

Effects of White and Black Garlic Extracts on Multiple Cancer Cells and Fibroblast Cells After Irradiation

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ABSTRACT

Purpose: Garlic, is one of the oldest cultivated plants in the Liliaceae family. It has been used as a medicine for thousands of years. White and black garlic contain a variety of beneficial minerals, vitamins and many other substances used for human health. It is also used to treat a wide range of diseases including blood pressure and cholesterol, cancer, anti-inflammatory, antioxidant, wound healing, asthma, arthritis, chronic fever, tuberculosis, rhinitis, malaria, severe skin diseases, digestive disorders, diabetes, kidney stones, anemia, jaundice and epilepsy. The aim was to investigate the effects of garlic extract on irradiated colon cancer, neuroblastoma cancer cells and fibroblasts.

Methods: In the study, colon, brain cancer, and fibroblast cells were replicated in a culture environment and treated for 24 hours with white garlic and fermented black garlic extracts. Cell viability was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test. All results were statistically analyzed with GraphPad Prism (Version 8.0.3).

Results: According to the results, the determined doses of garlics were treated with 24-hour garlic doses, after which the cells were first given 2 Gray radiation doses for combined radiation therapy. The MTT test determined cell vitality and evaluated the results through statistical analysis.

Conclusion: According to our study, we have concluded that white garlic has a protective effect on radiation damage, while black garlic increases cancer cell death.

Keywords: white and black garlic extract, cancer cells and fibroblast cells, irradiation

ÖZET

Amaç: Sarımsak, Liliaceae ailesindeki en eski yetiştirilen bitkilerden biridir ve binlerce yıldır tıbbi amaçlarla kullanılmaktadır. Beyaz ve siyah sarımsak, insan sağlığı için faydalı olan birçok mineral, vitamin ve diğer bileşenleri içermektedir. Kan basıncı ve kolesterol, kanser, anti-enflamatuvar, antioksidan, yara iyileşmesi, astım, artrit, kronik ateş, tüberküloz, rinit, sıtma, ciddi cilt hastalıkları, sindirim sistemi bozuklukları, diyabet, böbrek taşı, anemi, sarılık ve epilepsi gibi birçok hastalığın tedavisinde kullanılmaktadır. Bu çalışmanın amacı, sarımsak ekstraktlarının ışınlanmış kolon kanseri, nöroblastoma kanser hücreleri ve fibroblastlar üzerindeki etkilerini araştırmaktır.

Yöntemler: Çalışmada, kolon, beyin kanseri ve fibroblast hücreleri kültür ortamında çoğaltılmış ve beyaz sarımsak ile fermente siyah sarımsak ekstraktlarıyla 24 saat boyunca muamele edilmiştir. Hücre canlılığı, 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolyum bromid (MTT) testi kullanılarak belirlenmiştir. Tüm sonuçlar GraphPad Prism (Sürüm 8.0.3) ile istatistiksel olarak analiz edilmiştir.

Bulgular: Sonuçlara göre, belirlenen sarımsak dozlarıyla 24 saatlik bir muamele sonrası hücrelere 2 Gray radyasyon dozu uygulanmıştır. Kombine radyoterapi uygulaması sonrası MTT testi ile hücre canlılığı ölçülmüş ve sonuçlar istatistiksel olarak değerlendirilmiştir.

Sonuç: Çalışmamıza göre, beyaz sarımsağın radyasyon hasarına karşı koruyucu bir etkisi olduğu, siyah sarımsağın ise kanser hücrelerinin ölümünü artırdığı sonucuna varılmıştır.

