

1-1-2010

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AVCI, ASLIHAN; SUNGUROĞLU, ASUMAN; ERGÜDER, İMGE B.; AKAY, GÜVEM GÜMÜŞ; DEVRİM, ERDİNÇ; BAYDIN, PINAR ÖZKAL; VAROL, NURAY; and DURAK, İLKER (2010) "Effects of aqueous garlic extract on oxidant/antioxidant status in 32D and 32Dp210 cell lines," *Turkish Journal of Medical Sciences*: Vol. 40: No. 6, Article 8. <https://doi.org/10.3906/sag-0901-33>
Available at: <https://journals.tubitak.gov.tr/medical/vol40/iss6/8>

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Effects of aqueous garlic extract on oxidant/antioxidant status in 32D and 32Dp210 cell lines

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Aim: To investigate the possible effects of aqueous garlic extract on the oxidant/antioxidant status and apoptosis in 32D (wild type mouse myeloid cell = normal) and 32Dp210 (BCR-ABL fusion gene (+) mouse myeloid cell = Chronic Myelocytic Leukemia cells) cell lines.

Materials and methods: Aqueous garlic extract (10% w/v) was added into the cell line media with 2 different final concentrations (0.4% and 1%). At 0 h and at 24, 48, and 72 h later, the oxidant (malondialdehyde (MDA) level, and xanthine oxidase (XO) enzyme activity) and antioxidant (superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) enzyme activities) parameters were measured in the cell lines.

Results: It was observed that the garlic extract caused no change in the XO and antioxidant enzyme activities, but it increased the MDA level in the 32D cell line. However, significant increases were found in the MDA level, XO, and antioxidant enzyme activities in the 32Dp210 cell line treated by the garlic extract. Additionally, it was shown that garlic extract had antiproliferative and apoptotic effects on both cell lines. The most effective apoptotic dose was found to be 0.4% (w/v), and at this concentration the death risk of the 32Dp210 cell line was calculated at 2.08 times higher than that of the 32D cell line.

Conclusion: It has been suggested that garlic directly causes oxidant stress in the 32D cell line owing to its own oxidant ingredients, and that the oxidant stress created by garlic in the 32Dp210 cell line might occur through increased XO activity and/or its oxidant ingredients. Additionally, antioxidant enzyme activities were found to increase in the 32Dp210 cell line; it would seem that this compensatory change could not prevent the oxidant stress created. We think that the oxidant potential of garlic extract might play a part in its possible anticancer potential, previously supposed by several investigators.

Key words: Garlic, mouse myeloid cell line, oxidant/antioxidant status

32D ve 32Dp210 hücre serilerinde aköz sarımsak ekstresinin oksidan/antioksidan durum üzerine etkileri

Amaç: Bu çalışmada aköz sarımsak ekstresinin 32D ve 32Dp210 hücre serilerinde oksidan/antioksidan ve apoptoz üzerine olası etkilerinin araştırılması amaçlandı.

Yöntem ve gereç: Bu amaçla aköz sarımsak ekstresinden (%10luk w/v) iki farklı konsantrasyonda (% 0,4 ve % 1) hücre ortamına eklendi. 0, 24, 48 ve 72 saat sonra oksidan belirteç olarak malondialdehit (MDA) düzeyi ve ksantin oksidaz (XO) enzim aktivitesi ve antioksidan olarak süperoksit dismutaz, glutatyon perokidaz ve katalaz (SOD, GSH-Px ve CAT aktiviteleri) enzim aktiviteleri hücre serilerinde ölçüldü.

Bulgular: Sarımsak ekstresinin 32 D hücre serisinde XO ve antioksidan enzim aktivitelerinde bir değişikliğe yol açmadığı fakat MDA düzeyini arttırdığı gözlemlendi. Bununla birlikte sarımsak ile tedavi edilen 32 Dp210 hücre serisinde MDA düzeyi, XO ve antioksidan enzim aktivitelerinde önemli artışlar bulundu. Ek olarak her iki hücre serisinde sarımsak

Received: 29.01.2009 – Accepted: 17.05.2010

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ekstresinin antiproliferatif ve apoptotik etkileri gösterildi. 32 Dp210 hücre serisinde en efektif apoptotik doz % 0,4 (w/v) olarak bulundu ve ölüm riski bu konsantrasyonda 32 D hücre serisinden 2,08 kat daha yüksek bulundu.

