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Research Article

Garlic Extract Triggers Cytotoxicity, DNA Damage, and Cell Cycle Arrest in Human Leukemic Cells *via* Oxidative Stress

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ABSTRACT

Garlic (*Allium sativum*) is one of the oldest medicinal plants that has received great attention throughout ancient and modern history. It has been used for the prevention and treatment of a wide variety of ailments, including cancer, cardiovascular disease, and hepatic and microbial infections. Although garlic shows potential in the prevention and treatment of cancer and other diseases, little is known about its therapeutic mechanisms of action. Here, we hypothesized that Garlic Extract (GE) induces cytotoxicity, DNA damage, and cell cycle arrest in Human Leukemia (HL-60) cells through oxidative stress. To test our hypothesis, HL-60 cells were treated with different concentrations (2 mg/mL, 4 mg/mL, and 6 mg/mL) of GE for 24 hours. Cell viability and cell morphology were analyzed by MTT, trypan blue, and Acridine Orange and Propidium Iodide (AO/PI) assays, respectively. The extent of oxidative stress was measured by the lipid peroxidation and glutathione peroxidase assays. The degree of DNA damage was evaluated by single-cell gel electrophoresis. Cell cycle arrest was assessed by the Cellometer Vision. Data obtained from the MTT assay, trypan blue dye, and AO/PI dye assessment indicated that GE significantly reduced the number of live cells in a concentration-dependent manner, showing a gradual increase in the loss of viability in GE-treated cells. MTT assay results also revealed that GE inhibits the viability of HL-60 cells, resulting in an IC_{50} of 4.72 mg/mL. Tests for oxidative stress indicated significant increases ($p<0.05$) in the production of Malondialdehyde (MDA) and activity of glutathione peroxidase in GE-treated cells compared to the control group. Data obtained from the comet assay indicated that GE causes DNA damage in HL-60 cells in a concentration-dependent manner. GE treatment modulated cell cycle progression in HL-60 cells, leading to an arrest at the G2/M phase. In summary, our current investigation underscores that GE effectively curbs cell proliferation, induces DNA damage, and enforces cell cycle arrest of HL-60 cells, with these primary effects associated with oxidative stress. These findings contribute to a deeper understanding of the potential therapeutic role of GE in combating acute promyelocytic leukemia and other health challenges.

Keywords: HL-60 cells; Garlic extract; Cytotoxicity; Oxidative stress; Cell cycle arrest; DNA damage

Abbreviations: GE: Garlic Extract; MDA: Malondialdehyde; HL: Human Leukemia; FBS: Fetal Bovine Serum; AO: Acridine Orange; PI: Propidium Iodide

INTRODUCTION

Garlic (*Allium sativum*) now cultivated around the world is natively from Central Asia. It is a popular spice and a remedy for a variety of ailments. It has been used worldwide both for its nutritional and medicinal properties. Many cultures incorporate garlic into their culinary traditions, not only for its distinctive flavor but also for its potential health benefits, such as boosting the immune system and

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reducing blood pressure. Additionally, garlic is often celebrated for its antioxidant properties, making it a staple in both traditional and modern herbal medicine. It plays an important role in the treatment of human diseases and is becoming a significant resource for drug development. It is ranked the highest among all herbal remedies consumed for its health benefits [1,2]. Throughout history, garlic is used to lower cholesterol and blood glucose, inhibit the formation of blood clots improve the immune system and treat a wide variety of diseases including asthma, leprosy, diarrhea, rheumatism, dermatitis, Chronic inflammatory bowel disease, abdominal pain, and bacterial infections [3-11]. Several pharmacological studies demonstrated that garlic possesses several medicinal properties including immune enhancement, cardioprotective, hepatoprotective, hypolipidemic, antioxidant, antiviral, antibacterial, anti-diabetic, anti-hypertensive, and anti-cancer [12-16]. The reputation of garlic as an effective remedy for tumors extends back to the Egyptian codex Ebers of 1550 B.C. [17,18].

The anticancer effects of garlic are attributed to a high concentration of its Organosulfur Compounds (OSCs) including Diallyl Sulfide (DAS) Diallyl Disulfide (DADS), Diallyl Trisulfide (DATS), and allicin which is the chief biologically active component of garlic [13,19,20]. These OSCs originating from garlic inhibit cell growth, induce cell cycle arrest, and stimulate the mitochondrial apoptotic pathway in numerous cancer cells [21-24]. Also, bioactive components of garlic have demonstrated anticancer properties by displaying significant anticarcinogenic actions *via* several mechanisms, including induction of oxidative stress, DNA damage, cell cycle arrest, apoptosis of cancer cells, and activation of angiogenic cascade [25-28]. The bioactive components of garlic are responsible for its healing properties. *In vitro* and *In vivo* studies demonstrated that garlic and its derivatives suppress carcinogenesis and inhibit the proliferation of several cancer cells including colorectal, esophageal, gastric, lung, prostate, and skin cancer [29-33]. Although numerous studies indicate that garlic has medicinal properties against several types of cancers, its detailed molecular mechanisms of action against leukemia remain unknown.