Anahtar Kelimeler: beyaz ve siyah sarımsak ekstraktı, kanser hücreleri ve fibroblast hücreleri, ışınlama

The vegetable plant garlic is a member of the Alliaceae family. Garlic is a common spice with numerous health benefits primarily due to its various bioactive compounds such as organic sulfides, flavanoids and polysaccharides (1-4). Because of its many health-promoting qualities, garlic has been utilized in traditional medicine throughout history and across the globe (3, 5, 6). Garlic is a perennial plant that grows in both warm and temperate climatic zones (7, 8). It is one of the most commonly used herbal treatments. When you look at cancer and its side effects, vitamin C and potassium appear to be at the forefront. There are some herbal products and plants that contain the most of these substances and are used as alternatives to cancer treatment. Among them are propolis, garlic, curcumin, and rosehip (9). Scientific studies have identified a variety of health benefits, such as immunomodulatory, liver-protective, kidney-protective, anti-allergic, antioxidant, and anti-cancer properties (10-15). Numerous studies conducted in the last few decades have uncovered its amazing biological properties, which include anti-bacterial, anti-inflammatory, anti-cancer, antioxidant, immunity-boosting, anti-diabetic, and anti-obesity qualities (3). Research on black garlic, a derivative of garlic that has more flavonoids and stronger antioxidant qualities than fresh garlic, has been increasing (3).

Cancer ranks as one of the foremost causes of mortality worldwide. For many cancer forms, cancer therapy remains a problem despite scientific advancements. Patients who get chemotherapy frequently have unpleasant side effects that lower their quality of life. It frequently has an impact on the decline in health as well. Scientists have been studying the role of flavonoids, primarily antioxidants, found in plants in order to find innovative and efficient ways to promote anticancer therapy, particularly with regard to minimizing side effects. To discover novel compounds with anti-cancer qualities and therapeutic potential, extensive research is being done. The development of cancer can be influenced by both internal and external factors. A significant contributor to cancer is the impact of free radicals, including reactive oxygen species produced within the human body (8, 15). Chronic inflammation can lead to increased proliferation, and oxidative stress is frequently linked to neighboring cell alterations that foster the growth of cancer. Owing to this connection, black garlic's anti-inflammatory and antioxidant qualities also function as indirect anti-cancer defenses (8). While numerous epidemiological studies provide promising evidence regarding the role of garlic

in the etiology of gastric cancer, the pharmacological mechanism by which garlic may inhibit gastric cancer is not yet clear. In our study, extracts prepared from geographically indicated Taşköprü garlic and fermented black garlic added to cancer cells medium cultured in vitro and the effects of radiation examined at the cellular level.

Material and Methods

Cell Culture

The ATCC CRL-2266 human neuroblastoma cell line and the HT29 human colon cancer cell line from Thermo Fisher Scientific were the cancer cell lines while the healthy cell line used in this investigation was the ATCC CRL-1459 fibroblast cell line. CRL-2266 and HT29 were selected as standard models for neuroblastoma and colon cancer, respectively, while CRL-1459 represented healthy cells to assess selective toxicity. These well-characterized cell lines ensure reliable and reproducible results, enabling a comprehensive evaluation of treatment effects on both cancerous and healthy cells. The cells came from a global cell bank that is widely utilized. The cells were cultured in RPMI 1640 medium enriched with 10% serum. The conditions of the cells' living environment are replicated in this culture media. Trypan blue staining was used to evaluate cell viability under a microscope. Conditions for conducting experiments were such that cell viability was greater than 95%.

Preparation of Garlic Extracts

Commercially purchased Taşköprü garlic and black garlic were extracted by dissolving them in water or oil. The Lowry method was used for protein quantification in the extractions. Protein quantification was performed for standardization purposes, as each measurement group was expected to contain the same amount of protein content. Measurements were taken according to the protocol of a commercial kit for protein quantification; samples containing 5, 10, 20, 30, 40, and 50 micrograms of protein were prepared. A review of the literature was carried out to establish the appropriate dosages of Taşköprü garlic and black garlic for use in the experiments (16, 17). To assess cytotoxicity in MTT cell viability experiments, both types of garlic were applied to cell lines at doses of 1 mg, 2.5 mg, 5 mg, and 7.5 mg for a duration of 24 hours. Based on the experimental results, a dose of 5 mg of garlic was decided upon for use.

