mL and 150 µg/mL.

Results: 21.9% more ROS was observed in K562R cells. It was observed that the amount of ROS formed in the cells to which SO was applied was 28.8% less in K562R cells and 23.8% in K562S cells. In combined applications, ROS was decreased by 67.56% in K562R cells and by 60.9% in K562S cells. The effects of SO on autophagic activation were observed by fluorescence microscopy.

Conclusion: SO increased autophagic activation compared with ponatinib in K562R cells and decreased autophagic activation compared with imatinib in K562S cells. Expression levels of Atg4A, LC3α/β and Atg5 indicate that autophagy is induced and ROS formation is reduced in combined applications.

Keywords: K562R, K562S, Sweetgum oil, ROS, autophagy

ÖZ

Amaç: Liquidambar orientalis ağacının yaralı gövdesinden elde edilen ve yerel olarak "Sweetgum yağı (SO)" olarak adlandırılan reçineli bir eksüda olan SO'nun antibakteriyel, antioksidan, antiseptik ve anti-enflamatuvar özellikleri birçok çalışmada bildirilmiştir.

Yöntemler: Bu çalışmada, dirençli K562R ve duyarlı K562S hücre hatlarında ROS oluşumundaki farklılıkları belirlemek ve reaktif oksijen türlerinin (ROS) otofaji üzerindeki etkilerini gözlemlemek için SO ile kombine edilmiş sitotoksik imatinib ve ponatinib dozu uygulanmıştır. Hücre hatlarında sitotoksisite, ROS oluşumu, ROS'ye bağlı DNA hasarı, otofaji ve Atg4A, Atg5, LC3α/β proteinlerinin ekspresyon seviyeleri araştırıldı. Sitotoksisite çalışmalarında, SO'nun K562R ve K562S hücrelerindeki IC_{50} değerleri 250 µg/mL ve 150 µg/mL olarak belirlendi.

Bulgular: K562R hücrelerinde %21,9 daha fazla ROS gözlendi. SO uygulanan hücrelerde oluşan ROS'nin K562R hücrelerinde %28,8, K562S hücrelerinde ise %23,8 daha az olduğu gözlendi. Kombine uygulamalarda K562R hücrelerinde ROS'nin %67,56, K562S hücrelerinde ise %60,9 azaldığı görülmüştür. SO'nun otofajik aktivasyon üzerindeki etkileri floresan boyalarla boyanarak floresan mikroskopi ile gözlemlendi.

Sonuç: K562R hücrelerinde SO'nun ponatinibe göre otofajik aktivasyonu artırdığı, K562S hücrelerinde ise imatinibe göre otofajik aktivasyonu azalttığı gözlenmiştir. Atg4A, LC3α/β ve Atg5 proteinlerinin ekspresyon seviyeleri kombine uygulamada otofaji indüksiyonunun sağlandığını ve ROS oluşumunun azaldığını göstermektedir.

Anahtar Sözcükler: K562R, K562S, Sığla yağı, ROS, otofaji

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INTRODUCTION

Chronic myeloid leukemia (CML) is a myeloproliferative tumor in which a monoclonal, multipotent hematopoietic progenitor cell is caused by reciprocal translocation and subsequent proliferation. Because of the resulting Philadelphia chromosome, the oncogene BCR-ABL fusion protein exhibited increased tyrosine kinase activity (1). Additional chromosome numbers change from CML to conversion from chronic phase to acute phase. Extensive chromosome G-banding studies reveal host analytical coincidence and often an extra-health condition of trisomy 8, isochromosome 17q, trisomy 19, or Philadelphia chromosomes. The cell line K562, which was derived from CML, has been widely used since its first review in 1975 (2). The Philadelphia chromosome is present in 90% of CML cases. The remaining parts exhibit complex translocations (3).

Imatinib is a tyrosine kinase inhibitor that is used as first-line therapy for CML and is highly effective for treating CML patients (4,5). With the use of imatinib, it has been demonstrated that approximately 80% of CML patients in the chronic phase achieve complete cytogenetic remission during the 12-month treatment period (6). Expenditures of chronic phase CML are prolonged, and imatinib resistance develops, making the patient resistant to imatinib treatment (7,8). Ponatinib has been approved for the treatment of patients with CML resistant or intolerant to previous TKI therapy and patients with Ph+ (Philadelphia Chromosome) Acute Lymphoblastic Leukemia (9). Solutions reported by O'Hare et al. (10) in which parts of BCR-ABL T315I refills can be stored in ponatinib treatment-resistant CML patients.

Sweetgum oil (SO) is obtained from the liquidambar orientalis mill (Hamamelidaceae) and has been used as antiulcerogenic in Turkish folk medicine for centuries (11). The antibacterial activities of SO with *in vitro* techniques performed by Sağdiç et al. (12). Antioxidant activity was determined using the DPPH test by Topal et al. (13). The antioxidants of SO Suzek et al. (14) were studied *in vivo* in a summarized manner. Since SO has protective properties and acts as an antioxidant; thus, they are modified by the arrangement of their molecular tissues (14).

Liquidambar orientalis is widespread in the southwestern coastal regions of Türkiye in Köyceğiz, Fethiye, Marmaris, and Ula (14). SO contains 45% cinnamic acid. Cinnamic acid is a phenolic plant that has antioxidant, antibacterial, and anti-inflammatory properties produced with plant extracts containing cinnamic acid and propolis. However, some changes from these replaceable cinnamic acids are also observed to be protected from lipid peroxidation and damage by various oxidative components. Although there is no detailed information about its pharmacokinetics in the human body, SO has antioxidant, anti-inflammatory, and antimicrobial effects based on its cinnamic acid content (15).

Extraction of Sweetgum Oil

In order to extract oil from the sweetgum tree, the bark on the injured parts of the trees is first chipped and thinned. With a tool called a spoon, the wounds called veins are opened. The process of rejuvenating the wounds after a week is called sur. Two weeks after the procedure, the fat accumulated in the veins is removed with a spoon. This process lasts from mid-July to October's end. Chips containing bark and wood with oil are boiled in copper pots for 30-90 minutes with water. Then, SO is extracted by compressing it with a needle (15).