This research aimed to elucidate the pathways involved and assess the potential therapeutic applications of garlic in leukemia treatment. By examining the biochemical changes induced by garlic extract, we hope to provide insight into its role as a natural agent in cancer therapy and contribute to the development of more effective treatment strategies.

MATERIALS AND METHODS

Cell line and culture

The human promyelocytic leukemia cell line was purchased from the American Type Culture Collection-ATCC (Manassas, VA). In the laboratory, cells were stored in the liquid nitrogen until use. They were next thawed by gentle agitation of their containers (vials) for 2 minutes in a water bath at 37°C. After thawing, the content of each vial of cells was transferred to a 25 cm^2 tissue culture flask, diluted with up to 10 mL of RPMI 1640 containing 1 mmol/L L-glutamine (GIBCO/BRL, Gaithersburg, MD) and supplemented with 10% (v/v) Fetal Bovine Serum (FBS) and 1% (w/v) penicillin/streptomycin. The 25 cm^2 culture flasks (2 × 10^6 viable cells) were observed under the microscope, followed by incubation in a humidified 5% CO_2 incubator at 37°C. Three times a week, they were diluted under the same conditions to maintain a density of 5 × 10^5/mL and harvested in the exponential phase of growth. The cell viability was assessed by the trypan blue exclusion test (Life Technologies) and manually counted using a hemocytometer.

Chemicals and media

Growth medium Iscove's Modified Dulbecco's Medium (IMDM) containing L-glutamine was purchased from American Type Culture Collection-ATCC (Manassas, VA). FBS, Phosphate Buffered Saline (PBS), and an MTT assay kit were obtained from Sigma Chemical Company (St. Louis, MO). Trypan blue cell viability dye was purchased from Lonza Inc. (Walkersville, MD, USA). Propidium Iodide (PI) was purchased from BD Biosciences (Pharmingen, Becton Dickinson Co., San Diego, CA, USA). The lipid peroxidation kit was obtained from Abcam (Cambridge, MA). The comet assay kit was obtained from Trevigen (Gaithersburg, MD, USA).

Preparation of garlic extract

We prepared garlic extract by homogenizing the required amount of garlic powder in an appropriate volume of distilled water to make a stock concentration of 100 mg/mL. This mixture was incubated overnight at 4°C. The homogenate was centrifuged at 3000 × g for 10 minutes and filtered to remove particulate matter; then the supernatant fraction was used for the assays.

Cell Treatment and assessment of the effects of GE on HL-60 cells

For this experiment, HL-60 cells (5 × 10^5 cells/mL) in suspension were treated with three concentrations (2, 4, and 6 µg/mL) of GE for 24 hours. After treatment, the control sample and treated cells were washed twice with PBS. For the MTT assay, 20 µL of the MTT solution were added to each of the 96-well plates containing untreated and treated cells. Then cells were incubated for 3 hours at 37°C. After incubation, the absorbance was read at 490 nm using a Biotex Model microplate reader. For the trypan blue dye exclusion assay, 10 µL of trypan blue (dye) was added to 100 µL of cell suspension taken out from each sample. The sample was gently mixed, and 20 µL of cell suspension was loaded into the counting chamber of the Cellometer Vision, and both cell concentration and viability were determined using the Cellometer Vision software as previously described [34]. For AO/PI staining, 50 µL of AO/PI dye were added into 1 mL of HL-60 cells in 12-well plates and incubated overnight at 37°C. Cells were examined and photographed under an Olympus fluorescent microscope.

Analysis of oxidative stress by lipid peroxidation assay

Lipid peroxidation is a chain reaction, which, once started, proceeds through three main steps referred to as initiation, propagation, and termination. It is a biochemical process that leads to the formation of byproducts including MDA and 4-hydroxynonenal. The extraction and measurement of MDA were performed using the lipid peroxidation MDA assay kit (Abcam, Cat. No. ab118970) to detect the level of MDA following the manufacturer's protocol with few modifications [35]. In summary, the cells were seeded at 2 × 10^6 HL-60 cells/mL untreated as a control, and cells treated with GE were then incubated in a total volume of 10 mL of growth medium for 24 hours. After the incubation period, cells were harvested in 15 mL tubes, followed by low-speed centrifugation (1,250 rpm for 6 minutes). The cell pellet was lysed in 200 µL MDA lysis buffer on ice with 2 µL BHT (100X) for 5 minutes. The

mixture was further centrifuged at 13000 × g for 10 minutes to remove insoluble material. TBA reagent was added to the samples (150 µL) and standards, then was incubated in the water bath set at 95°C for 1 hr, and was cooled on ice for 10 minutes. The sample and the standards (100 µL) were transferred to a 96-well plate, and the absorbance was read using the spectrophotometer microplate reader at a 532 nm wavelength. The standard curve was generated and used to calculate the MDA production in the treated and untreated samples.

