In vitro cytotoxicity and genotoxicity/ antigenotoxicity evaluation of encapsulated black garlic extracts on A549 cells.

Çiğdem İnci Aydemir[1], Hatice Elif Temiztürk[2], Gökçe Taner[3]

[1]Department of Biotechnology, Bursa Technical University, Graduate Education Institute, Bursa, Türkiye
[2]Department of Bioengineering, Bursa Technical University, Graduate Education Institute, Bursa, Türkiye
[3]Department of Bioengineering, Bursa Technical University, Faculty of Engineering and Natural Sciences, Bursa, Türkiye

Journal of Bursa Faculty of Medicine
e-ISSN: 2980-0218

Original Article

Clinical Sciences

Received
March 13, 2024
Accepted
April 19, 2024
Published online
May 4, 2024

J Bursa Med 2024;2(2) 59-68

ABSTRACT

Objectives: Black garlic is produced by fermenting fresh garlic under controlled temperature and humidity conditions for an extended period. Due to its sweeter taste and lack of pungent odor compared to fresh garlic, black garlic is easier to consume. Moreover, the increase in bioactive compounds such as polyphenols and flavonoids during fermentation has sparked interest in studying the health effects of black garlic. It is known that different fermentation and extraction methods can lead to variations in biological activities. Therefore, analyzing the effectiveness of black garlic processed by different methods is of critical importance. In our study, we investigated the cytotoxic, genotoxic, and antigenotoxic effects of different concentrations of encapsulated black garlic capsule extract (BGC) on lung cancer cells.

Methods: The A549 cell line was used to investigate the effects of BGC. Cells treated with BGC at different concentrations (10, 25, 50, 100, 125, 250, 500, and 1000 µg/mL) for 24 hours were subjected to MTT and NRU assays to examine the cytotoxic effects. Alkaline comet assay was performed to investigate genotoxic and antigenotoxic effects. For antigenotoxicity analysis, cells pretreated with BGC were exposed to H2O2 to explore the protective effects of BGC.

Results: According to the MTT results, cell viability remained at 90% even at concentrations higher than 125 µg/mL. However, in the NRU analysis, viability decreased to less than 70% at concentrations ranging from 50 µg/mL. Comet assay results revealed significant increases in tail length and tail intensity at different concentrations (specifically, at 250 µg/mL and above and at 50 µg/mL and 100 µg/mL, respectively). However, tail moments did not show any significant differences at any concentration. Additionally, BGC significantly reduced H_2O_2-induced DNA damage.

Conclusions: Our research demonstrated that BGC reduces the viability of lung cancer cells and can have genotoxic effects. Additionally, its protective effect against oxidative damage was shown at the DNA level. Based on these data, further research can be conducted on the use of BGC against cancer.

Keywords: Black Garlic, Antigenotoxicity, Cytotoxicity, Comet Assay

How to cite this article
Aydemir Çİ, Temiztürk HE, Taner G. In vitro Cytotoxicity and Genotoxicity/Antigenotoxicity Evaluation of Encapsulated Black Garlic Extracts on A549 Cells. J Bursa Med 2024;2(2);59-68

Address for correspondence
Çiğdem İnci Aydemir, Bursa Technical University, Graduate Education Institute, Bursa, Türkiye.
E-mail: incicigdem@gmail.com

Available at https://dergipark.org.tr/tr/pub/bursamed

Garlic (Allium sativum L.) is a plant widely used in global cuisine and traditional medicine. It is consumed in various forms, such as fresh, dried, pureed, and powdered. The biological functions of garlic have been investigated in numerous studies. It contains many bioactive compounds, including organic sulfides, saponins, phenolic compounds, and polysaccharides [1]. Various biological and pharmacological effects, such as antimicrobial, antioxidant, anticancer, antidiabetic, antiallergic, cardiovascular protective, anti-inflammatory, immunomodulatory, and antiobesity effects, have been demonstrated [1, 2]. However, the characteristic taste and odor of garlic may limit its consumption. Black garlic is obtained through the controlled fermentation of fresh garlic. It has a sweeter taste, is dark brown or black in color, and is odorless compared to fresh garlic. Although there is no standard method for fermentation process, fresh garlic is fermented at high temperatures (60-90 °C) and 60-90% humidity for 10-80 days during production [3]. The chemical composition of black garlic is different from that of fresh garlic after fermentation. Black garlic contains relatively high levels of antioxidants such as polyphenols, flavonoids, pyruvate, S-allyl-cysteine (SAC), S-allyl-mercapto-cysteine (SAMC), and 5-hydroxymethylfurfural (5-HMF) [3-6].

In scientific studies, black garlic has been shown to have various beneficial health effects, such as anticancer, anti-inflammatory, immunomodulatory, cardioprotective, nephroprotective, hepatoprotective, antidiabetic, and antiobesity effects [1]. In terms of hepatoprotective effects in a liver damage model, black garlic has been shown to prevent an increase in AST and ALT levels related to carbon tetrachloride-induced liver damage and to suppress hepatic damage induced by D-galactosamine. However, no changes were observed in the levels of alkaline phosphatase (ALP), a marker of hepatobiliary damage [7]. Another study revealed that treatment with black garlic extract decreased AST, ALT, ALP, and malondialdehyde (MDA) levels in a mouse model of acute hepatitis, and significant anti-inflammatory effects were identified in the mice [8]. Black garlic and yeast-fermented black garlic exhibit hepatoprotective, nephroprotective, hypolipidemic, and antiobesity effects in obese mice. However, no hypoglycemic effect was observed in the study [9]. The effects of fresh garlic and BG-ethanol extracts on immune cells obtained from the blood of 21 volunteers were examined. Garlic extract significantly influenced the proliferation, TNF-α level, and NO production of primary immune cells, with slight differences observed between garlic extract and BG. BG was determined to be a much stronger immunostimulant than raw garlic extract [10].