ROS are cellular molecules produced as a byproduct of mitochondrial oxidative metabolism or NADPH oxidase enzymes. ROS consists of radical and non-radical oxygen species including superoxide anion (O_2-), hydrogen peroxide (H_2O_2), and hydroxyl radical (-OH) (16). ROS at normal concentrations; they act as important second messengers that play a role in various signal transduction events that regulate the growth, proliferation, and differentiation of cells (17). ROS can cause DNA damage and thus induce transformation. Transformed cells are widely accepted to be associated with cancer because of their higher ROS production than normal cells. Increased ROS production; In addition to being associated with genomic instability and DNA damage, ROS performs a signaling function to promote cell proliferation and migration and contributes to leukemic cell transformation (16).

ROS and autophagy are key regulators of cellular homeostasis in the human body. Autophagy cooperates with ROS to maintain cellular homeostasis. Autophagy can be induced by ROS and inhibits ROS-induced damage to cells and tissues (17). Both oxidative stress and autophagy are described as protective and harmful pathways in response to cellular stressors. Direct redox-based regulation of autophagy occurs through H_2O_2-inhibited oxidation of Atg4 by H_2O_2, which suppresses delipidation of LC3-II. More slowly and indirectly, oxidative stress also regulates the transcription of Beclin 1 and LC3. The overproduction of ROS carries the risk of serious damage to the mitochondria and must be partially removed. This process occurs via selective autophagic degradation of damaged mitochondrial fragments, which is called "mitophagy" (18,19).

The aim of this study was to use SO, which has antioxidant activity reported in the literature, to eliminate ROS caused by the use of chemotherapeutic agents and thus prevent DNA damage in the K562R CML cell line, which has developed resistance to imatinib in the advanced stages, which is used as first- line treatment in the treatment of CML patients. to investigate its usage.

MATERIALS AND METHODS

SO, imatinib, and ponatinib were purchased commercially.

Cell Lines

The K562R cell line obtained from the pleural fluid of a 53-year-old female patient in the CML blast crisis period was grown in RPMI 1640 medium containing 10% FBS (Fetal Bovine Serum), 2 mM L glutamine, 1% penicillin/streptomycin and 1 µM imatinib at 37 °C in a 5% CO_2 incubator. The K562S cell line, an erythroid-myeloid precursor cell line, derived from a 53-year-old female patient in terminal blast crisis was grown in RPMI 1640 broth containing 10% FBS, 2 mM L glutamine, and 1% penicillin/streptomycin at 37 °C in a 5% CO_2 incubator.

Cytotoxicity Studies

Cytotoxic effects were determined using the methylthiazole diphenyl tetrazolium (MTT) method, which is a cell proliferation test based on the measurement of metabolic activity. An increase in mitochondrial succinate dehydrogenase activity is observed in cells proliferating according to their activities in mitochondria. MTT (Glentham Life

Sciences) dye is catalyzed by mitochondrial succinate dehydrogenase and reduced to dark blue formazan salts. Formazan formation occurs only in living cells with active mitochondria.

ROS Detection

An ABP ROS Assay Kit (catalog number: A057) was used for ROS detection. ROS generation was induced by incubation with 105 cells/mL chemicals. Negative controls were prepared without an inducing agent. For the positive control, a 50 mM tert-butyl hydroperoxide (TBHP) stock solution was prepared by adding 3.2 µL of 70% TBHP into 496.8 µL of phosphate buffered salt solution (PBS). The final concentration was then adjusted to 200 µM and incubated for 30-60 min under normal growth conditions. Dilute the H2DCFDA stock solution 1:1000 in pre-warmed buffer [Hanks' Balanced Salt solution (HBSS) or HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)] to detach cells from the growth medium to provide a final working concentration of 10 µM dye. Cells were incubated at 37 °C for 10-30 minutes. After removing the loading buffer, the cells were washed three times with prewarmed buffer (HBSS or HEPES). Cells were returned to pre-warmed growth medium and incubated at 37 °C for 10-20 minutes. It was immediately observed by fluorescence microscopy.

Determination of DNA Damage

DNA laddering tests were performed to determine DNA damage. Cells treated with imatinib, ponatinib, SO, or combined doses at IC_{50} values determined by the MTT assay and cells from the control group without the drug were incubated for 24 h. After incubation, the media from the flasks were collected into a 1.5 mL collection tube and centrifuged at 13,200 g and 40 °C for 5 min, and the supernatant was discarded. Flasks were washed with PBS, scraped, collected in the same collection tube, and centrifuged at 13,200 g for 2 min. After discarding the supernatant, 600 µL Lyzis Buffer (100 µL 10 mM tris-HCl + 200 µL 0.1 M EDTA + 500 µL 0.5% SDS + 7500 µL dH2O) was added to the pellet and left on ice for 1 h. After 1 h, the sample was centrifuged at 13,200 g for 10 min. The upper phase was taken into a new collection tube, 5 µL of RNase and 4 µL of Proteinase K were added and it was waited at 37 °C for 1 h. We centrifuged the mixture at 13,200 g for 15 min by adding phenolchloroform at a ratio of 1:1. The supernatant was taken into a new collection tube, and 1/10 of the volume of 3M Ammonium acetate pH: 5.2 and 2 times the volume of absolute ethanol were added. The samples were centrifuged at 13200 g for 2 min after incubating at 200 °C overnight. After the ethanol had dried, the pellet was dissolved in 25 µL of TE (Tris-EDTA). DNA sample was then mixed at a ratio of 1:5 in 150 mL of 3% agarose gel and loading dye were performed at 70 V for 120 min.