Analysis of oxidative stress by glutathione peroxidase assay

We measured the Glutathione Peroxidase (GPx) activity using a glutathione peroxidase assay kit from Calbiochem-EMD Biosciences in Gibbstown, NJ. Cells were treated with and without GE according to the aforementioned concentrations for 24 hours. The cells were harvested, rinsed twice with PBS, and digested in 1 mL of ice-cold 50 mM Tris-HCl, 5 mM Ethylenediamine Tetraacetate (EDTA), and 1 mM DTT. Cellular debris was removed by centrifugation, and lysate was used to determine GPx activity. The absorbance was measured at 340 nm using the Lab Systems Multiskan Ascent microplate reader. The rate of the reaction was determined by constructing a standard curve as a function of absorbance versus time, and the GPx activity was expressed as nmol/minute/mL.

Analysis for DNA damage by comet assay

Garlic extract genotoxicity in treated and untreated HL-60 cells was analyzed by alkaline single-cell gel electrophoresis (comet) assay as described earlier with few modifications using a comet assay kit for single gel electrophoresis from Trivegen (Gaithersburg, MD, USA) [36]. Cells were counted (10,000 cells/well), and aliquots of 100 µL of the cell suspension were placed in each well of 12-well plates, then treated with 100 µL aliquots of media for the control and GE with the final concentrations of 2, 4, and 6 mg/mL respectively, and incubated in 5% CO_2 at 37°C for 24 hours. After incubation, the cells were centrifuged, washed two times with cold PBS free of calcium and magnesium, and resuspended in 100 µL PBS. In a 2 mL test tube, 50 µL of the cell suspension and 500 µL of melted LMAgarose were mixed, and 75 µL was immediately pipetted into a pre-warmed comet slide. The side of the pipette tip was used to gently spread the agarose/cells completely over the sample area. The slides were placed flat in the dark at 4°C for 30 minutes to allow the mixture to solidify and then were immersed in pre-chilled (in ice) lysis solution at 4°C for 40 minutes. The slides were removed from the lysis solution, tapped gently, and immersed in alkaline solution, pH>13, for 40 minutes at room temperature in the dark. The slides were washed twice for 5 minutes with Tris-borate-EDTA (TBE). The slides were electrophoresed in a horizontal gel apparatus at low voltage (300 mA, 25 V) for 20 minutes. The slides were placed in 70% ethanol for 5 minutes, removed, tapped, and air-dried overnight. The slides were stained with SYBR Green stain designed for the Comet assay and were allowed to air dry at room temperature for six hours. SYBR Green-stained Comet slides were viewed with an Olympus fluorescence microscope and analyzed using LAI's Comet Assay Analysis System Software (Loates Associates, Inc., Westminster, MD).

Analysis of cell distribution across different stages by the cell cycle analysis

The cell cycle analysis was done with PI staining to find out how many cells were in each stage of the cell cycle for both control and GE-treated HL60 cells. The cells were seeded into 6-well plates at the density of 6 × 10^6 cells/well and treated with various concentrations (0, 2, 4, and 6 mg/mL) of GE for 24 hours. Then, the cells were harvested, washed twice with 200 µL of 1X PBS, and fixed in 500 µL of ice-cold methanol for 30 minutes.

The cells were centrifuged at 1250 rpm for 8 minutes, then the supernatant was removed, and the pellet was resuspended in 150 µL of PI solution (Nexcelom Biosciences, Lawrence, MA) and was incubated for 40 minutes as described previously [37]. Cells were centrifuged again to remove the supernatant and resuspended in 200 µL 1X PBS, then analyzed using the Cellometer Vision CBA Software (Nexcelom Biosciences, Lawrence, MA).

Statistical analysis

Experiments were performed in triplicate. Data were presented as means ± SDs. Statistical analyses were done using one-way Analysis Of Variance (ANOVA Dunnett's test) for multiple samples, or Student's paired t-test was used to analyze the difference between the controls and garlic extract-treated cells. All p-values<0.05 were considered statistically significant.

RESULTS

Cytotoxic efficacy of garlic extract on human leukemia HL-60 cells

Garlic is ranked the highest among all herbal remedies consumed for its health benefits. As such, we tested the cytotoxic effects of GE treated with HL-60 cells by the trypan blue exclusion test using the Cellometer Vision. Our data revealed after treatment that GE gradually reduced the viability of HL-60 cells in a dose-dependent manner (Figures 1 and 2). As seen in Figures 1 and 2, GE inhibits the growth of HL-60 cells (green color) and increases the death of HL-60 cells (red color) with increasing concentration of GE.