Studies have been conducted on the anticancer properties of BG both *in vitro* and *in vivo*. Regularly administering aged garlic extract to BALB/c mice implanted with fibrosarcoma cells increases IFN-γ and IL-4 production in splenocytes, specific cytotoxicity against fibrosarcoma cells, and strengthens the immune system, inhibiting tumor growth [11]. BG extracts from different fermentation periods have been shown to have cytotoxic effects on the HL60 leukemia cell line, albeit to a lesser extent than fresh garlic. The IC50 value for fresh garlic was determined to be 0.03 mg/mL, whereas for BG extracts, it was calculated to be 0.7 (fermented for 32 days) and 0.9 (fermented for 45 days) mg/mL [12]. SAMC found in BG reduces colorectal cancer cell viability *in vitro* in a dose- and time-dependent manner via the JNK and p38 pathways [13]. Furthermore, in colon cancer animal models, BG therapy reduces proliferative activity in adenoma and adenocarcinoma lesions [14].

Oxidative stress occurs as a result of an imbalance between reactive oxygen species (ROS) and free radicals generated during cellular metabolism and the antioxidant mechanisms. Elevated levels of ROS and free radicals can lead to DNA damage and alter various signaling pathways. Therefore, oxidative stress is known to be associated with many types of cancer. Different dietary habits, such as inadequate or excessive food intake, can trigger inflammatory and oxidative states and lead to certain pathophysiological conditions [15]. Moreover, it is known that biologically active molecules found in foods can support the prevention of many diseases. For instance, there are numerous reports on the anticancer effects of flavonoids, which possess strong anti-inflammatory and antioxidant properties [16]. Garlic, which contains a variety of phytochemicals, has emerged as an important plant with antioxidant effects. Studies have demonstrated that the antioxidant activities of garlic and its active components are influenced by garlic type and processing method. It has been shown that short-term fermented black garlic (13 days) exhibits better physicochemical qualities and greater biological activity than long-term fermented black garlic (32 and 45 days) and even white garlic [12]. The antioxidant activity of black garlic is greater than that of raw garlic according to DPPH (2,2-diphenyl-1-picrylhydrazyl), which is a method of measuring antioxidant activity, ABTS (2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfon-

ic acid)), FRAP (Ferric Reducing Antioxidant Power), H2O2 scavenging, and Fe2+ chelation analyses [17–19]. Additionally, different extraction methods also influence their biological activities. It has been reported that the DPPH radical scavenging activity of the distilled water extract of BG is higher than that of other extracts, while the Fe2+ chelating activities of the ethanol and chloroform extracts are greater than those of the distilled water extracts [17]. During a 30-day period, mice fed with BG or BG residue showed decreased levels of MDA, while SOD and GSH-Px activities significantly increased [20]. Pretreatment with black garlic significantly reduces tert-Butyl hydroperoxide (tBHP)-induced damage in rat clone-9 hepatocytes. BG facilitates the reversal of decreased antioxidant enzyme activity in hepatocytes induced by tBHP [21].

The biological activity of black garlic is influenced by various factors, such as fermentation conditions, raw material quality, and extraction methods. In our study, we aimed to investigate the effects of the encapsulated form of black garlic aqueous extract on cells and compare them with the data available in the literature. We examined the cytotoxic, genotoxic, and antigenotoxic effects of the encapsulated black garlic extract (BGC) on the human lung adenocarcinoma cell line A549.

METHODS

Preparation of the encapsulated black garlic extract

In our study, BGC produced by Cinar and colleagues in 2022, with a detailed methodology published, were utilized [22].

Sample Preparation

The BGCs were dissolved in cell culture medium at 37°C in a water bath. Serial dilutions were prepared at concentrations of 10, 25, 50, 100, 125, 250, 500, and 1000 µg/mL.

Cell Culture

The A549 cell line, which is commercially available for cytotoxicity, genotoxicity, and antigenotoxicity analyses, was used. The cells were cultured in RPMI (Roswell Park Memorial Institute) medium supplemented with 10% FBS (Fetal Bovine Serum) and penicillin-streptomycin, seeded into a 75 cm^2 culture flask with culture medium and maintained in a 37°C incubator under standard conditions with 5% CO_2. After reaching 80% confluence, they were harvested by trypsinization. The cells were seeded at a density of 10^4 cells/well in 96-well plates for the MTT (a method to measure cell viability) and NRU assays and at a concentration of 10^5 cells/well in 12-well plates for the comet assay. After 24 hours, the culture medium was removed, and the culture wells were supplemented with BG-containing media at predetermined concentrations. For the negative control, standard culture media was utilized, while for the positive control, 0.1% Triton X-100 was applied. The culture was sustained for another 24 hours in this manner, after which toxicity analyses were performed.

Cytotoxicity analysis

The potential cytotoxic effects of BGC extracts on cells were assessed using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and neutral red uptake (NRU) assays. Cells treated with BGC for 24 hours in 96-well plates were exposed to MTT solution (5 mg/mL) and incubated for 4 hours. Subsequently, the cells were washed with PBS, and the formazan crystals were dissolved in DMSO. The absorbance at 570 nm was measured for evaluation.

For the NRU assay, after the medium was discarded and the cells were washed with PBS, prewarmed NR solution (50 µg/mL NR in medium) was added to the wells. The mixture was then incubated for 3 hours at 37°C. The cells were washed with PBS three times. After removing the excess dye, a fixation solution (50% methanol, 1% acetic acid, and 49% dH_2O) was added. The plates were shaken for 20 minutes, and measurements in each well were conducted at a wavelength of 540 nm.

Genotoxicity and Antigenotoxicity Analyses

The alkali comet assay method was employed for the evaluation of genotoxic and antigenotoxic effects. Cells treated with varying concentrations (50, 100, 250, 500 and 1000 µg/mL) of BGC for 24 hours were collected by trypsinization and suspended in PBS. In the antigenotoxicity groups, the cells were treated with 50 µM/mL H_2O_2 on ice for 5 minutes. After the treatment period, the cells were centrifuged and resuspended in PBS. A negative control sample (DPBS) and a positive control (50 µM/mL H_2O_2 alone) were also included in the experiments. The cell suspensions were mixed with 0.65% LMA (low melting point agarose) and embedded in slides precoated with 1% NMA (normal melting point agarose). The slides were solid-

ified on ice, after which the coverslips were removed. Subsequently, the slides were incubated in cold fresh lysis solution (2.5 M NaCl, 100 mM EDTA, 100 mM Tris-base, 1% sodium sarcosinate, pH 10.0) supplemented with 1% Triton X-100 and 10% DMSO for 2 hours at 4°C. After incubation, the slides were transferred to cold electrophoresis buffer (1 mM sodium EDTA and 300 mM NaOH, pH 13.0) to enable DNA unwinding, after which the samples were kept at 4°C for 20 minutes. Electrophoresis was performed for 20 minutes at 21 V and 620 mA. Neutralization was achieved by washing three times in 0.4 M Tris–HCl (pH 7.5) for 5 minutes at room temperature. Next, the slides were incubated in an alcohol series (50%, 75%, and 98%) for 5 minutes each.