Hoecsht (33342) staining was performed to detect DNA damage. Cells were cultured not to exceed $1x10^6$ cells/mL. The test reagents were applied to the cells and incubated for 24 h. The cells were then separated from the medium and washed 2 times with 1X assay buffer. The supernatants were discarded and suspended with 100 µL of Hoecsht (33342) dye. After incubation at 37 °C for 30 min, the cells were washed with 1X assay buffer. It was suspended in 100 µL of 1X assay buffer. One drop of the cell suspension was applied on 1 slide and covered with a coverslip, and images were taken under a fluorescence microscope.

Determination of Autophagy

A CYTO-ID Autophagy Detection Kit (catalog no: ENZ-51031) was used to detect autophagy. Cells were cultured not to exceed $1x10^6$ cells/mL. Cells were collected via centrifugation, and test reagents and positive and negative controls were applied to the cells. After 24 h of incubation, cells were removed from the medium and washed 2 times with 1X assay buffer. The supernatant was discarded and suspended with 100 µL microscopy dual detection reagent. The cells were incubated at 37 °C for 30 min. Cells were washed with 1X assay buffer and suspended with 100 µL of 1X assay buffer. One drop of the cell suspension was applied on 1 slide and covered with a coverslip, and images were taken under a fluorescence microscope.

Immunoblotting Method

From the cell lysates, the protein concentrations determined by the Bradford test were taken into a new collection tube so that each protein sample contained an equal amount of protein. The proteins were denatured by adding 2X laemmli buffer to each collection tube at a ratio of 1:1 and incubating for 5 min at 95 °C. After denaturation, the samples were placed on ice. After the cell lysates, whose protein concentrations were determined, were loaded into the gel wells, the proteins were sorted by first passing through the loading gel and then through the dissociative gel via electric current. In order to detect the presence of the desired protein on the gel, the proteins listed according to their molecular weights were passed to the membrane with the help of an electric current. In order to show the protein sought on the membrane with the help of specific antibodies, primary and secondary antibody markings were made, and the desired proteins were shown.

Statistical Analysis

The data obtained are the average of at least 3 replicates. Data were analyzed using Graph Pad Prism 9.0 and Image J program. Independent t-tests were used for comparisons.

RESULTS

Cytotoxicity Studies

Because K562R cells are imatinib-resistant, cytotoxicity assays were performed on cells grown both with and without imatinib. To determine the effect of DMSO (dimethyl sulfoxide), in which the active ingredients of SO and imatinib are dissolved, on cell viability, cells were treated with DMSO, and it was observed that there was no significant effect on cell viability. The IC_{50} value of SO was determined as 250 µg/mL in both cases. The IC_{50} of ponatinib was determined as 0.015 µM in growth medium containing imatinib and 0.020 µM in growth medium without imatinib. In their combined application, cytotoxicity experiments were also performed in both imatinib-containing and imatinib-free growth media. In order to minimize the side effects of ponatinib, a lower dose of ponatinib was found to be