Treatment of Cells with Radiation

The cells were cultured in a medium to achieve >95% viability and a density of 1×10^6 cells/ml. Initially, cell viability was assessed using the MTT method to determine the dose-dependent effect. Briefly, 2×10^4 cells were seeded into each well of a 96-well plate, and cell viability was evaluated 24 hours after treatment with 1 mg, 2.5 mg, 5 mg, and 7.5 mg doses of black and white garlic extracts using the IC₅₀ assay. Following the IC₅₀ experiments, the effective dose was determined to be 5 mg.

To evaluate the effects of garlics and/or 2 Gy radiation, HT29, SH-SY5Y and CCD-18Co cells were treated with 5 mg of both black and white garlic extracts, followed by irradiation (Elekta Synergy, Swedish) with a 2 Gy dose of radiation 24 hours later. The 2 Gy radiation dose is the dose that triggers apoptosis in tumor cells (18, 19). The cell cycle returns to normal 24 hours after 2 Gy irradiation. Our examination was conducted 24 hours post-irradiation to investigate normal cell damage. Cell viability was assessed using the MTT assay 24 hours after irradiation. The results were presented as the mean \pm SEM of four independent experiments.

Determination of Cell Viability

Cell proliferation was determined using the MTT method. The method is based on the reduction of the yellow tetrazolium salt to form purple formazan crystals by metabolically active cells. Living cells contain oxidoreductase enzymes that reduce MTT to formazan, which is insoluble in aqueous solutions. Formazan crystals were dissolved in a solubilization solution, and

the absorbance of the resulting colored solution was measured at 540 nm using a spectrophotometer.

Statistical Analysis

In this study, the statistical analysis of the data was performed using the GraphPad (GraphPad Software, 8.0 Version, Boston, USA) statistical analysis tool. Two-way ANOVA (analysis of variance) is an analytical technique that allows the simultaneous evaluation of two or more factors that have an effect on the dependent variable. This analysis was applied to assess the statistical significance between experiment groups by evaluating the interaction between factors. To perform group comparisons, a non-parametric test was conducted, followed by a Two-way ANOVA test and Post Hoc Tukey's multiple comparison analysis.

Results

IC₅₀ dose determination experiments of white and black garlic on neuroblastoma, colorectal cancer and fibroblast cells

Initially, to determine the dose-dependent effect, cell proliferation was determined 24 hours later in cells treated with extracts at doses of 1 mg, 2.5 mg, 5 mg, and 7.5 mg using the MTT assay. Cell proliferation assay results are shown in Fig1. and Fig2.

According to the experimental results, the 5 mg dose was determined to have the best effect, and a 5 mg dose was used for both black and white garlic in all experiments (Fig1 and Fig2).

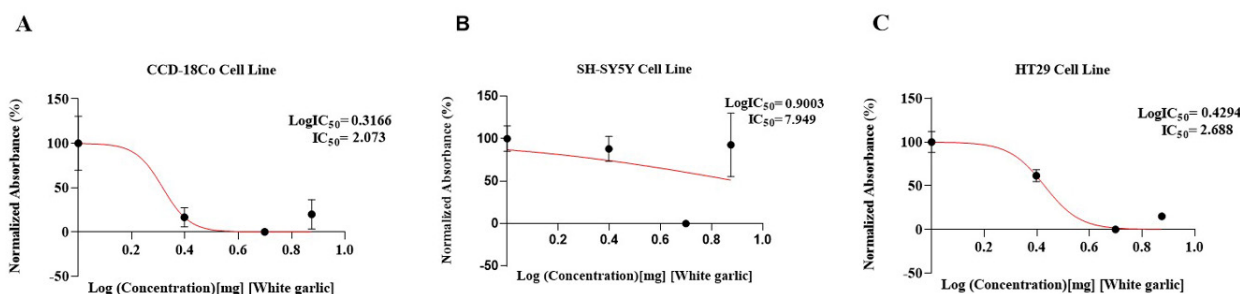


Figure 1: The IC₅₀ values of white garlic in inhibiting the proliferation of SH-SY5Y, HT29, and CCD-18Co cells were determined. Results are presented as the mean \pm SEM of four independent experiments (White garlic concentrations: 1 mg, 2.5 mg, 5 mg, and 7.5 mg; Black garlic concentrations: 1 mg, 2.5 mg, 5 mg, and 7.5 mg)