The dried agarose gel blocks were stained with ethidium bromide (20 µg/mL in dH2O) and covered with a coverslip. The migration of DNA was quantified using Comet Assay IV analysis software (Perceptive Instruments Ltd.), and the results are reported in terms of DNA tail length, DNA tail intensity (% tail DNA), and DNA tail moment. At least 200 cells from two replicate slides were scored at 20X for each experiment.

Statistical analysis

The statistical analysis was conducted using IBM SPSS 22 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). The study results are expressed as the mean ± standard error. Differences between the means of data were compared by the one way variance analysis (ANOVA) test and post hoc analysis of group differences by least significant difference (LSD) test. p Values of less than 0.05 were considered as statistically significant.

RESULTS

Cytotoxicity analysis

MTT and NRU assays were used to investigate the cytotoxic effects of BGC on A549 cells. MTT assay revealed that the cell viability decreased to less than 90% at concentrations higher than 125 µg/mL, although viability did not decrease to less than 70% within the applied concentration range (Figure 1).

The NRU assay results showed higher alterations in cell viability. Although cell viability did not decrease below 50% at any concentration, viability decreased to less than 70% at concentrations ranging from 50

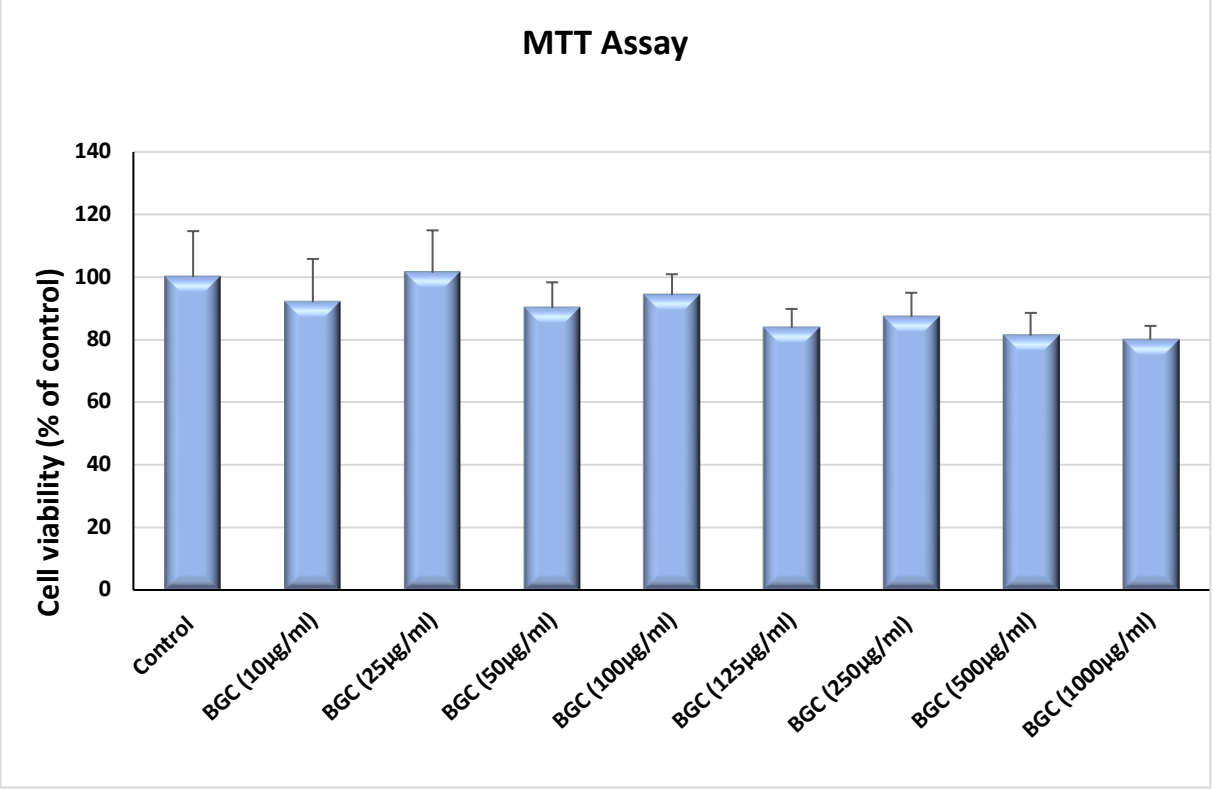

Figure 1. Cell viability rates compared to the control using the MTT assay following the treatment of A549 cells with different concentrations of BGC