Sonuç: Sonuç olarak, sarımsağın 32 D hücre serisinde kendi oksidan içeriğinden dolayı oksidan strese yol açtığı ve 32 Dp210 hücre serisinde XO artışı ve kendi oksidan içeriğinden ötürü oksidan stres oluşturduğu öne sürülmektedir. Ek olarak 32 Dp210 serisinde antioksidan enzimlerin arttığı fakat bu kompensatuvar değişikliğin oksidan stres oluşumna karşı korumadığı bulundu. Biz sarımsak ekstresinin daha önceden çeşitli araştırmacılar tarafından da öne sürülen antikanser etkisinde onun oksidan potansiyelinin rol oynayabileceğini düşünüyoruz.

Anahtar sözcükler: Sarımsak, fare miyeloid hücre serisi, oksidan/antioksidan durum

Introduction

In garlic extracts (*Allium sativum* L.) the presence of 2 main classes of antioxidant components, namely flavonoids (1-4) and sulfur-containing compounds, such as diallyl sulfide and trisulfide, and allyl-cysteine have been reported. CML (Chronic Myeloid Leukemia) is a myeloproliferative disorder that is characterized by the Philadelphia (Ph) chromosome. The Ph chromosome is found in approximately 95% of CML patients. This chromosome is caused by reciprocal translocation t(9;22)(q34;q11.2), which results in the BCR-ABL fusion gene and produces a fusion tyrosine kinase (FTK). Oncogenic tyrosine kinases are thought to induce, either directly or indirectly, a critical repertoire of transforming events, namely uncontrolled cell growth, genomic instability, and protection of DNA-damaged cells from apoptosis (2).

Apoptosis is a physiologically programmed mechanism, by which cells die. It is characterized by chromatin condensation, membrane blebbing, cell shrinkage, and DNA fragmentation. These changes in cells undergoing apoptosis are easily recognizable using electron or light microscopy. Internucleosomal DNA fragmentation may be detected by gel electrophoresis (3).

Reactive oxygen species (ROS) originated from mitochondria induces molecular signaling, such as p53. In mitochondria-dependent apoptosis, molecular signaling returns to mitochondria, then triggers the release of critical apoptotic activators of cell death. It means that oxidant stress may induce apoptosis in cancerous and/or noncancerous cells.

Garlic is a plant commonly used for seasoning food in many different cultures of the world, and its medicinal properties have been known since ancient times (3). In general, garlic has been used throughout

the world to treat coughs, toothaches, earaches, dandruff, hypertension, hysteria, diarrhea, dysentery, diphtheria, vaginitis, and many other conditions (5). Epidemiological studies have shown that enhanced garlic consumption is closely related to reduced cancer incidence, including esophageal, mammary, skin, pulmonary, forestomach, colon, and lung (6-10). In vitro studies indicate that garlic has antiproliferative and apoptotic effects on different cancer cell lines including HL60, DU145, PC3, BGC823, and HCT15. However, there are no reports on whether or not it affects CML cell lines in vitro.

In the present study, the aim was to investigate the possible effects of aqueous garlic extract on oxidant/antioxidant status and cell death in 32D (wild type mouse myeloid cell line = normal) and 32Dp210 (BCR-ABL fusion gene (+) mouse myeloid cell line = Chronic Myelocytic Leukemia cells) cell lines.