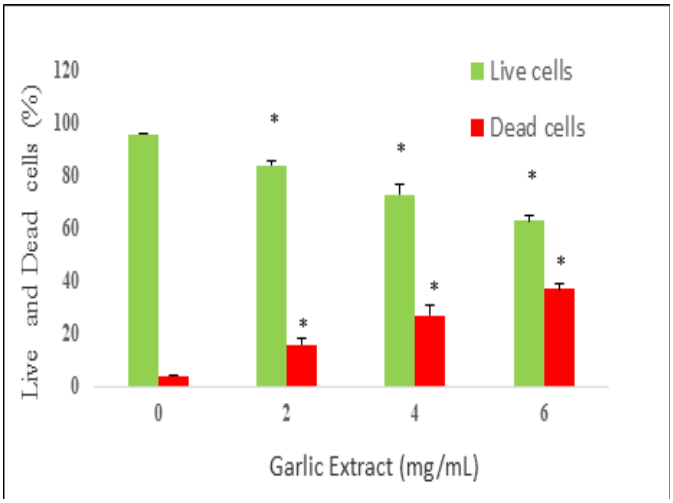

Figure 1: Cytotoxic effects of garlic extract on human leukemia (HL-60) cells. HL-60 cells were treated with various concentrations of garlic extract (2, 4, and 6 mg/mL) for 24 hours as indicated in the Materials and Methods. Cell viability was determined based on the trypan blue exclusion test. Each point represents a mean ± SD of 3 experiments. Significantly different (p<0.05) from the control, according to the ANOVA Dunnett's test.

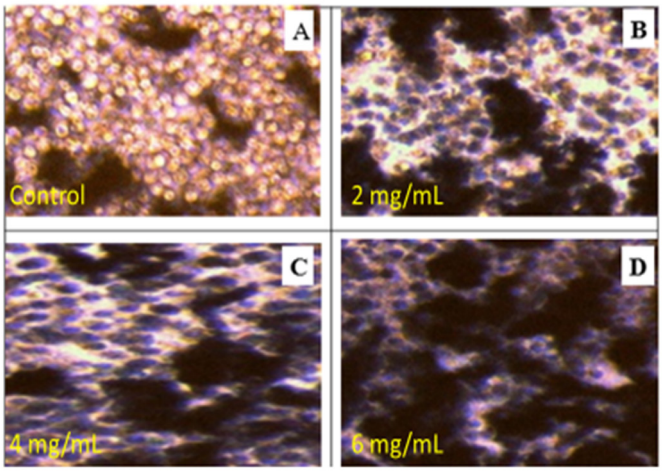

Figure 2: Representative fluorescence images of HL-60 cells stained with trypan blue. The dye does enter the cells when the membrane is compromised. Gradual intensity of blue color is noticeable in the GE-treated samples (2 mg/mL-6 mg/mL compared to the control).

To assess the alterations of HL-60 cell morphology, untreated and treated cells with GE were observed under a microscope (Figure 2). As observed in Figure 2, the trypan blue dye did not penetrate the plasma membrane of untreated HL-60 cells (control) because the cells are healthy and their plasma membranes are undamaged. However, the trypan blue dye significantly penetrated the plasma membrane of treated HL-60 cells and got into the cytoplasm in a concentration-dependent manner because the cell membrane was damaged.

To confirm that GE effectively decreases the cell viability, the antiproliferative effect of GE was determined by the MTT assay. Data generated from the MTT assay showed that the viability of HL-60 cells was significantly ($p<0.05$) decreased as compared to the control cells in a concentration-dependent manner (Figure 3). The present finding clearly demonstrates that HL-60 cells were sensitive to GE treatment, with the IC_{50} value that caused a 50% loss of the HL-60 cell viability computed to be 4.72 mg/mL upon 24 hours of treatment.

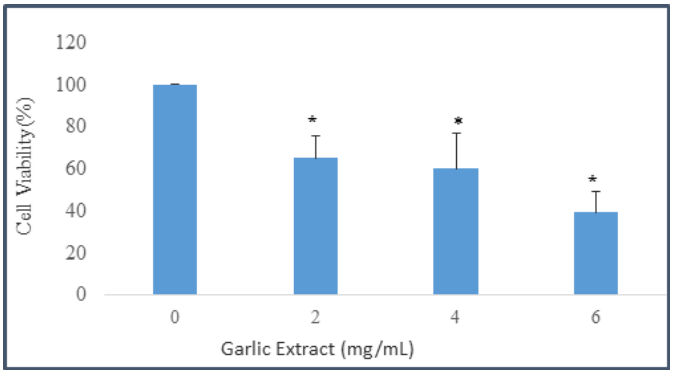

Figure 3: Cytotoxic effect of garlic extract on human HL-60 cells. HL-60 cells were treated with different concentrations of GE at 0, 2, 4, and 6 mg/mL for 24 hours as described in the Materials and Methods section. Data are expressed as means ± SD (n=3). **Note:** *Significantly different ($p<0.05$) from the control, according to the Dunnett's test.