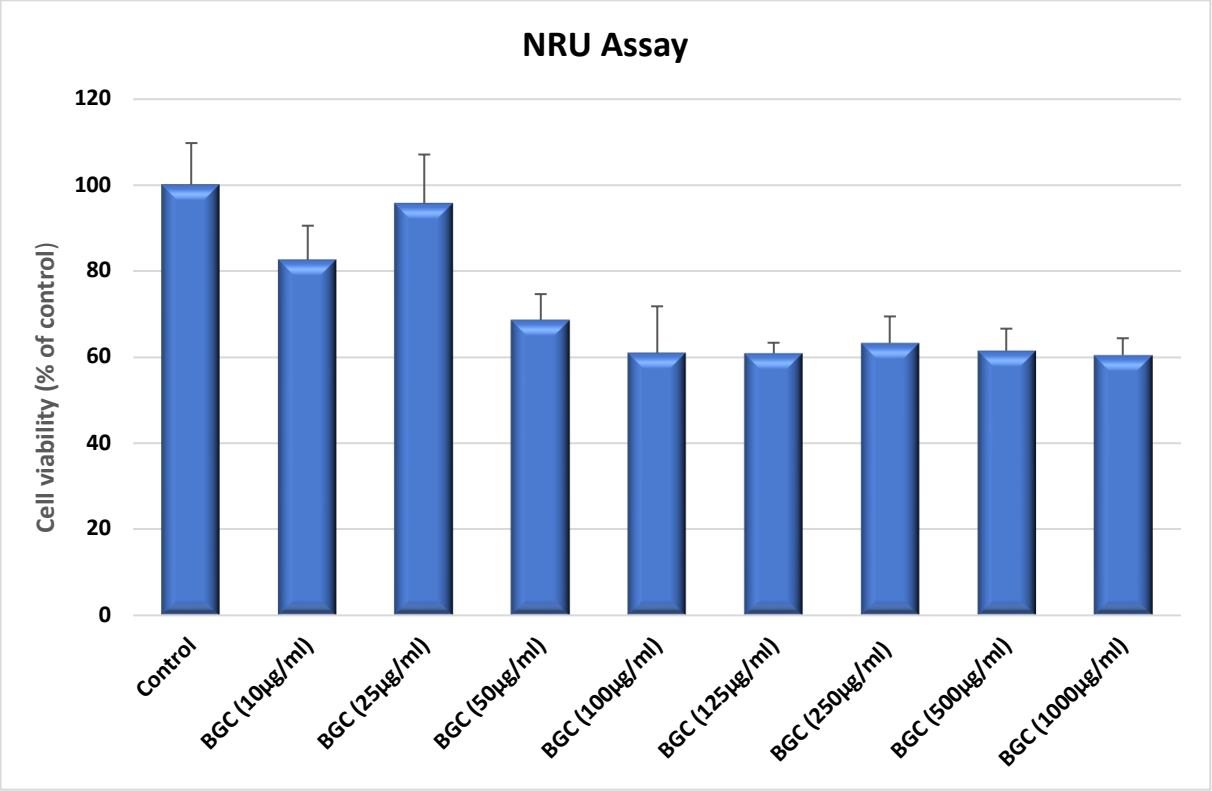

Figure 2. Cell viability rates compared to the control using the NRU assay following the treatment of A549 cells with different concentrations of BGC

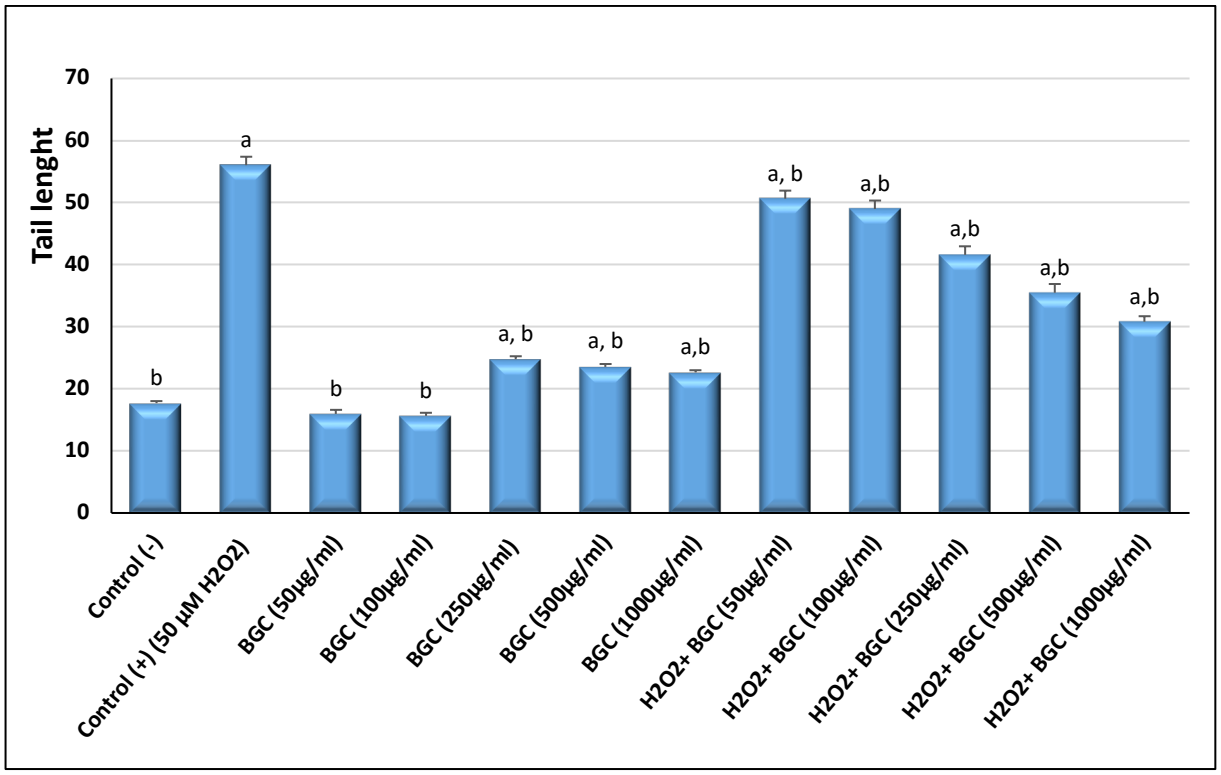

Figure 3. DNA damage, expressed as DNA tail length in the A549 cells treated with different concentrations of BGC and H_2O_2 (a: significant difference compared with Control (-), b: significant difference when compared with Control (+))

µg/mL (Figure 2).

Genotoxicity and Antigenotoxicity Analyses

The comet assay results for genotoxicity and antigenotoxicity analysis are presented in Figures 3-5, including tail length, tail intensity, and tail moment. Tail length increased significantly in the genotoxicity analysis groups when concentrations reached 250 µg/mL and above (Figure 3). Tail intensity measurements showed an increase at concentrations of 50 µg/mL and 100 µg/mL (Figure 4). When compared to the untreated control group, no differences were found in the tail moment values among the groups (Figure 5).

Pretreatment with BGC resulted in significant decreases in terms of tail length, tail intensity, and tail moment at all concentrations of H2O2-induced oxidative damage compared to the control (Figure 3-5). DNA damage was higher than in the untreated negative control but lower than in the control treated with H_2O_2.