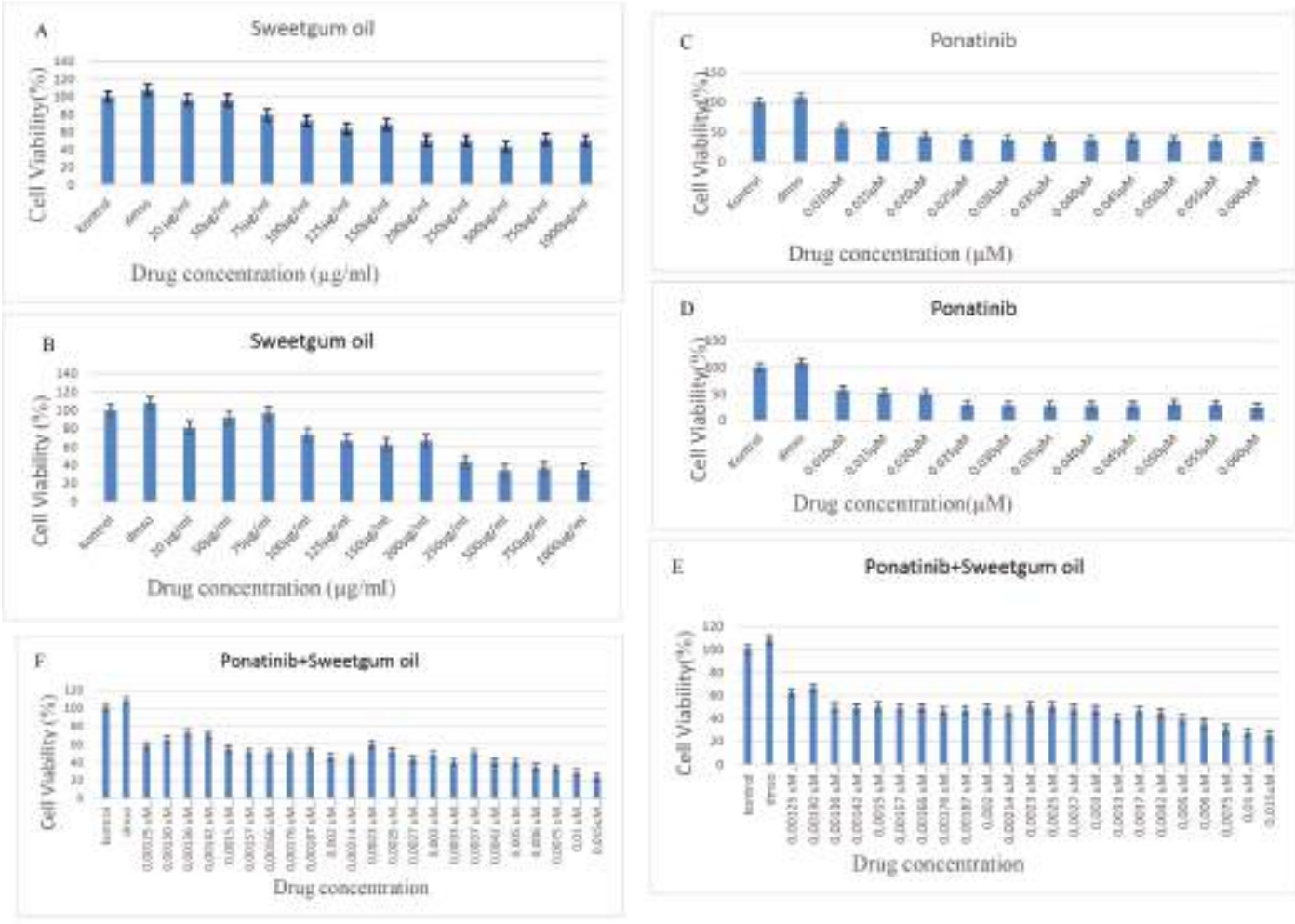


Figure 1. MTT results in K562R cells (A) Sweetgum oil MTT results in growth medium containing 1 µM imatinib in K562R cell line (IC_{50} value 250 µg/mL) (B) Sweetgum oil MTT results in growth medium containing imatinib in K562R cell line (IC_{50} value 250 µg/mL) (C) Containing 1 µM imatinib Ponatinib MTT results in growth medium (IC_{50} value 0.015 µM) (D) Ponatinib MTT results in growth medium without imatinib (IC_{50} value 0.020 µM) (E) Ponatinib and Sweetgum oil combined MTT results in growth medium containing 1 µM imatinib (IC_{50} value 0.0015 µM Ponatinib + 150 µg/mL Sweetgum oil) (F) Combined MTT results of ponatinib and Sweetgum oil in imatinib-free growth medium (IC_{50} value 0.0037 µM Ponatinib + 150 µg/mL Sweetgum oil).

effective (0.0015 µM instead of 0.0037 µM) when 150 µg/mL SO was used in the presence of imatinib, and the group containing low doses of ponatinib and imatinib was used for future experiments. The group was named combined 1. In order to assess the effect of imatinib in the experiments, another combined dose was also applied, and it was termed combined 2 (Figure 1-5).

In K562S cells, the IC_{50} value of SO was determined as 150 µg/mL, and the IC_{50} value of imatinib was determined as 10 µM. The combined IC_{50} value was 2 µM imatinib + 100 µg/mL SO. A smaller amount of SO was sufficient in K562S cells compared with K562R cells.

ROS Detection

It was determined that the amount of ROS formed in the group treated with SO in K562R cells was 28.8% less than the group treated with ponatinib. When combined applications are examined, ROS is 67.56% lower in combined applications than in single applications of SO (Figure 2).

In K562S cells, on the other hand, it was observed that the ROS formed in the group treated with SO was 23.8% lower than that in the group administered imatinib. In the combined application, the ROS was 60.9% less than that in the single application of SO (Figure 6).

Detection of DNA Damage

DNA breaks were visualized using agarose gel electrophoresis and Hoecsht (33342) dye using a fluorescence microscope 24 h after the application of determined doses of imatinib, ponatinib, and SO to K562R and K562S cells. It was observed that DNA damage increased with imatinib and ponatinib treatment compared with the control and SO groups (Figures 3, 7).

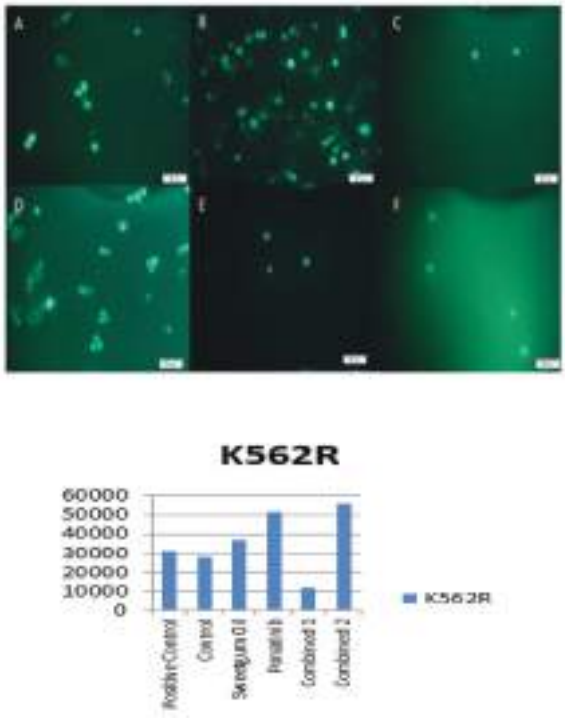


Figure 2. ROS graph of ROS in K562R cells with fluorescent microscope images (40x) (A) Positive control (B) Control (C) Sweetgum oil (D) Ponatinib (E) 0.0015 µM ponatinib + 150 µg/mIL Sweetgum oil (F) 0.0037 µM ponatinib + 150 µg/mL Sweetgum oil.

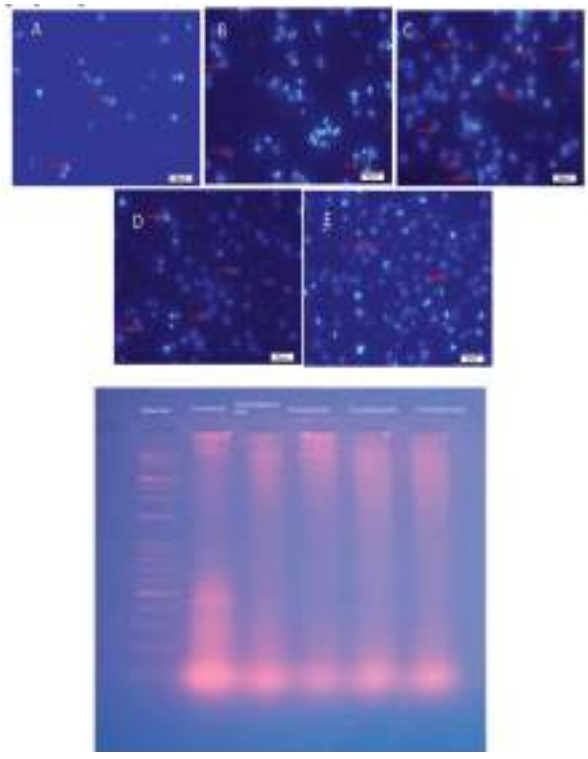


Figure 3. Demonstration of DNA breaks in K562R cells by fluorescent microscope (40x) and showing DNA breaks by agarose gel electrophoresis A) Control (group without any chemical application) B) Sweetgum oil C) Ponatinib D) 0.0015 µM Ponatinib + 150 µg/mL Sweetgum oil (E) 0.0037 µM ponatinib + 150 µg/mL Sweetgum oil (Arrows indicate DNA breaks).