To further confirm that GE effectively decreases the viability of HL-60 cells, we performed AO/PI double staining. As seen in Figure 4, the untreated cells (A-Control) retained normal cell morphology and attached firmly to the culture plates with a random orientation. However, the treated cells with 2, 4, and 6 µg/mL of GE showed remarkable cell damage, showing decreases in cell number, rounding effects, reduction in cell size, detachment from the substratum, hydropic degeneration of cytoplasm, and more apoptotic bodies. Significant morphological changes were observed for the highest concentration tested, presenting features of necrosis such as a loss of membrane integrity, no vesicle formation, and complete lysis, as compared to the control cells. These findings suggest that increasing concentrations of GE have a pronounced cytotoxic effect on the cells, leading to both apoptotic and necrotic pathways. Further investigation is warranted to explore the underlying mechanisms of GE's action and to evaluate its potential implications for therapeutic interventions.

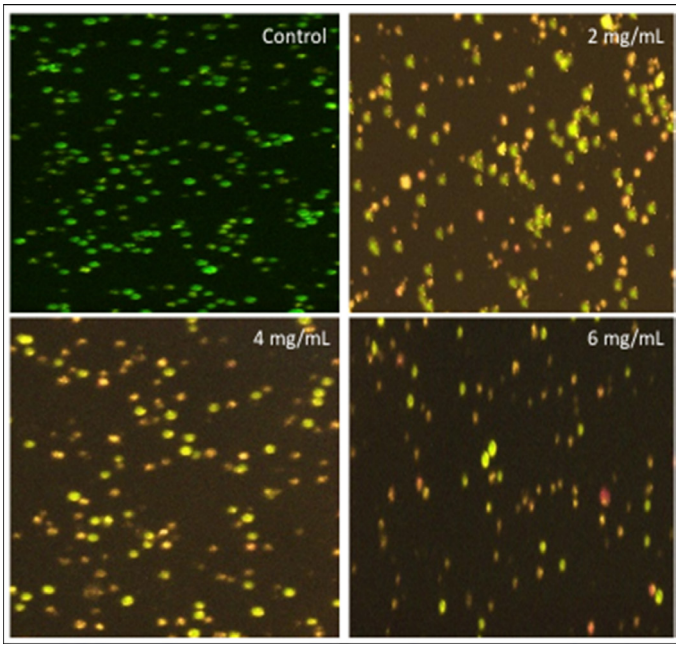

Figure 4: Representative fluorescence images of HL-60 cells stained with AO/PI. Live cells are stained in green, and dead cells are stained in red. Control cells (0 mg/mL), 2 mg/mL, 4 mg/mL, and 6 mg/mL of the treatment were compared to assess the cytotoxic effects. The increasing concentrations of the treatment resulted in a higher proportion of red-stained cells, indicating a dose-dependent increase in cell death.

Garlic extract induces oxidative stress in HL-60 cells

To determine whether oxidative stress plays a role in the GE-induced antiproliferative effect against HL-60 cells, we measured the levels of lipid peroxidation and glutathione peroxidase activity in HL-60 cells. The results showed a significant increase in lipid peroxidation levels and a decrease in glutathione peroxidase activity as the concentration of garlic extract increased. These findings suggest that oxidative stress may indeed be a key mechanism through which garlic extract exerts its cytotoxic effects on HL-60 cells. Results from the lipid peroxidation assay demonstrated that GE significantly ($p<0.05$) increased the production of malondialdehyde (a by-product of lipid peroxidation and biomarker of oxidative stress) in HL-60-treated cells compared to the control (Figure 5). Upon 24 h of treatment, the MDA values were 0.217 ± 0.008, 0.514 ± 0.018, 0.613 ± 0.016, and 0.682 ± 0.018 nmol in 0, 2, 2.4, and 6 mg/mL of GE, respectively.

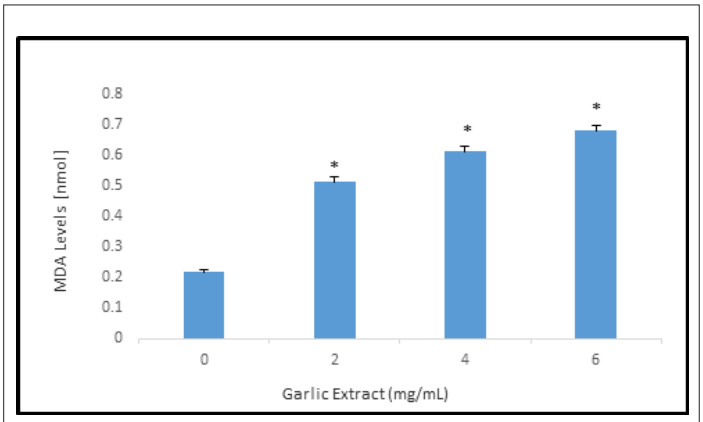

Figure 5: Effect of different concentrations of garlic extract on MDA production in untreated and treated HL-60 cells for 24 hours. The concentrations that were found to be statistically significantly different ($p<0.05$) compared to the control are denoted according to ANOVA Dunnett.