DISCUSSION

Black garlic is a fermented type of garlic. The sensory characteristics of BG transform at the end of the aging process. The product is provided as an alternative for people who find fresh garlic to have a strong smell and irritating taste. Furthermore, after fermentation, there are significant changes in the bioactive properties of BG. There are numerous studies regarding its anti-inflammatory, antioxidant, anticancer, hepatoprotective, and hypolipidemic properties [1]. The ethanol extract of BG inhibited growth and induced apoptosis in HT29 colon cancer cells by inhibiting the PI3K/Akt pathway. Growth inhibition occurred both in a dose- and time-dependent manner. Incubation with 100 mg/mL BG at the highest dose tested inhibited 46.7±4%, 55.2±3%, and 63.9±5% of the cells at 24, 48, and 72 hours, respectively [23]. These studies suggest that black garlic may have potential health benefits beyond its mild taste and smell. Additionally, the fermentation process of black garlic enhances its nutritional profile by increasing the levels of certain beneficial compounds, such as S-allyl cysteine.

The properties of black garlic are influenced by many factors, such as fermentation conditions, processing technologies, and the quality of the raw garlic used. For instance, Bae et al. demonstrated that changing the fermentation temperature from 40 to 85°C resulted in a decrease in the amount of S-allyl cysteine

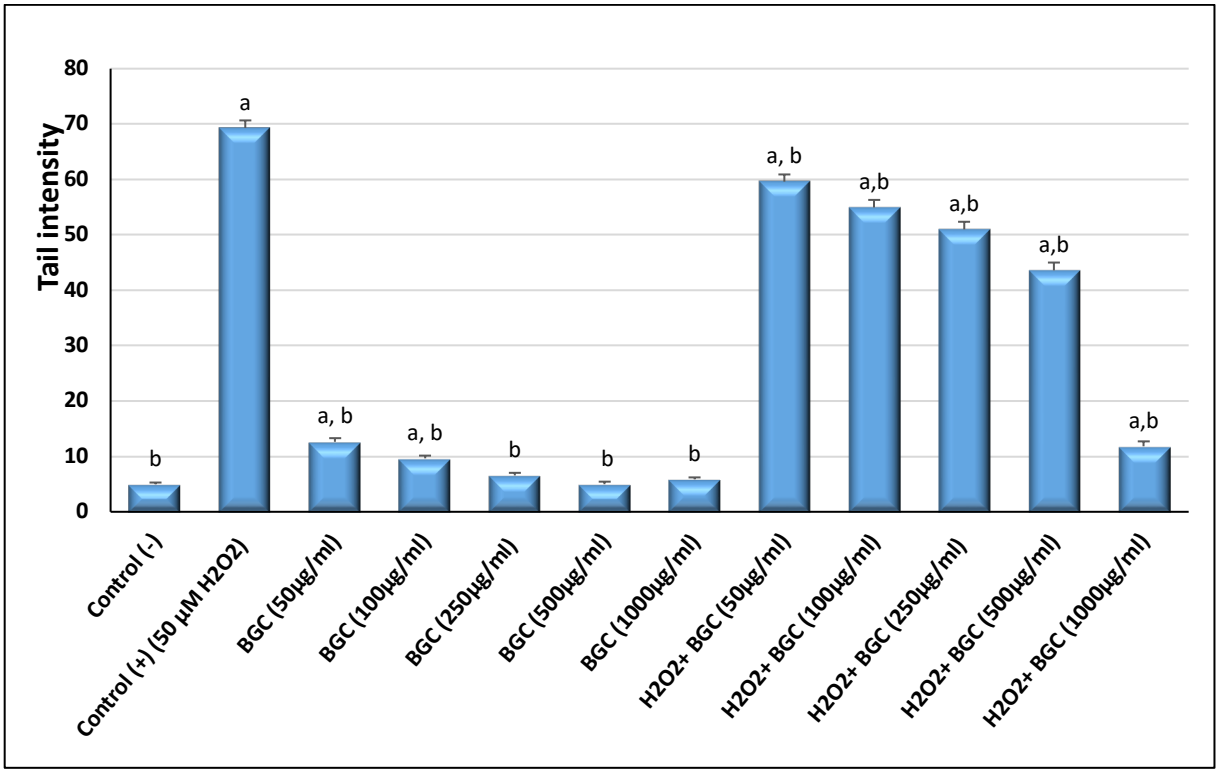

Figure 4. DNA damage, expressed as DNA tail intensity in the A549 cells treated with different concentrations of BGC and H2O2 (a: significant difference compared with Control (-), b: significant difference when compared with Control (+))