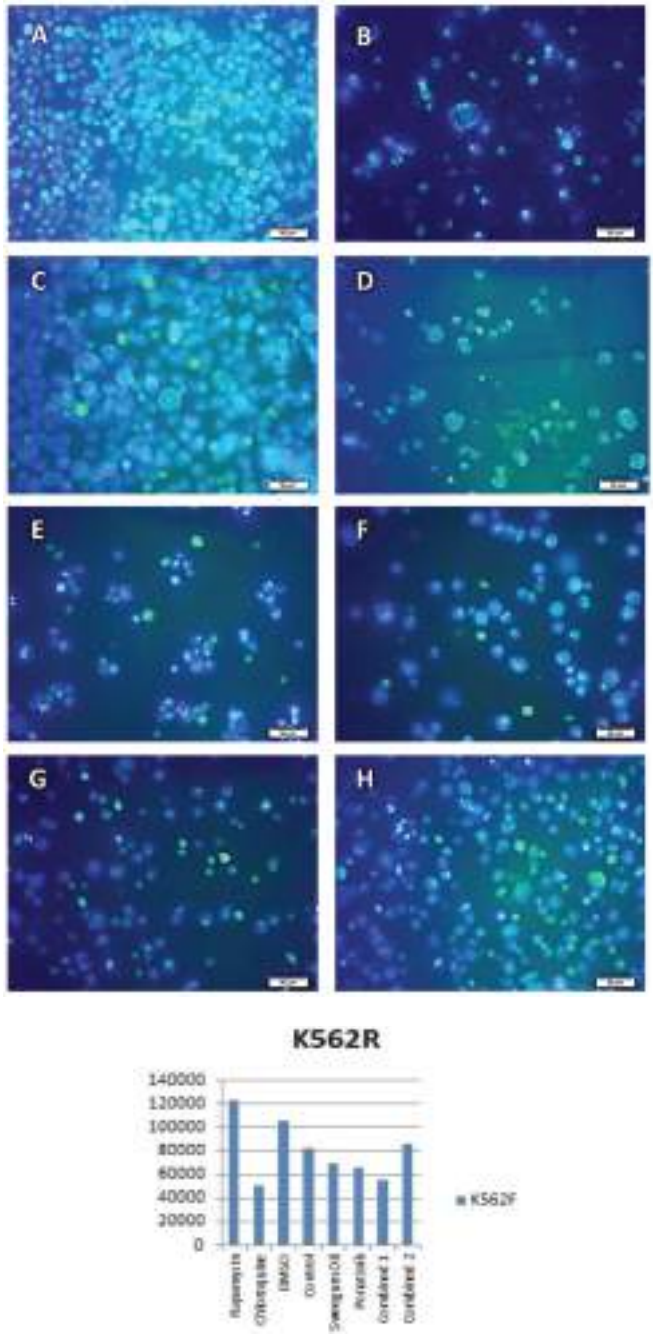
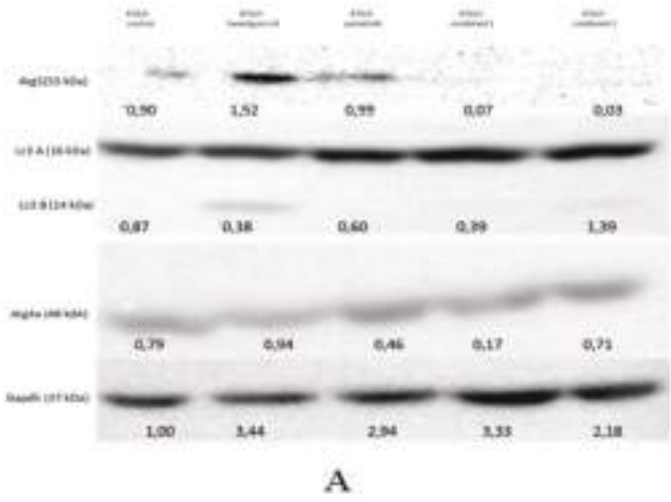


Figure 4. Graph of autophagic activation with fluorescent microscope images of autophagy formed in K562R cells at x40 magnification (A) Rapamycin (B) Chloroquine (C) DMSO (D) Control (E) Sweetgum oil (F) Ponatinib (G) 0.0015 µM Ponatinib + 150 µg/mL Sweetgum oil (H) 0.0037 µM ponatinib + 150 µg/mL Sweetgum oil.

Autophagy Determination

After K562R and K562S cells were incubated with the drugs at the indicated doses for 24 h, the kit procedure was applied, and images were obtained using a fluorescence microscope. It was observed that SO increased autophagic activation in K562R cells compared with ponatinib and decreased autophagic activation in K562S cells compared with imatinib (Figures 4, 8).



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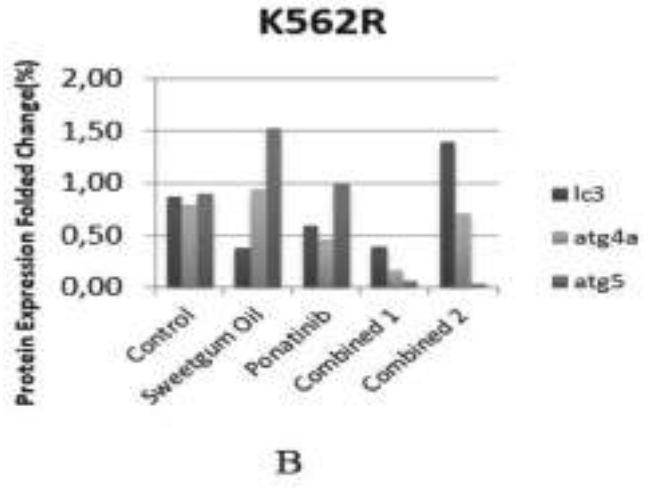

B

Figure 5. (A) Western blot images of Atg5, Atg4A, LC3α/β and GAPDH proteins in K562R cells (B) Folded change graph of Atg5, Atg4A, LC3α/β and GAPDH proteins in K562R cells.