Materials and methods

Cell culture preparation

The 32D and 32Dp210 cell lines (5×10^6 cells/mL in 6 well plates) were cultured in RPMI-1640 medium supplemented with 20% heat inactivated fetal calf serum, 2 mM L-Glutamine, and antibiotics (1,000,000 U/mL penicillin, and 1 g/mL streptomycin) at 37 °C under a humidified 5% CO₂ atmosphere. Both cell lines were treated with garlic at a final concentration of 1% (w/v) and 0.4% (w/v) at 0, 24, 48, and 72 h. Untreated cells were used as a negative control for each condition.

Enzymatic assays

Analyses were performed as described in the references for xanthine oxidase (XO), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) and malondialdehyde (MDA)

analyses, respectively (11-15). Results were expressed as international unit/mL (GSH-Px and CAT). One unit for SOD activity was expressed as the enzyme protein amount causing a 50% inhibition in NBT reduction rate. The MDA level was given in nmol/mL nmol/million cells. The CAT activity was determined by measuring the absorbance decrease of hydrogen peroxide (H₂O₂) at 240 nm. The GSH-Px activity was measured by following changes in the NADPH absorbance at 340 nm. Xanthine oxidase activity was determined by measuring the uric acid formation from xanthine at 293 nm. In the activity calculations, extinction coefficients of uric acid, H₂O₂, and NADPH were used for XO, CAT, and GSH-Px enzymes, respectively.

Extract preparation

Aqueous garlic extract (10%, w/v) was prepared as described previously (16), using a special kind of garlic (Kastamonu Garlic). The antioxidant potential value of the extract measured with the method described (17) was 596.4 nmol/mL h, which is equivalent to the antioxidant potential value of approximately 1 g of ascorbic acid measured with the same method.

MTT assay for cell viability

The cell proliferation of cultured cells was determined by Cell Proliferation Kit I MTT (ROCHE, IN, USA) according to the manufacturer's instructions. In brief, cells were incubated with the MTT solution for approximately 4 h in 96 well plates. After this incubation period, a water-insoluble formazan dye formed. After solubilization, the formazan dye was quantitated by measuring absorbances at OD550, and OD690 with a SOFTMax Pro 3.12 program.

Apoptosis determination

Apoptosis was examined morphologically (fragmented/condensed nuclei with intact cytoplasm) by light microscopy. Briefly, cells were washed with PBS (pH 7.3), fixed with methanol and acetic acid (3:1), and then stained with 5% Giemsa. For each concentration and duration, 1000 cells were scored from each sample in order to find the percentage of apoptosis.

Apoptosis was also confirmed by DNA laddering using an Apoptotic DNA Ladder Kit (ROCHE, IN,

USA) and analyzed by agarose gel electrophoresis. DNA isolated from Imatinib Mesylate treated 32Dp210 cells was used as appositive control. DNA in the gels was visualized under UV light after staining it with ethidium bromide.

Statistical analyses

The antiproliferative effects of different garlic concentrations were compared by the Kruskal-Wallis Test. The chi-square test was used to determine the effect of concentration differences on apoptosis. P values less than 0.05 were considered statistically significant. In order to calculate the death risk of different concentrations in both cell lines, the odds ratio test was used.

Results

As seen from the tables, significant increases in the MDA level, XO, and antioxidant enzyme activities were found in the 32Dp210 cell line treated with the garlic extract (Tables 1 and 2). However, the garlic extract caused no change in the XO and antioxidant enzyme activities, but it led to an increased MDA level in the 32D cell line (Tables 3 and 4). Morphological examination indicated apoptosis in the 32D and 32Dp210 cell lines treated with 2 different concentrations of fresh aqueous garlic extract (Figure 1A). The DNA ladder pattern also confirmed the apoptosis of both cell lines (Figure 1B).

Figure 2A represents the antiproliferative effects of different aqueous garlic extract concentrations on the 32D cell line at 0, 24, 48, and 72 h. All of the concentrations were found to be significantly different ($P < 0.001$) in respect to their antiproliferative and apoptotic effects on the 32D cell line. For this cell line, there was a significant decrease in the cell number at concentrations of 0.4% (w/v), and 1% (w/v) after 24 h of treatment (Figure 2A). However, maximum apoptotic effect was seen when cells were treated with 0.4% (w/v) of garlic extract (Figure 2B). Therefore, the most effective apoptotic concentration was found to be 0.4% (w/v) for 32D cells.