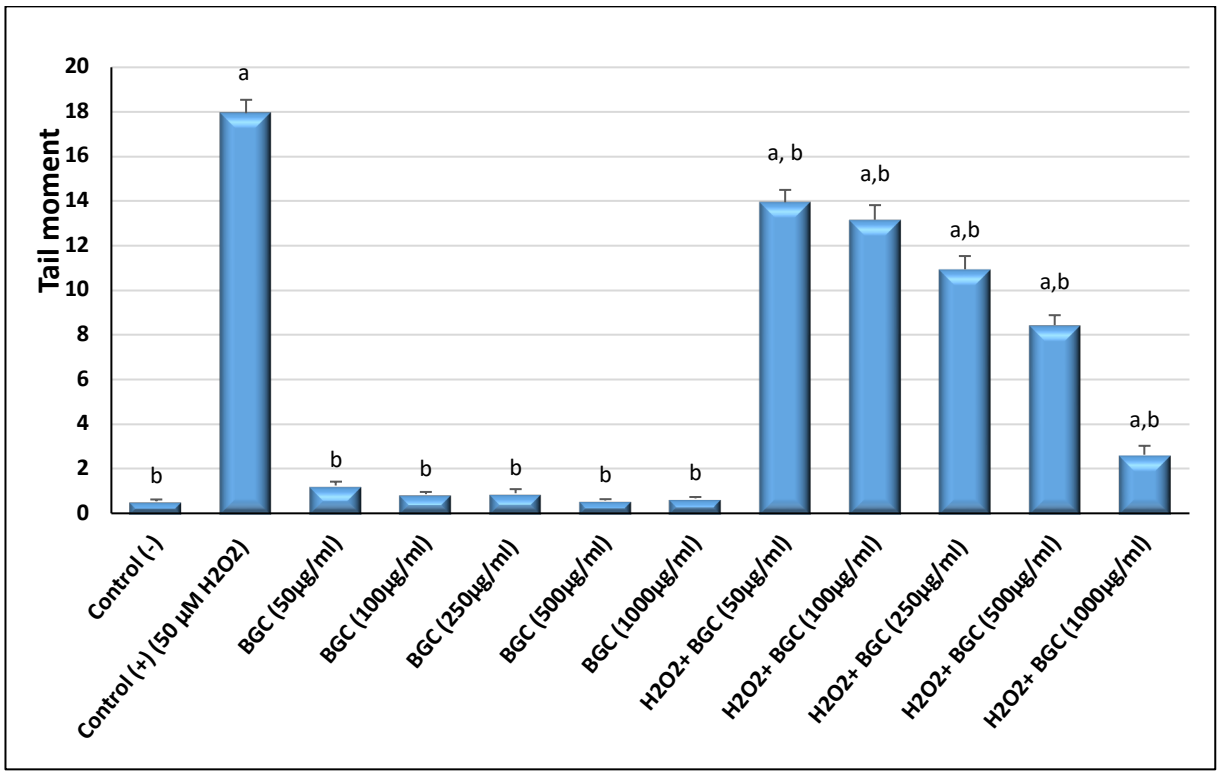

Figure 5. DNA damage, expressed as DNA tail moment in the A549 cells treated with different concentrations of BGC and H_2O_2 (a: significant difference compared with Control (-), b: significant difference when compared with Control (+))

(SAC), which is thought to be the most significant antioxidant of BG [24]. In a study investigating the 35-day aging process of black garlic, it was determined that the highest antioxidant activity was achieved on the 21st day. Along with their antioxidant activity, the quantities of polyphenols and flavonoids significantly increased on the 21st day [25]. In contrast, another study reveals that a 13-day fermentation time provides more biological activity than more prolonged fermentation periods [12].

The antiproliferative effect of BG may depend on the type of cell. The effects of the methanol extract of BG on different cancer cells (human hepatocellular carcinoma (HepG2) and human leukemia (U937)) were examined, revealing that BG dose-dependently inhibited cancer cells after 48 hours of incubation [26]. The IC50 values varied according to the cell type, with IC50 values of 0.8 mg/mL and 2.0 mg/mL observed for HepG2 and U937 cells, respectively [10]. Although the cytotoxic effect of BG on the A549 cell line has been previously investigated, this study focused on the cytotoxic, genotoxic, and antigenotoxic effects of the encapsulated form of BG on these cells.

Cytotoxic effects of BGC

The effects of BGC on cell viability were evaluated *in vitro* using the human lung cancer cell line A549. In the present study, 8 different concentrations ranging from 10 to 1000 µg/mL were used. After 24 hours of BGA treatment, the viability of the A549 cells was still greater than 80% at the concentrations used. Therefore, according to the MTT results, up to a concentration of 1000 µg/mL, BGA had no cytotoxic effect on A549 cells. These findings are consistent with those of a study conducted in 2023 involving various lung cancer cell lines, including A549 cells. Farhat et al. conducted a study comparing various forms of garlic, including BG, to assess their antioxidant and antiproliferative effects across different lung cancer cell lines. Their findings indicated that, in comparison to other forms, such as garlic powder, black garlic, water and alcohol extracts of fresh garlic, and commercial garlic supplements, BG demonstrated reduced antioxidant and antiproliferative efficacy. MTT analysis further revealed that BG had no impact on lung cancer cell lines (27). In another study investigating the anticancer effects of ethanol extracts of fresh garlic and BG on MCF-7, AGS, A549, and HepG2 cells, both extracts were found to be effective on all cell lines. However, the MCF-7 and HepG2 cells were more sensitive than the A549 and AGS cells were. The cells were treated with the extracts for 24, 48, or 72 hours,

and the highest inhibition was observed at 48 hours [10].

BG has been shown to inhibit various types of cancer cells. The cytotoxic effect of hexane extract of BG on U937 leukemia cells has been demonstrated through MTT analysis [28]. It has been determined that apoptosis is induced in cells through the caspase cascade. The hexane, chloroform, and ethyl acetate extracts of BG individually exert antiproliferative effects on breast cancer cells (T47D) [29]. The effects of BG on gastric cancer were examined, revealing that BG triggers apoptosis in SGC-7901 cells in a dose-dependent manner. Antitumor effects have also been observed in *in vivo* studies [30]. A study comparing the antioxidant properties of different garlic forms, including black garlic, reported that the greatest antiproliferative effect on ovarian cancer cell lines was achieved with fresh garlic extracts. There was no significant relationship between the potential to inhibit proliferation and the phenolic or flavonoid content, indicating that phenolics may not contribute significantly to the antiproliferative effects of garlic [31].

The results of the NRU assay conducted to investigate the effect of BGC on the proliferation of A549 cells differed from the MTT results. During the MTT assay, metabolically active cells reduce tetrazolium salts to form blue-colored formazan crystals. Neutral red is taken up by undamaged cells and shows lysosomal accumulation within the cell. Cytotoxicity analyses often yield contradictory results, as observed in this study. Putnam et al. compared four different toxicity assays (NRU, MTT, kenacid blue, and LDH) and found that during short-term incubations initiated from 3 hours onward, NRU provided more sensitive results than MTT. MTT analysis yielded results similar to those of other assays during 18- and 24-hour incubations [32]. In another study in which the viabilities of HTC (Human T-cell leukemia cell) and HepG2 cells were measured after incubation in cadmium chloride for different durations (3, 5, 8, and 24 h), the EC50 values at 24 hours were measured via NRU and MTT analyses as follows: for HTC cells, 20 µM ± 3.31 and 100 µM ± 14.47; for HepG2 cells, 8 µM ± 0.21 and 15 µM ± 5.03 (33). Furthermore, the EC50 values for HepG2 cells in the 3-, 5-, and 8-hour groups were assessed using both MTT and NRU, whereas the EC50 values for HTC cells in these groups were determined using only NRU. According to studies utilizing BG, Purev et al., 48 hours of incubation had the strongest inhibitory effect [10]. As a result, it is possible that the 24-hour incubation time we chose in our study was too short to demonstrate any effects in the MTT test. The NRU results revealed a decrease in cell viability, which was consistent with the findings of other publications suggesting that the test is more sensitive for shorter incubations.