Immunoblotting Method

In the western blotting experiment, the expression of LC3-I in K562R cells decreased in the SO-treated group compared with the control group. Cytosolic LC3-I is converted to LC3-II by adding phosphatidetetanolamine (PE), which means that autophagy is induced. An increase in Atg5 expression in SO means that autophagy is activated. As ROS levels decreased, Atg4A expression increased in the group treated with SO (Figure 5).

It was observed that LC3-I expression in K562S cells increased with the administration of SO and imatinib compared with that in the control group and decreased with the combined administration. As in K562R cells, SO appears to increase Atg5 expression in K562S cells. Autophagy induction appears to be achieved by combined treatment. Since ROS levels decreased in K562S cells, Atg4A expression also increased in the SO-treated group (Figure 9).

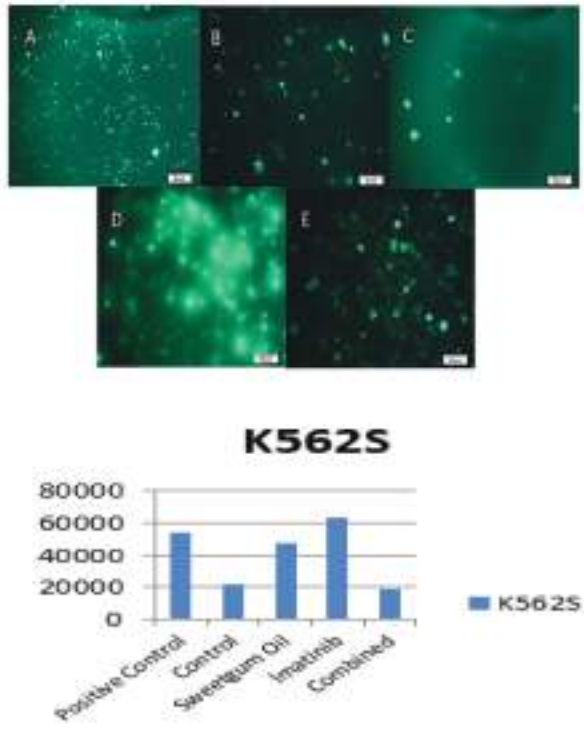

Figure 6. ROS plot of ROS in K562S cells with fluorescent microscope images (x40) (A) Positive control (B) Control (C) Sweetgum oil (D) Imatinib (E) 2 µM imatinib + 100 µg/mL Sweetgum oil.

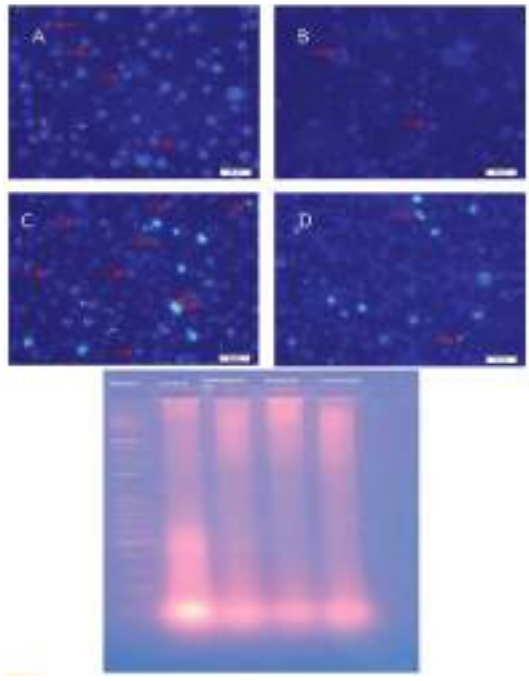

Figure 7. Demonstration of DNA breaks in K562S cells by fluorescent microscopy (x40) and DNA fragmentation by agarose gel electrophoresis (A) Control (the group that did not apply any chemicals) (B) Sweetgum oil (C) Imatinib (D) 2 µM imatinib + 100 µg/mL Sweetgum oil (arrows indicate DNA breaks).