Figure 3A shows the antiproliferative effect of different aqueous garlic extract concentrations on the 32Dp210 cell line at 0, 24, 48, and 72 h. All of the concentrations were found to be significantly different

Table 1. Enzymes and MDA levels in the 32Dp (Cancerous) cell line (0 and 24 h).

Parameters	0 h			24 h		
	No extract	0.4%	1%	No extract	0.4%	1%
Garlic ext. conc.						
XO μ IU/mL cell	5.80 \pm 3.07	6.88 \pm 1.54	6.52 \pm 1.02	5.86 \pm 2.76	8.49 \pm 0.68	10.62 \pm 1.89*
SOD U/mL cell	2.66 \pm 1.43	2.04 \pm 0.45	3.02 \pm 0.70	1.92 \pm 0.52	3.98 \pm 0.70	4.48 \pm 1.75
GSH-Px IU/mL cell	0.06 \pm 0.03	0.04 \pm 0.01	0.07 \pm 0.01	0.06 \pm 0.03	0.08 \pm 0.03	0.09 \pm 0.06
CAT	0.37 \pm 0.17	0.37 \pm 0.52	0.73 \pm 0.35	0.23 \pm 0.01	0.57 \pm 0.28	1.62 \pm 0.23
MDA nmol/mL cell	1.29 \pm 0.15	1.77 \pm 0.70	1.03 \pm 0.29	0.75 \pm 0.12	2.53 \pm 0.68	3.65 \pm 0.60*

(Significant differences were marked (). No extract group versus maximum (1%) concentration)

Table 2. Enzyme activities and MDA levels in the 32Dp (Cancerous) cell line (48 and 72 h).

Parameters	48 h			72 h		
	No extract	min	max	No extract	min	max
Garlic ext. conc.						
XO μ IU/mL cell	5.64 \pm 0.70	11.13 \pm 4.76	15.5 \pm 0.82*	5.78 \pm 1.19	13.05 \pm 5.91*	13.33 \pm 4.65*
SOD U/mL cell	1.99 \pm 0.80	3.18 \pm 2.50	3.60 \pm 1.48	2.08 \pm 0.46	3.54 \pm 0.78	3.67 \pm 1.56
GSH-Px IU/mL cell	0.04 \pm 0.01	0.11 \pm 0.04	0.11 \pm 0.02	0.05 \pm 0.01	0.10 \pm 0.04	0.07 \pm 0.01
CAT	0.11 \pm 0.15	1.22 \pm 1.23	1.29 \pm 1.26	0.63 \pm 0.37	1.97 \pm 2.26	1.24 \pm 0.17
MDA nmol/mL cell	0.71 \pm 0.15	2.41 \pm 0.53	3.26 \pm 0.99*	0.63 \pm 0.35	1.76 \pm 0.58	4.05 \pm 1.94*

(Significant differences were marked (). No extract group versus maximum (1%) concentration)

Table 3. Enzyme activities and MDA levels in the 32D (Non-cancerous) cell line (0 and 24 h).

Parameters	0 h			24 h		
	No extract	min	max	No extract	min	max
Garlic ext. conc.						
XO μ IU/mL cell	4.71 \pm 0.51	7.61 \pm 2.56*	6.52 \pm 0.01*	5.57 \pm 1.66	9.07 \pm 1.82*	11.97 \pm 2.82*
SOD U/mL cell	2.34 \pm 0.88	2.99 \pm 0.38	3.39 \pm 0.51	2.67 \pm 0.12	2.61 \pm 0.98	3.75 \pm 1.24
GSH-Px IU/mL cell	0.06 \pm 0.03	0.05 \pm 0.03	0.06 \pm 0.03	0.06 \pm 0.03	0.07 \pm 0.04	0.07 \pm 0.03
CAT	0.37 \pm 0.17	0.85 \pm 0.17	1.34 \pm 0.52	0.99 \pm 0.20	0.76 \pm 0.53	0.67 \pm 0.47
MDA nmol/mL cell	1.05 \pm 0.19	1.34 \pm 0.0	1.68 \pm 0.06	0.82 \pm 0.36	1.18 \pm 0.16	2.90 \pm 0.76*