Genotoxic and Antigenotoxic Effects of BGC

The genotoxic and antigenotoxic effects of BGC were examined using the comet assay. Single-cell gel electrophoresis is commonly used for the detection of single- and double-strand breaks in DNA. The applied alkaline comet analysis enables direct measurement of DNA damage at the cellular level. A549 cells were treated with different doses of BGC for 24 hours. Subsequently, the cells were harvested and divided into two groups. One group was subjected to comet analysis directly for genotoxicity evaluation, while the other group was treated with H_2O_2 before analysis for antigenotoxicity evaluation. According to the results, there was a significant increase in tail intensity (at 50 and 100 µg/mL) and tail length (at 250, 500, and 1000 µg/mL) in cells treated with BGC compared to those in the control group across different concentration applications. On the other hand, BGC did not significantly differ at any concentration when the tail moment was analyzed. These findings suggest that BGC may induce DNA damage in A549 cells, as evidenced by the increase in tail intensity and length. However, further investigations are needed to determine the exact mechanism of DNA damage caused by BGC and its potential implications for cellular function. The effects of black garlic extracts with different fermentation durations and fresh garlic on the genomic integrity of HL60 cells were investigated by DNA laddering, which revealed that only fresh garlic caused mild DNA fragmentation at low concentrations [12].

When examining the groups induced by oxidative damage, it was observed that H_2O_2 had genotoxic effects on the cells, while BGC had protective effects against this damage. With all administered concentrations of BGC, the outcome of oxidative damage was significantly reduced in terms of tail length, tail intensity, and tail moment. Our findings are consistent with our prior research, demonstrating that the BGC utilized in our investigation did not demonstrate any genotoxic effects on human lymphocytes at concentrations of 50, 100, or 200 µg/mL. Furthermore, BGC exhibited a protective antigenotoxic effect against the induction of micronuclei by Mitomycin C, a drug known for its ability to inhibit cell proliferation [22]. In a study conducted with the mouse macrophage line

RAW264.7, the effects of different forms of garlic on H2O2-induced oxidative damage were compared. It was determined that black garlic extract exhibited stronger antioxidant activity than fresh garlic. Upon investigating the content responsible for this effect, it was suggested that pyruvate, which is known to increase in quantity during the fermentation process, might be responsible [18]. Further analysis revealed that black garlic extract also had relatively high levels of other antioxidants, such as SAC, allicin, polyphenols and alkaloids which could contribute to its stronger antioxidant activity [3,6]. These findings suggest that the fermentation process involved in producing black garlic may enhance its antioxidant properties compared to those of fresh garlic. Studies have demonstrated the antioxidant activity of black garlic both *in vitro* and *in vivo*. Even at lower doses, black garlic has more antioxidant activity than fresh garlic [18]. The type of black garlic, processing method, and extraction method are important factors influencing its antioxidant capacity. Polyphenol content has been shown to increase with higher temperatures and decreasing humidity during the processing of black garlic. The optimal protocol for preserving antioxidant capacity involves 75°C and 85% relative humidity over a period of 8 days [34]. Water extracts of black garlic show more antioxidant activity than alcohol extracts when extraction techniques are compared [27].

CONCLUSION

In this study, we evaluated the encapsulated black garlic which we found in our previous studies that it has much higher antioxidant capacity and biological activities compared to fresh garlic and is also safe to consume due to its anti-genotoxic effect on human lymphocytes (22), for its in vitro cytotoxic, genotoxic and antigenotoxic properties on A549 cell line. According to our results, it was determined that BGC had cytotoxic and genotoxic effects depending on its concentration, on the other hand, it had protective effects against DNA damage caused by hydrogen peroxide. Considering that powerful antioxidants and antigenotoxic agents can also be anticarcinogenic, it should not be ignored that these agents can be of great benefit in cancer prevention. However, this in vitro study was planned for only a single cancer cell type. Further studies such as in different cancer cells and in comparison, with healthy cells and also in vivo studies are needed to clearly determine the potential of BGC for its use against cancer.

Acknowledgments

Authors would like to thank Prof. Dr. Rasim Alper Oral and Research Assistant Hüseyin Demircan from the Food Engineering Department of Bursa Technical University for the production of encapsulated black garlic used in this study, and especially for Assoc. Prof. Dr. Aycan Yiğit Çınar for providing the product.

Conflict of Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' Contribution

Study Conception: ÇIE, HET, GT; Study Design: ÇIE, HET, GT; Literature Review: ÇIE, GT; Critical Review: ÇIE, GT; Data Collection and/or Processing: ÇIE, HET, GT,; Analysis and/or Data Interpretation: ÇIE, GT; Manuscript preparing: ÇIE, GT.

REFERENCES

1. Shang A, Cao SY, Xu XY, Gan RY, Tang GY, Corke H, et al. Bioactive Compounds and Biological Functions of Garlic (Allium sativum L.). Foods 2019;8(7):246.
2. Kilic-Buyukkurt O, Kelebek H, Bordiga M, Keskin M, Selli S. Changes in the aroma and key odorants from white garlic to black garlic using approaches of molecular sensory science: A review. Heliyon 2023;9(8).
3. Stępień AE, Trojniak J, Tabarkiewicz J. Anti-Cancer and Anti-Inflammatory Properties of Black Garlic. Int J Mol Sci 2024;25(3):1801.
4. Kim JS, Kang OJ, Gweon OC. Comparison of phenolic acids and flavonoids in black garlic at different thermal processing steps. J Funct Foods 2013;5(1).
5. Sato E, Kohno M, Hamano H, Niwano Y. Increased anti-oxidative potency of garlic by spontaneous short-term fermentation. Plant Foods for Human Nutrition 2006;61(4).
6. Toledano Medina MA, Pérez-Aparicio J, Moreno-Rojas R, Merinas-Amo T. Evolution of some physicochemical and antioxidant properties of black garlic whole bulbs and peeled cloves. Food Chem 2016;199.
7. Shin JH, Lee CW, Oh SJ, Yun J, Kang MR, Han SB, et al. Hepatoprotective effect of aged black garlic extract in rodents. Toxicol Res 2014;30(1).
8. Tsai JC, Chen YA, Wu JT, Cheng KC, Lai PS, Liu KF, et al. Extracts from fermented black garlic exhibit a hepatoprotective effect on acute hepatic injury. Molecules 2019;24(6).
9. Jung YM, Lee SH, Lee DS, You MJ, Chung IK, Cheon WH, et al. Fermented garlic protects diabetic, obese mice when fed a high-fat diet by antioxidant effects. Nutrition Research 2011;31(5).
10. Purev U, Chung MJ, Oh DH. Individual differences on im-