DISCUSSION

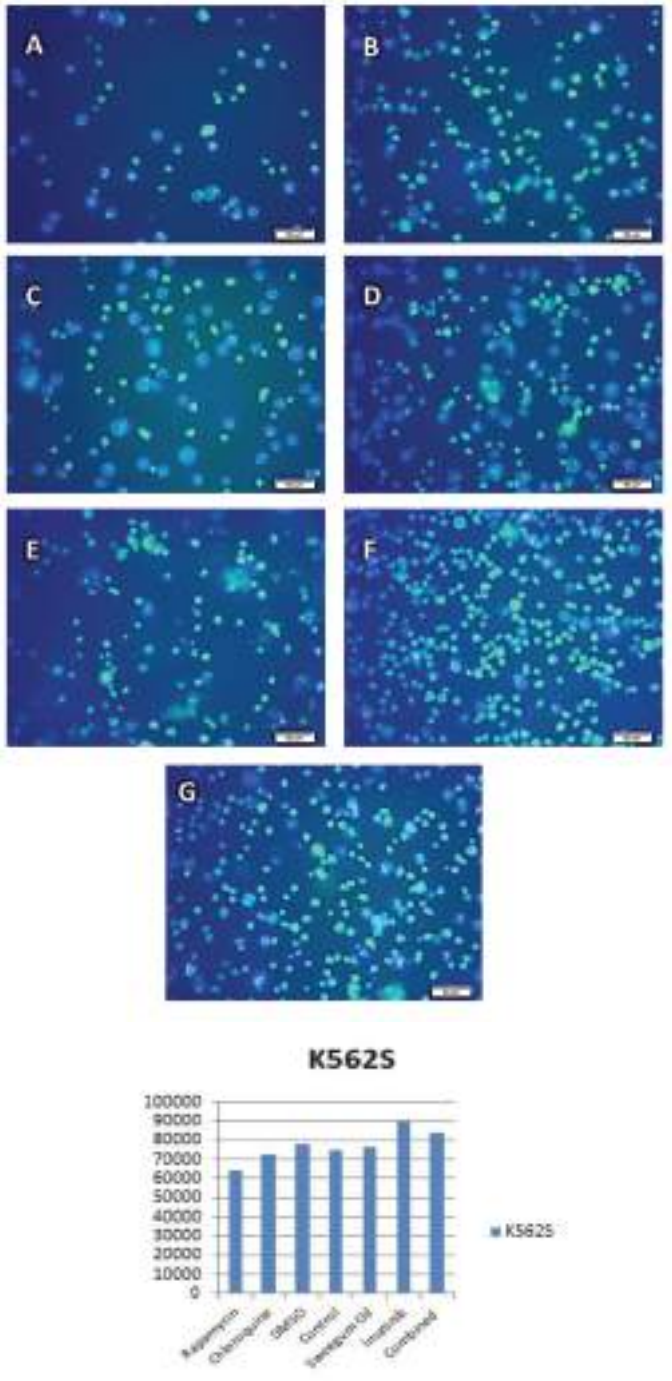

Figure 8. Graph of autophagic activation with fluorescent microscope images of autophagy formed in K562S cells at x40 magnification (A) Rapamycin (B) Chloroquine (C) DMSO (D) Control (E) Sweetgum oil (F) Imatinib (G) 2 µM imatinib + 100 µg/mL Sweetgum oil.

In this study, the effects of imatinib resistant K562R cell line and sensitive K562S cell lines, the effects of ROS on DNA resulting from the use of chemotherapeutic agents, and the effects of ROS on autophagy were investigated. Antioxidant activity of SO2 [Topal et al. (13) DPPH test]. Suzek et al. (14) extensively investigated the antioxidant activity of SO2 *in vivo*.

In this study, the MTT assay was performed to determine the

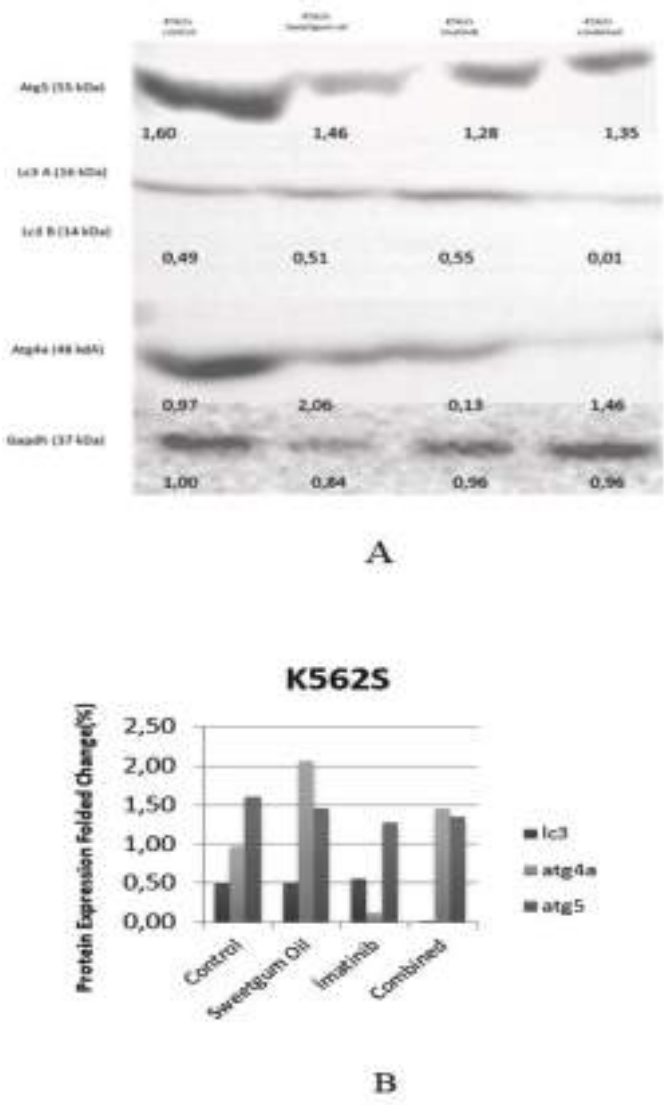

Figure 9. (A) Western blot images of Atg5, Atg4A, LC3α/β and GAPDH proteins in K562S cells (B) Folded change graph of Atg5, Atg4A, LC3α/β and GAPDH proteins in K562S cells.

cytotoxicity of the drug and SO in cell lines. Because of the MTT assay, a decrease in the viability of K562R and K562S cells was observed with increasing doses of SO (Figure 1, 10). In a previous study, it was shown that some substances in SO can have cytotoxic effects and can be a source of oxidative stress. In this study, it was emphasized that SO exerts its cytotoxic properties through DNA damage and is the source of the antimicrobial effect of SO (20). It was stated in a previous study that SO is a plant oil with antioxidant and oxidative effects (21). When SO was used, ROS formation in K562R and K562S cells was lower compared with ponatinib and imatinib. When combined applications were examined, ROS levels were decreased in K562R and K562S cells compared with the single application of SO. In this case, the application of SO together with tyrosine kinase inhibitors to the K562R and K562S cell lines has more positive effects on ROS than single application (Figure 2, 6). In addition, TKI- resistant cells may have higher ROS production than TKI-sensitive cells (22). When ROS production was compared between K562R cells showing