(Significant differences were marked (). No extract group versus maximum (1%) concentration)

($P < 0.001$) in respect to their antiproliferative and apoptotic effects on the 32Dp210 cell line. The number of cells started to reduce immediately after treatment with different garlic concentrations. Both of the garlic concentrations resulted in a minimum number of viable cells after 48 h of treatment (Figure 3A). Maximum apoptotic effect was obtained when 32Dp210 was treated with 0.4% (w/v) of garlic concentration (Figure 3B). Therefore, the most

effective apoptotic and concentration was also found to be 0.4% (w/v) for 32Dp210 cells.

In addition, 1% (w/v) garlic treatment had the same effect on 32D and 32Dp210 cell proliferation ($P = 0.977$). On the other hand, 0.4% (w/v) of garlic concentration caused different effects on the proliferation of each cell line ($P = 0.003$). As can be seen from Figure 3A, after a 48 h exposure to 0.4%

Table 4. Enzyme activities and MDA levels in the 32D (Non-cancerous) cell line (48 and 72 h).

Parameters	48 h			72 h		
	No extract	min	max	No extract	min	max
Garlic ext. conc.						
XO μ IU/mL cell	6.01 \pm 0.87	8.38 \pm 3.17*	12.8 \pm 1.41*	8.03 \pm 6.09	6.08 \pm 0.19	10.4 \pm 3.22
SOD U/mL cell	3.15 \pm 0.65	3.58 \pm 0.95	3.79 \pm 0.72	2.68 \pm 0.99	2.74 \pm 0.34	4.07 \pm 1.46
GSH-Px IU/mL cell	0.08 \pm 0.02	0.07 \pm 0.02	0.07 \pm 0.03	0.06 \pm 0.02	0.08 \pm 0.03	0.08 \pm 0.03
CAT	0.77 \pm 0.38	0.34 \pm 0.10	0.75 \pm 0.26	0.70 \pm 0.40	0.39 \pm 0.26	1.68 \pm 0.65
MDA nmol/mL cell	0.78 \pm 0.17	1.69 \pm 0.59	3.38 \pm 0.95*	1.17 \pm 0.31	1.17 \pm 0.31	3.31 \pm 0.73*

(Significant differences were marked (). No extract group versus maximum (1%) concentration)