munostimulatory activity of raw and black garlic extract in human primary immune cells. Immunopharmacol Immunotoxicol 2012;34(4):651–60.
11. Tabari M, Ebrahimpour S. Effect of aged garlic extract on immune responses to experimental fibrosarcoma tumor in BALB/c mice. Indian J Cancer 2014;51(4).
12. Medina MÁT, Merinas-Amo T, Fernández-Bedmar Z, Font R, Del Río-Celestino M, Pérez-Aparicio J, et al. Physicochemical characterization and biological activities of black and white garlic: In vivo and in vitro assays. Foods 2019;8(6).
13. Zhang Y, Li HY, Zhang ZH, Bian HL, Lin G. Garlic-derived compound S-allylmercaptocysteine inhibits cell growth and induces apoptosis via the JNK and p38 pathways in human colorectal carcinoma cells. Oncol Lett 2014;8(6).
14. Jikihara H, Qi G, Nozoe K, Hirokawa M, Sato H, Sugihara Y, et al. Aged garlic extract inhibits 1,2-dimethylhydrazine-induced colon tumor development by suppressing cell proliferation. Oncol Rep 2015;33(3).
15. Saha SK, Lee S Bin, Won J, Choi HY, Kim K, Yang GM, et al. Correlation between oxidative stress, nutrition, and cancer initiation. Vol. 18, International Journal of Molecular Sciences 2017.
16. Forni C, Rossi M, Borromeo I, Feriotto G, Platamone G, Tabolacci C, et al. Flavonoids: A myth or a reality for cancer therapy? Molecules 2021;26(12).
17. Jang HJ, Lee HJ, Yoon DK, Ji DS, Kim JH, Lee CH. Antioxidant and antimicrobial activities of fresh garlic and aged garlic by-products extracted with different solvents. Food Sci Biotechnol 2018;27(1).
18. Jeong YY, Ryu JH, Shin JH, Kang MJ, Kang JR, Han J, et al. Comparison of anti-oxidant and anti-inflammatory effects between fresh and aged black garlic extracts. Molecules 2016;21(4).
19. Zhang Z, Lei M, Liu R, Gao Y, Xu M, Zhang M. Evaluation of Alliin, Saccharide Contents and Antioxidant Activities of Black Garlic during Thermal Processing. J Food Biochem 2015;39(1).
20. Xiong F, Dai CH, Hou FR, Zhu PP, He RH, Ma H Le. Study on the ageing method and antioxidant activity of black garlic residues. Czech Journal of Food Sciences 2018;36(1).
21. Lee KC, Teng CC, Shen CH, Huang WS, Lu CC, Kuo HC, et al. Protective effect of black garlic extracts on tert-Butyl hydroperoxide-induced injury in hepatocytes via a c-Jun N-terminal kinase-dependent mechanism. Exp Ther Med 2018;15(3).
22. Cinar A, Altuntas S, Demircan H, Dundar AN, Taner G, Oral RA. Encapsulated black garlic: Comparison with black garlic extract in terms of physicochemical properties, biological activities, production efficiency and storage stability. Food Biosci 2022;50.
23. Dong M, Yang G, Liu H, Liu X, Lin S, Sun D, et al. Aged black garlic extract inhibits HT29 colon cancer cell growth via the PI3K/Akt signaling pathway. Biomed Rep 2014;2(2):250–4.
24. Bae SE, Cho SY, Won YD, Lee SH, Park HJ. Changes in S-allyl cysteine contents and physicochemical properties of black garlic during heat treatment. LWT 2014;55(1).
25. Choi IS, Cha HS, Lee YS. Physicochemical and antioxidant properties of black garlic. Molecules 2014;19(10):16811–23.
26. Bontempo P, Stiuso P, Lama S, Napolitano A, Piacente S, Altucci L, et al. Metabolite Profile and In Vitro Beneficial Effects of Black Garlic (Allium sativum L.) Polar Extract. Nutrients 2021;13(8).
27. Farhat Z, Scheving T, Aga DS, Hershberger PA, Freudenheim JL, Hageman Blair R, et al. Antioxidant and Antiproliferative Activities of Several Garlic Forms. Nutrients 2023;15(19).
28. Park C, Park S, Chung YH, Kim GY, Choi YW, Kim BW, et al. Induction of apoptosis by a hexane extract of aged black garlic in the human leukemic U937 cells. Nutr Res Pract 2014;8(2).
29. Permatasari E, Farida, Widiyanto S. Cytotoxic effects and apoptosis of solo black garlic (Allium sativum L.) extract on T47D breast cancer cell Line. In: AIP Conference Proceedings. American Institute of Physics Inc 2020.
30. Wang X, Jiao F, Wang QW, Wang J, Yang K, Hu RR, et al. Aged black garlic extract induces inhibition of gastric cancer cell growth in vitro and in vivo. Mol Med Rep 2012;5(1):66–72.
31. Furdak P, Pieńkowska N, Kapusta I, Bartosz G, Sadowska-Bartosz I. Comparison of Antioxidant and Antiproliferative Effects of Various Forms of Garlic and Ramsons. Molecules 2023;28(18).
32. Putnam KP, Bombick DW, Doolittle DJ. Evaluation of eight in vitro assays for assessing the cytotoxicity of cigarette smoke condensate. Toxicology in Vitro 2002;16(5).
33. Fotakis G, Timbrell JA. In vitro cytotoxicity assays: Comparison of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride. Toxicol Lett 2006;160(2).
34. Sun YE, Wang W. Changes in nutritional and bio-functional compounds and antioxidant capacity during black garlic processing. J Food Sci Technol 2018;55(2).