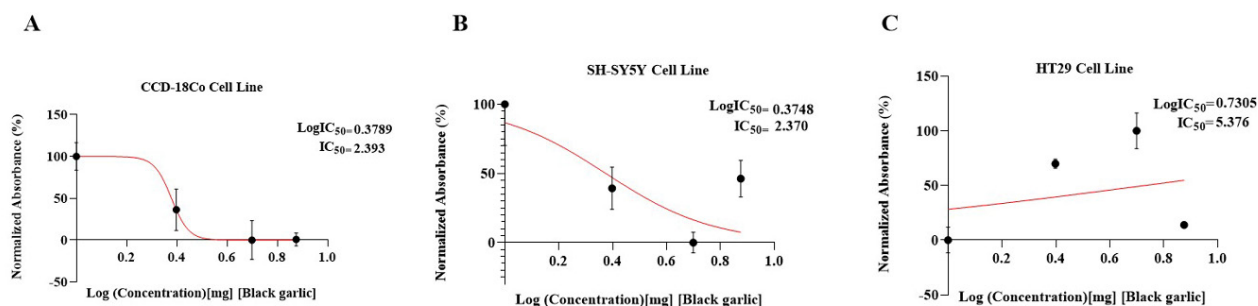


Figure 2: The IC₅₀ values of black garlic in inhibiting the proliferation of SH-SY5Y, HT29, and CCD-18Co cells were determined. Results are presented as the mean \pm SEM of four independent experiments (White garlic concentrations: 1 mg, 2.5 mg, 5 mg, and 7.5 mg; Black garlic concentrations: 1 mg, 2.5 mg, 5 mg, and 7.5 mg).