Glutathione peroxidase activity

To validate that GE induces oxidative stress in HL-60 cells, we evaluated the activity of glutathione peroxidase. We observed that GE significantly increases the activity of glutathione peroxidase in treated HL-60 cells compared to the control. The control sample absorbance was 0.289 ± 0.056 at the wavelength of 340 nm. The optical density readings for 2 mg/ml, 4 mg/ml, and 6 mg/ml were 0.891 ± 0.122, 0.207 ± 0.122, and 0.946 ± 0.03, respectively (Figure 6).

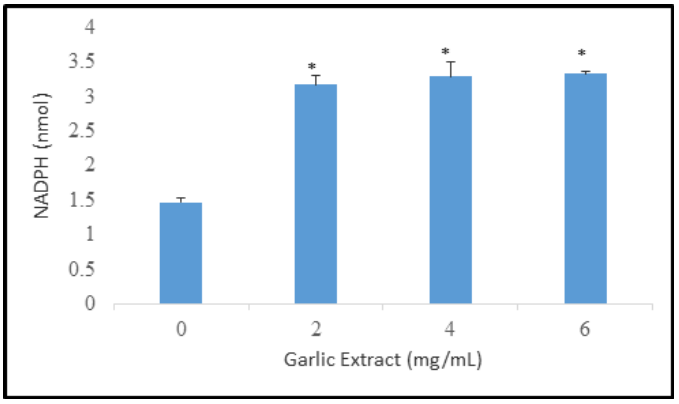

Figure 6: Glutathione peroxidase production in HL-60 cells treated with GE. Cells were treated with 0, 2, 4, and 6 mg/ml of GE, respectively, for 24 hours. The treated samples were found to be significantly different ($p<0.05$) from the control according to ANOVA Dunnett's test.

Genotoxic effect of garlic extract on HL-60 cells

In this research, we used the comet assay technique to study GE-induced DNA damage in treated HL-60 cells. Representative comet assay images of control and GE-treated cells using SYBR Green stain are presented in Figure 7. Our data showed a gradual increase in the mean values of comet tail length, tail moment, and percentages of DNA cleavage in HL-60 cells with increasing concentrations of GE, indicating that GE treatment is able to induce DNA damage in human leukemia cells in a concentration-dependent manner.

As seen in Figure 7, the image (A) represents untreated HL-60 cells and serves as the control or baseline. Image A shows cells with no or minimal DNA damage will appear as compact, round shapes. Image (B) shows HL-60 cells treated with garlic extract at a concentration of 2 mg/mL for 24 hours, showing some level of DNA damage compared to the control. The images (C) represent HL-60 cells treated at a concentration of 4 mg/mL, showing more pronounced comet tails, suggesting greater DNA damage. The image (D) displays HL-60 cells treated with the highest concentration of garlic extract, 6 mg/mL, revealing more substantial DNA damage, with longer and more prominent comet tails compared to the control.

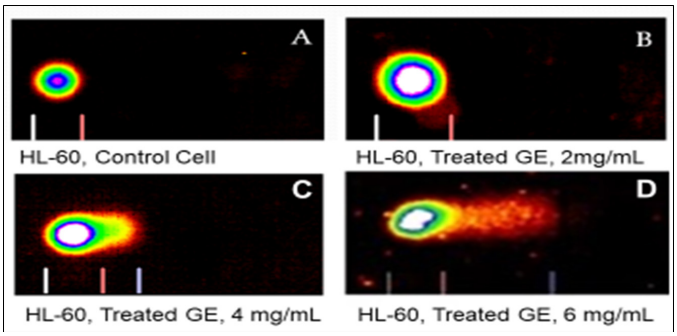

Figure 7: SYBR Green comet assay with images representing untreated (A-control) and garlic extract-treated Human Leukemia (HL-60) cells at 2 mg/mL (B), 4 mg/mL (C), and 6 mg/mL (D) for 24 hours.