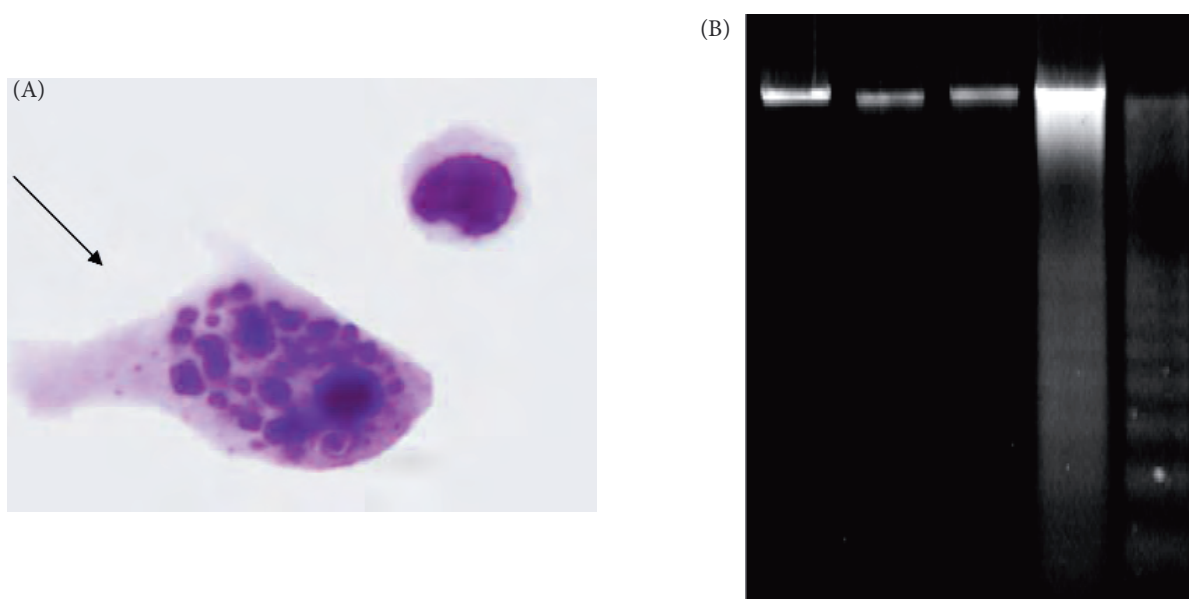


Figure 1. (A) 32Dp210 cell exposed to 0.4% (w/v) fresh aqueous garlic extract for 24 h. Arrow indicates the cell with typical apoptotic morphology.

(B) 1% agarose gel electrophoresis of DNA from 0.4% (w/v) garlic extract treated 32D (lane B) and 32Dp210 (lane D) cells. Lane A: DNA from untreated control 32D cells, Lane C: DNA from untreated control 32Dp210 cells, Lane E: DNA from Imatinib Mesylate treated 32Dp210 cells (Positive control).

(w/v) garlic, 32Dp210 cells have almost completely died. However, 32D cells were still viable even after a 72 h treatment (Figure 2B). It was calculated that at 0.4% (w/v) concentration the death risk of the 32Dp210 cell line was 2.08 times higher than the death risk of the 32D cell line.

Discussion

Garlic is widely consumed worldwide. Fresh garlic contains water, carbohydrates, proteins, fiber, fat, various amino acids, minerals, and vitamins (18-20).

Additionally, garlic includes sulfur compounds, especially allicin, diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), and ajoene. When garlic is cut or crushed, the clove's membrane is disrupted, and S-allylcysteine (SAC) sulfoxide is converted enzymatically into allicin by allinase (21). Allicin is responsible for the typical odor of garlic, but is unstable and converts readily into mono-, di-, and trisulfides, as well as other compounds such as ajoene. The total allicin yield has been determined as 2.5 mg/g of fresh crushed garlic, or about 5-20 mg per clove. Experimental and

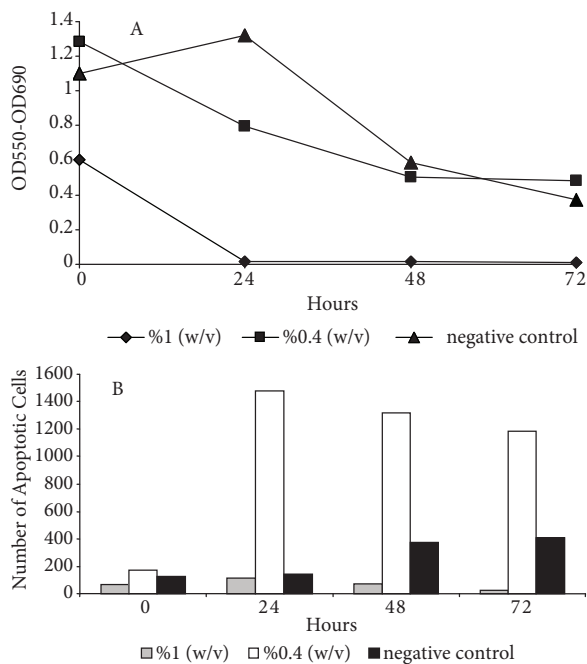


Figure 2. Cell proliferation (A) and apoptosis rates (B) of 32D cells after 0.4% (w/v), and 1% (w/v) fresh aqueous garlic extract treatment for 72 h.