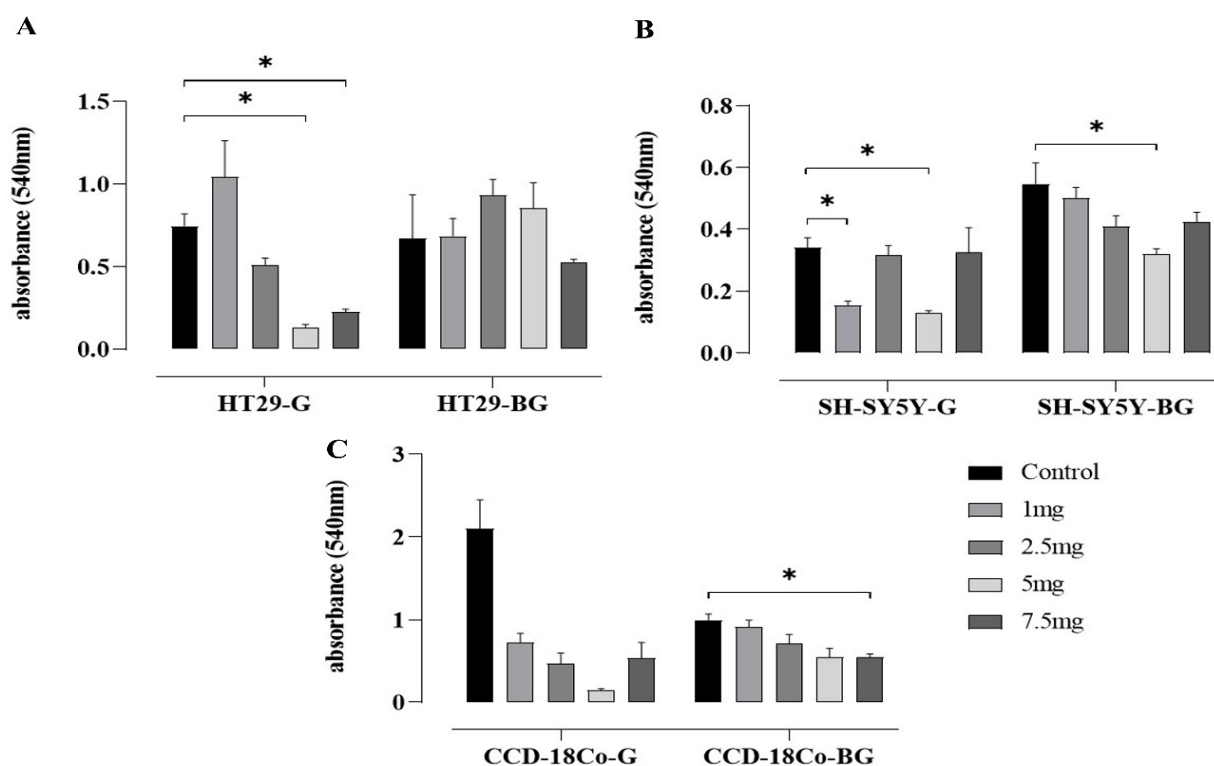


Figure 3: Determination of the cell proliferation data in SH-SY5Y, CDD-18Co, and HT29 cells. The MTT assay is used to determine the IC₅₀ dose of white and black garlic. Cells were incubated with 1 mg, 2.5 mg, 5 mg, and 7.5 mg of white and black garlic for 24 hours (Abbreviations: **G**: white garlic; **BG**: Black garlic).

The effects of white and black garlic on cell proliferation in each cell line are shown comparatively according to increasing doses (Fig3).

In all cell lines, the most effective dose of white garlic was observed to be 5 mg ($p < 0.05$). Similarly, although not statistically significant, the 5 mg dose of black garlic generally showed an inhibitory effect in CCD-18Co, HT29, and SH-SY5Y cells ($p < 0.05$) (Fig 3). Based on these results, a 5 mg dose was used in combination treatments with radiation in all cell lines.

The protective effect of white garlic (GE) on neuroblastoma, intestinal cancer, and fibroblast cells

The cells treated with 5 mg of white garlic extract for 24 hours. They were then irradiated with a 2 Gy dose of radiation, while no radiation was applied to the 1st and 2nd group cells. The effect of garlic was determined based on the results obtained from the MTT assay 24 hours later. White garlic caused a decrease in the proliferation of the HT29 cell line both alone and in combination with radiation ($p < 0.05$). Similarly, white garlic significantly

inhibited proliferation in SH-SY5Y neuroblastoma cells ($p < 0.05$). In healthy fibroblast cell lines used as a control, a 2 Gy radiation dose also significantly reduced viable cells ($p < 0.001$) (Fig4). Overall, when cells were treated with white garlic in combination with radiation, an effect on cell proliferation was observed.