Induction of cell cycle arrest

We analyzed the cell cycle profile using the Cellometer Vision software. Treatment of HL-60 cells with GE significantly decreases cell population at the G0/G1 phase from 38% in the control sample to 7.6% in the treated sample at 6 mg/mL (Figure 8). Meanwhile, we observed an increased cell population at the G2/M phase, indicating that more cells transitioned from S to G2/M in preparation for mitosis. Therefore, the increase of cell population at the G2/M phase and decrease of cell population at the G0/G1 phase suggested that GE caused cell cycle arrest at the G2/M checkpoint.

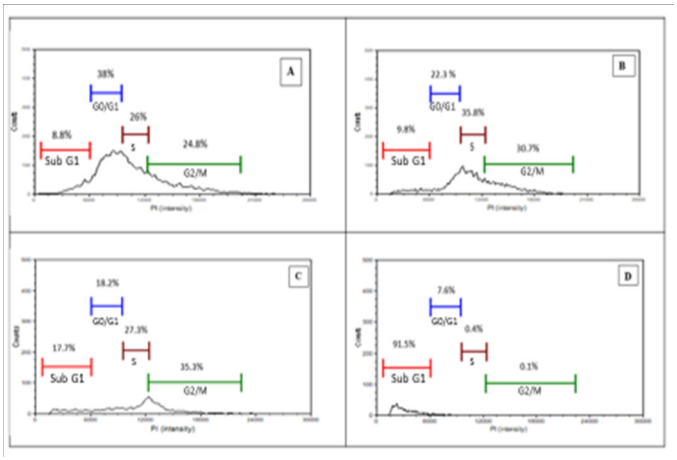

Figure 8: Cell cycle analysis of HL-60 cells treated with GE. Histogram of percentage of HL-60 cells in each phase, representative of (A) control, (B) 2 mg/mL, (C) 4 mg/mL, and (D) 6 mg/mL GE treatment. Three experiments were performed, and one representative experiment is shown.

Figure 9 below shows bar graph representing the percentage of cell cycle distribution from each phase generated from Figure 8. Sub-G1 in both Figures 8 and 9 indicates apoptotic cell death.

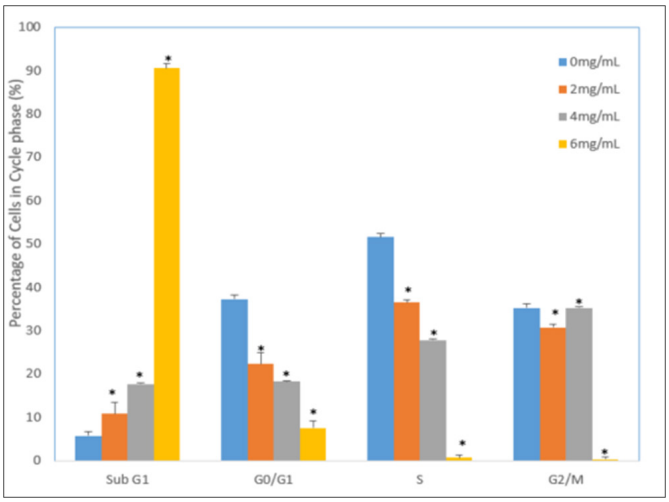

Figure 9: Bar graph showing percentage of cell cycle arrest in GE-treated HL-60 cells. Cell cycle distribution was measured by the propidium iodide staining method. Each point represents the mean ± standard deviation of three independent experiments. **Note:** * denotes a statistically significant difference between the control and the treated group according to ANOVA Dunnett's test ($p<0.05$).

DISCUSSION

Cytotoxic effect of GE on HL-60 human leukemia cells

The reputation of garlic as an effective remedy for tumors extends back to the Egyptian codex Ebers of 1550 B.C. [17]. Garlic and its constituents have been widely used as therapeutic agents for the prevention and treatment of cardiovascular diseases, viral infections, fungal infections, microbial infections, diabetes, cancer, and other metabolic diseases such as hyperlipidemia, hypertension, and atherosclerosis [38-40]. In the present study, we evaluated the cytotoxic effects of GE by means of the trypan blue exclusion test, MTT, and AO/PI assays. Our results demonstrated that garlic extract significantly ($p<0.05$) reduced the viability of HL-60 cells in a concentration-dependent manner (Figures 1-4). In addition, the trypan blue dye and AO/PI dye results revealed that GE induced morphological alterations characteristic of apoptosis and necrosis, such as cell shrinkage, blebbing of the plasma membrane, chromatin condensation, cytoplasmic swelling, cell membrane damage, organelle breakdown, and formation of apoptotic bodies in treated HL-60 cells compared to the control with intact cell morphology (Figures 2 and 4). In agreement with our observations, other studies have observed similar morphological alterations *in vitro* and *in vivo* [41,42]. A previous report from our laboratory demonstrated the anti-proliferation of GE in treated HL-60 cells for 12 hours [42]. Several studies conducted in the USA and China have shown an association between increased intake of raw garlic and reduced risk of cancers of the stomach, colon, esophagus, pancreas, blood, lung, and breast [43-45].