epidemiological studies provided evidence in support of the association between garlic intake and a reduced cancer risk, for example, esophageal, mammary, skin, pulmonary, stomach, colon, and lung tumors (21). Studies indicate that migration from native to adopted country, however, exposes an individual to the same cancer risk and incidence as that of others living in the adopted country. Because human beings are 99.1% identical in their genetic sequence, these differences in incidence cannot be attributed to the variation in their DNA sequence. In fact, if one twin is identified with breast cancer, the chance that the second twin will be diagnosed with breast cancer is 20%, indicating that the contribution of faulty genes to the pathogenesis of cancer is minimum. Instead, it is estimated that 75%-85% of all chronic illnesses and diseases are linked to lifestyle, and cannot be explained by differences in genetic makeup. For example, a positive correlation was found between smoking and lung cancer (22). Additionally, there is a negative correlation between consuming fruits and vegetables and colon cancer. Epidemiological studies have indicated that populations that consume food rich in fruits and

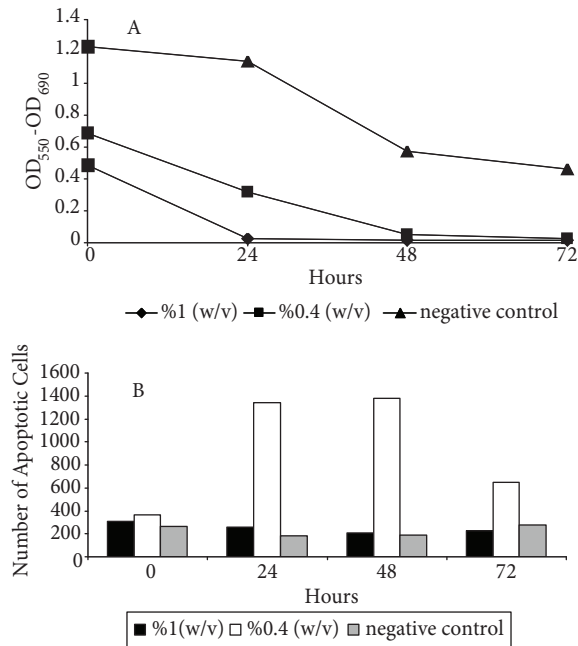


Figure 3. Cell proliferation (A) and apoptosis rates (B) of 32Dp210 cells after 0.4% (w/v), and 1% (w/v) fresh aqueous garlic extract treatment for 72 h.

vegetables have a lower incidence of cancer. A review of results from 206 human epidemiologic studies, and 22 animal studies has indicated an inverse relationship between the consumption of vegetables and fruits and the risk for cancers of the stomach, esophagus, lung, oral cavity, pharynx, endometrium, pancreas, and colon (23).

Diallyl disulfide (DADS) is a major constituent of garlic. Ling et al. investigated the effects of DADS in human gastric cancer MGC803 cells, and whether it was related to an alteration in ERK activity. They had shown that the growth of MGC803 cells was inhibited by DADS (24). Chemoprevention of colorectal cancer has become essential in the modern industrialized world, as cancer of the large bowel has become one of the major causes of cancer mortality, second only to lung cancer. Diet and nutrition clearly play a role in the etiology of colon cancer. Sengupta et al. have shown that garlic had a chemopreventive power in colon carcinogenesis. In their study, the protective activity of diallyl sulfide, lycopene, and theaflavin, important antioxidative ingredients of garlic, tomato, and black tea, respectively, was assessed during colon

carcinogenesis. The effect was observed on aberrant crypt foci, the preneoplastic lesion. As inhibition of cyclooxygenase-2 and inducible nitric oxide synthase activities is correlated with the prevention of colon cancer, the study continues with the determination of the change in the expression of these proteins. Following treatment, a significant reduction in the incidences of aberrant crypt foci (by 43.65% in diallyl sulfide, 57.39% in lycopene, and 66.08% in theaflavin group) was observed, which was in accordance with the reduced expression of cyclooxygenase-2 and inducible nitric oxide synthase. The effect of the intact source was found to be more pronounced than their components when used separately (25).