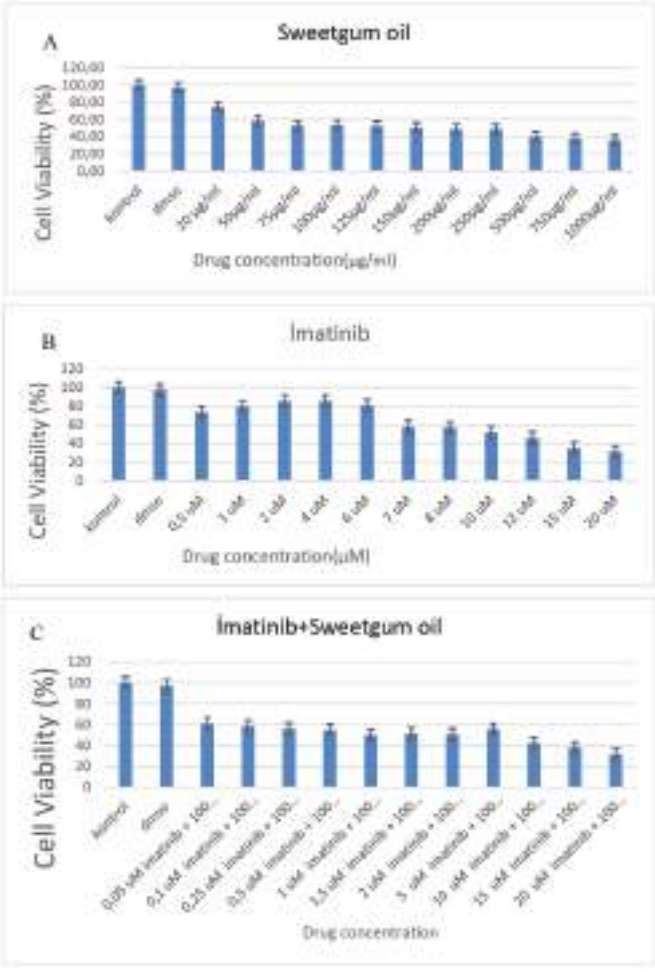

Figure 10. MTT results in K562S cells (A) MTT results of Sweetgum oil in K562S cell line (IC_{50} value 150 µg/mL) (B) MTT results of imatinib in K562S cell line (IC_{50} value 10 µM) (C) MTT results of combined imatinib and Sweetgum oil in K562S cell line (IC_{50} value 2 µM imatinib + 100 µg/mL Sweetgum oil).

TKI resistance and sensitive K562S cells not showing TKI resistance, more ROS was observed in K562R control cells compared with K562S control cells, which is consistent with the data in the literature. In this study, Hoecsht (33342) staining with the DNA laddering assay was performed to demonstrate ROS-induced DNA breakage and apoptosis (Figure 3, 7). More DNA breakage was observed in cells treated with imatinib and ponatinib in the K562R and K562S lines. These results demonstrate the relationship between ROS and DNA damage. Owing to its antioxidant properties, SO prevents the formation of ROS. ROS oxidizes cysteine amino acids in the catalytic region of Atg4A and inactivates Atg4A. It was observed that Atg4A expression was higher in cells treated with SO and decreased Atg4A expression in other groups with high ROS (Figure 5). An increase in Atg4A expression was observed in K562S cells treated with SO compared with the control group (Figure 9). These results support H2DCFDA and Hoecsht (33342) staining results. ROS and autophagy interact to maintain cellular homeostasis. Despite the increase in ROS in SO in K562R cells compared with control cells, it caused a decrease in autophagy activation, which was examined under fluorescence microscopy (Figure 4). The same is true for ponatinib. On the contrary, in K562S cells, autophagy increased with ROS increase (Figure 8). This suggests that the resistance formed in cells affects the working systems of ROS and autophagy.

When autophagy is induced in cells, it is converted to LC3-II by adding PE to cytosolic LC3-I and localizing to the LC3-II autophagosome membrane. Therefore, LC3-II expression is an indicator of autophagy (23). The expression levels of Atg5 and LC3 proteins were examined to assess autophagy. Under fluorescence microscopy, increased ROS levels suppressed autophagy in K562R cells. In autophagy, the elongation of the vesicle membrane and formation of a vesicle are catalyzed by the covalent attachment of the Atg12 protein to the Atg5 protein in the initial ubiquitin-like conjugation system. Atg5 bound to Atg12 associates with Atg16 and binds to the outer surface of the insulating membrane. Atg5 expression indicates membrane elongation. Atg5 expression in K562R and K562S cells was increased in the SO-treated group compared with the imatinib and ponatinib. autophagic activation is increased by SO in cells (20,23). In the western blot experiment, LC3-I expression was decreased in the SO group (Figure 5). It was observed that LC3-I expression in K562S cells increased with the administration of SO and imatinib compared with the control group, but decreased with the combined administration (Figure 9).

In studies using SO, it was shown that SO has cytotoxic and antitumor effects on K562R and K562S cells. SO reduced cell proliferation in a dose-dependent manner. Considering the results obtained in this study, which are in line with the literature, it can be seen that the chemotherapeutic agent causes DNA damage by creating oxidative stress while trying to kill cancer cells. At the same time, since antioxidants can exhibit oxidizing properties, attention should be paid to the dose of the antioxidant used. The use of antioxidants in cancer chemotherapy should not prevent the apoptosis or autophagic death of cancer cells. Elucidating signal transduction pathways and molecular mechanisms, including ROS and autophagy, that maintain homeostasis may provide new targets for cancer chemotherapy.

Study Limitations

Considering the results obtained in this study, which are in line with the literature, it can be seen that the chemotherapeutic agent causes DNA damage by creating oxidative stress while trying to kill cancer cells.

CONCLUSION

There is a need for studies examining the effects of SO on many biological processes, such as the cell cycle, homeostasis, migration, and angiogenesis, that support cancer chemotherapy.

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Ethics

Ethics Committee Approval: None.

Informed Consent: None.

Author Contributions

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