Garlic extract induces oxidative stress on HL-60 human leukemia cells

The ability of GE to modulate oxidative stress was estimated by measuring the level of lipid peroxidation products and the activity of glutathione peroxidase in HL-60 cells. Our results showed that GE significantly increases ($p<0.05$) the production of MDA levels and the activity of glutathione peroxidase in GE-treated cells compared to the control group. Our findings are consistent with previous studies revealing that garlic treatment induces oxidative stress, cell cycle arrest, and apoptosis of cancer cells [46-48].

Genotoxic effects of garlic extract on HL-60 human leukemia cells

To determine the ability of GE to cause genotoxic damage in leukemia cells, HL-60 cells were treated with different concentrations of GE, in the range of 0–6 mg/mL, for 24 hours, and the degree of DNA damage was quantified by means of LAI's Comet Assay Analysis System software (Loates Associates, Inc., Westminster, MD) after staining with SYBR Green. Our results showed that GE causes DNA single-strand breaks in treated HL-60 cells compared to the control cells, and there is a gradual concentration-response relationship. Several possible mechanisms may be involved in the induction of DNA damage. Previous studies in our laboratory have provided evidence that oxidative stress is involved in DNA damage induced by carcinogenic metal ions and medicinal plants [49-52].

Garlic extract induced cell cycle arrest in HL-60 human leukemia cells

To gain insight into whether the effect of GE is associated with cell cycle arrest, we assess the cell cycle analysis. We found that GE treatment induced cell population accumulation in the S phase at 2 mg/mL, as well as with 4 mg/mL in the G2/M phase. Meanwhile, the G0/G1 phase decreased in cell populations with increasing concentrations of GE. The increase of cell population at the S and G2/M phases and decrease of cell population at the G1/G0 phase suggested that GE caused cell cycle arrest at the G2/M checkpoint. A similar report indicated that *z*-ajoene displayed a progressive increase in the percentage of mitotic cells and was blocked at the G2/M phase after treatment with 10 microml/L *z*-ajoene on HL-60 cells [53]. Several research studies have indicated that natural products triggered apoptosis through cell cycle arrest in cancer cells [54-56]. In addition, a study indicated that the green tea constituent (-)-epigallocatechin-3-gallate induced growth inhibition, cell cycle dysregulation, and apoptosis of androgen-sensitive and androgen-insensitive human PCa cells [57]. Apigenin induced cell cycle arrest and apoptosis in xenograft prostate cancer model, which is found to be mediated through modulation of MAPK, PI3K-Akt, and loss of cyclin D1-associated retinoblastoma dephosphorylation in human PCa cells [58,59]. We found no study in the literature regarding cell cycle arrest caused by GE in HL-60 cells. Here, to the best of our knowledge, we report for the first time that GE causes cell cycle arrest at the G2/M checkpoint in HL-60 cells.

CONCLUSION

Many plant-derived natural products have been evaluated against a plethora of cancer cells. Due to their minimal toxicity, plant-derived anticancer agents have gained significant attention in recent years. Garlic and its bioactive compounds, such as allicin, have been studied for their potential health benefits, including anticancer properties, due to their minimal toxicity in normal cells. Here, we tested the cellular and molecular mechanisms by which GE induces cytotoxicity, oxidative stress, DNA damage, and cell cycle arrest in HL-60 cells. Our findings demonstrated GE significantly reduces the viability of HL-60 cells in a concentration-dependent manner, causing a 50% cell reduction at 4.72 mg/mL. We found that GE induces cytotoxicity, DNA damage, and cell cycle arrest in HL-60 cells through oxidative stress. Taken together, GE may represent a potential therapy for the prevention and/or treatment of acute

promyelocytic leukemia. However, while our study provides promising results, further research, including *in vivo* and clinical trials, would be necessary to confirm the safety and efficacy of GE as a therapeutic option for acute promyelocytic leukemia.

AUTHOR CONTRIBUTIONS

Conceptualization: Sylvianne Njiki, Clement G. Yedjou, and Paul B. Tchounwou; Methodology: Sylvianne Njiki, Keara Johnson, Jennifer N. Sims, and Ariane M. Chitoh, and Felicite K. Noubissi; Formal Analysis: Sylvianne Njiki, Keara Johnson, Jennifer N. Sims, and Ariane M. Chitoh, and Felicite K. Noubissi, and Barbara Graham; Investigation: Sylvianne Njiki, Keara Johnson, Jennifer N. Sims, and Ariane M. Chitoh, and Clement G. Yedjou; Supervision: Clement G. Yedjou, and Paul B. Tchounwou; Funding: Clement G. Yedjou, and Paul B. Tchounwou; Writing-Original Draft Preparation: All authors; Writing-Reviewing and Editing: All authors; All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

The data that support the present research study are included in the article.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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