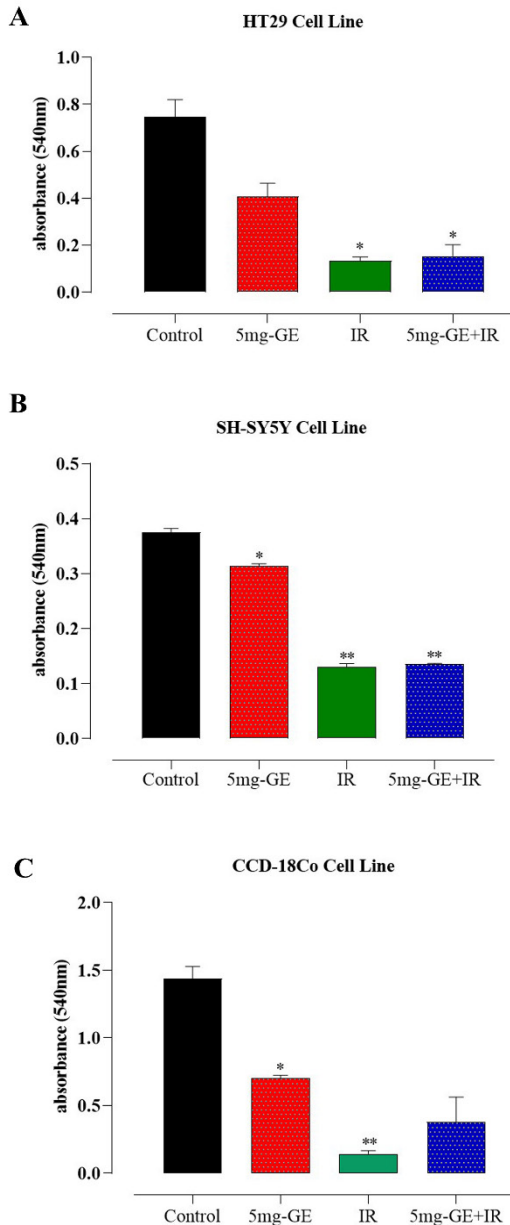


Figure 4: The effect of radiation in combination with white garlic on SH-SY5Y, CDD-18Co, and HT29 cells. The MTT assay demonstrates the combined effect of white garlic with radiation. Cells were incubated with 5 mg of white garlic for 24 hours followed by incubation with a 2 Gray radiation dose for an additional 24 hours (Abbreviations: GE, white garlic extract; IR, ionizing radiation)

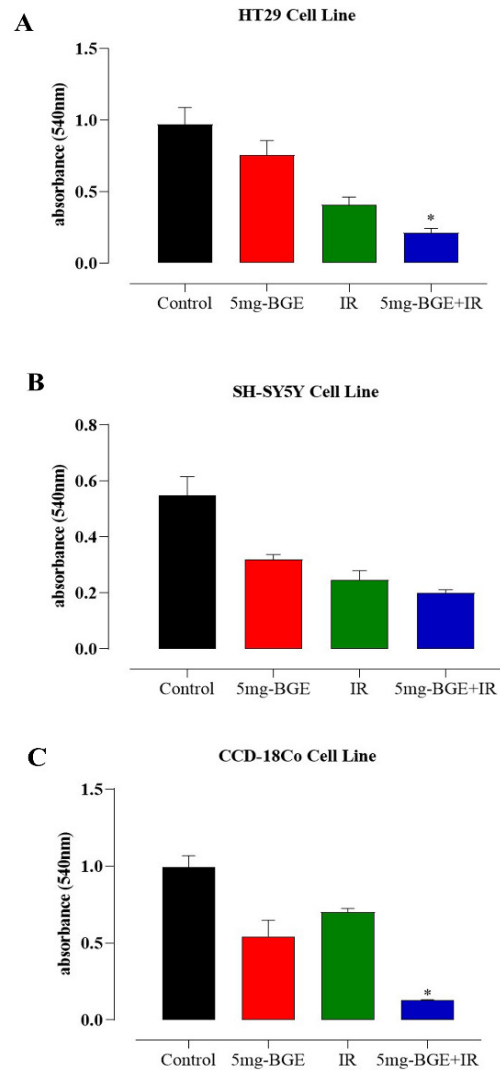


Figure 5: The effect of irradiation in combination with black garlic on SH-SY5Y, CDD-18Co, and HT29 cells. The MTT assay demonstrates the combined effect of black garlic with irradiation. Cells were incubated with 5 mg of white garlic for 24 hours followed by incubation with a 2 Gray irradiation dose for an additional 24 hours (Abbreviations: BGE, black garlic extract; IR, ionizing radiation).

The combined inhibitory effect of radiation and black garlic extract (BGE) on cancer cell death

Black garlic was found to exhibit inhibitory effects across all cell lines ($p > 0.05$). However, when black garlic was combined with a 2 Gy radiation dose, it was observed to reduce cell survival more significantly in all groups. This combination effect was more pronounced in HT29 cells, while it was less noticeable in brain cells ($p < 0.05$).