Wu et al. have shown that garlic extract inhibits proliferation and induces differentiation of HL-60 cells, and it has been shown that garlic extract has an antiproliferative effect on human lymphatic leukemia cells (26). Moreover, it has been reported that different garlic compounds, including diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), ajoene, allicin, S-allyl cysteine (SAC), and S-allylmercaptocysteine (SAMC), lead to apoptosis by upregulation of p53, Bax, activation of Caspase-3, -8, and -9, JNK, p38, ERK, downregulation of Bcl2, and the production of reactive oxygen species (ROS).

BCR-ABL oncoprotein, a critical determinant in the pathogenesis of CML, exhibits constitutive tyrosine kinase activity. This oncoprotein has been attributed anti-apoptotic activity, as it prevents apoptosis by upregulation of anti-apoptotic proteins such as Bcl-2 or Bcl-xL (27). Induction of the apoptosis by blocking the BCR-ABL oncoprotein with pharmaceuticals, such as Gleevec, has been one of the most attractive CML therapy strategies for many years.

The aim of the present study was first to examine whether fresh aqueous garlic extract was able to inhibit proliferation and induce apoptosis of the BCR-ABL positive cell line.

Aqueous garlic extract treatment has shown an antiproliferative effect on both the 32D and 32Dp210 cells at 2 different concentrations (Figures 2A and 3A). However, the number of apoptotic cells was significantly lower in cells treated with 1% (w/v) garlic than in cells treated with 0.4% (w/v) garlic for each cell line. Because of this, we thought that 1% (w/v)

garlic treatment might have led to cell death by mechanisms other than apoptosis. Therefore, the most effective apoptotic dose of aqueous garlic extract has been determined as 0.4% (w/v).

Consequently, 1% (w/v) garlic treatment has resulted in similar antiproliferative and apoptotic effects in both cell lines. However, as can be seen from Figures 2B and 3B, response of the 2 cell lines to the 0.4% (w/v) garlic treatment was different ($P = 0.003$). Upon treatment with garlic at this concentration, reduction in the cell number continued for 48 h in both cell lines. Although most 32Dp210 cells completely died, 32D cells were able to recover at the end of the 72 h exposure. In support of this observation, we found that at 0.4% (w/v) concentration the death risk of the 32Dp210 cell line was 2.08 times higher than the death risk of the 32D cell line. Therefore, our results support the idea that garlic triggers apoptosis selectively in cancer cells when compared with the normal cells.

Previous studies of garlic on many cancer species, such as prostate cancer, colon tumor cells, HK-60 human leukemia, lung cancer, neuroblastoma cells, and K-562 human leukemia cells were performed, but there was no study on Chronic Myeloid Leukemia (CML) in this regard. Thus, we aimed to investigate the effects of aqueous garlic extract on the 32Dp210 cell line, and to reveal the possible mechanism of the effects. For this aim, aqueous garlic extract (10% w/v) was added into the cell line media at 2 different final concentrations (0.4% and 1%). At 0 h, and at 24, 48, and 72 h later, oxidant (the MDA level and XO activity) and antioxidant enzyme activities were measured in the cell lines. It was observed that the garlic extract caused increases in the MDA level, and XO and antioxidant enzyme activities in the 32Dp210 cell line treated by the garlic extract.

It is concluded that garlic directly causes oxidation in the 32D cell line owing to its oxidant ingredients, and that the oxidant stress created by garlic in the 32Dp210 cell line might occur through increased XO activity and/or its oxidant ingredients.

Acknowledgements

The authors thank Dr. KI Mills, Wales College of Medicine, Cardiff, UK, for providing the 32D and 32Dp210 cell lines.

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