Discussion

In this study, we aimed to compare the effects of white and black garlic on irradiated cancer and normal cells. Our findings suggest that white garlic offers protective effects, while black garlic seems to exacerbate damage. A study conducted on MCF-7 and MDA-MB-361 cell lines demonstrated that mature black garlic extract suppressed the proliferation, migration, invasion, and metastasis of ER+ breast cancer cells (20). It was also proposed that black garlic suppresses the production of anti-apoptotic proteins MCL-1 and BCL-2, inducing apoptosis in ER+ breast cancer cells. The increase in reactive oxygen species (ROS) in cancer cells leads to the activation of JNK, which in turn reduces MCL-1 expression (20).

In our study, black garlic alone demonstrated the most significant dose-dependent reduction in cell viability in SH-SY5Y cell lines, indicating its inhibitory effect on cancer cell proliferation. Park et al. (21) reported that hexane extract from mature black garlic induces apoptosis in human leukemia cells (U937) through both intrinsic and extrinsic caspase-dependent apoptotic pathways. The application of aged black garlic extract (ABGE) also shows promising therapeutic potential in colon cancer treatment. A study by Dong et al. found that ABGE inhibited proliferation and triggered apoptosis in HT29 colon cancer cells (22). In our study, the combined effect of 5 mg black garlic and/or radiation had the most significant impact on HT29 colorectal cancer cell lines. This anticancer effect may be linked to the regulation of the PI3K/Akt signaling pathway, which involves an increase in PTEN expression and a decrease in Akt and p-Akt levels (22). Another study examined the effect of matured black garlic extract on colon cancer models induced by 1,2-dimethylhydrazine (DMH) in mice and found that it inhibited cell proliferation by reducing cyclin B1 and cdk1 expression, which was associated with the suppression of NF- κ B activity (23). In our study, 5 mg white garlic also exhibited inhibitory effects on HT29 and SH-SY5Y cell lines.

Another bioactive compound found in black garlic, S-Allyl-Mercapto-Cysteine (SAMC), has shown health-promoting properties. Studies by Zhang et al. (24) have demonstrated that SAMC induces apoptosis in the SW620 human colon cancer cell line via the JNK and p38 pathways, activating Bax and p53. Black garlic extract (BGE) has also been suggested to sensitize Lewis lung cancer cells to ionizing radiation, potentially through alterations in the cell cycle and modulation of Bax and Bcl-2 expressions (20).

Choromanska et al. assessed the toxic effects of diallyl disulfide (DADS) and garlic oil on glioma cells. Their findings revealed that DADS did not induce cytotoxicity in SW1783 and SW1088 glioma cell lines (25). Aged black garlic has been reported to protect against damage caused by ionizing radiation or toxic substances (26). A study by Kim et al. showed that black garlic extract did not exhibit cytotoxic effects when applied to RAW264.7 and RBL-2H3 cells (11).

In our study, both 5 mg of white and black garlic reduced cell viability in cancer cell lines. When a 2 Gy radiation dose was added, there was a significant decrease in cell viability. However, when 2 Gy radiation was applied alone in all cell lines, a greater reduction in cell viability was observed. Comparing all results, white garlic showed the greatest effect, while black garlic also exhibited a protective role. These findings suggest that garlic plays an important role in cancer treatment, especially when combined with radiation, and warrant further investigation.

Conclusion

In this study, black garlic exhibited inhibitory effects across all cell lines, and when combined with a 2 Gy radiation dose, it reduced cell survival in all groups. However, the effect was less pronounced in brain cells. These findings suggest that while black garlic combined with radiation contributes to increased damage in cancer cells, its impact varies depending on the cell type. As a result of these results highlight the potential therapeutic value of white garlic and suggest that further studies are needed to better understand the mechanisms involved and the possible clinical applications of black garlic in combination with radiation therapy.

Declarations

Funding

The authors declare they have no financial interests.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Ethics Approva

No ethical approval is required.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

Zehra Kanlı: Methodology, Performed the experiments, Analyzed the results, prepared figures, and conducted the Project. **Hülya Cabadak, İlknur Alsan Çetin, Zehra Kanlı, Banu Aydın:** Interpretation of data for the study. **Banu Aydın;** Supervision, Project administration, Methodology, Designed experiments, funding acquisition, conducted the project, all authors approved the final manuscript.

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