Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2008; 33 (3) ; 78–84.

Research Article [Araştırma Makalesi]

Yayın tarihi 19 Eylül, 2008 © TurkJBiochem.com

[Published online 19 September, 2008]

Effects of Water Soluble Garlic Extract on Human Leukemia HL60 Cell Lines

[Suda Çözünür Sarımsak Ekstresinin İnsan Lösemi HL60 Hücre Serisi Üzerine Etkileri]

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Kayıt tarihi: 29 Haziran 2008, Kabul tarihi: 25 Temmuz 2008

[Received: 29 June 2008, Accepted: 25 July 2008]

ABSTRACT

Purpose: Studies with garlic have shown the medicinal value of the plant by its antioxidant potential which is exerted by its organosulfur rich content. In this study, the aim is to determine any potential antioxidant efficacy of the less toxic water soluble extract of a widely cultivated garlic form in Turkey, in terms of alterations on the oxidant/antioxidant status of HL60 promyelocytic leukemia cell line.

Methods: Water soluble extracts of plant were prepared and aged for 1 hour to sixty days at constant pH and temperature. The toxicity was investigated by percent growth inhibition after treatment. To analyze the potential antioxidant efficacy of the extracts, cell homogenates were prepared from the treated and untreated control cells, and then the levels of antioxidant defense enzymes, cellular glutathione level, and superoxide production capacity were determined.

Results and Conclusion: In this study, the effective extract concentration was determined as 132 µg/mL and the toxic concentration was 1320 µg/mL. The analysis of homogenates prepared from cells exposed to 132 µg/mL resulted in the increases in activities of enzymes as 27.5 % for catalase, 160 % for superoxide dismutase, and 21 % for the glutathione peroxidase. Therefore, the antioxidant efficacy of extract at 132 µg/mL may be attributed to the regulation of antioxidant status via potential accumulation of hydrogen peroxide in cells.

Key Words: Allium sativum, water soluble garlic extract, acute promyeloctic leukemia, antioxidant defense

ÖZET

Amaç: Sarmısakla ilgili çalışmalarda bitkinin tıbbi değeri organik sülfürce zengin içeriğinin antioksidan potansiyeliyle gösterilmiştir. Bu çalışmada amaçlanan, yaygın olarak Türkiye'de tarımı yapılan sarmısak formundan hazırlanmış, suda çözünebilen düşük toksisiteli ekstrenin olası antioksidan etkinliğinin HL60 akut promiyelösitik lösemi hücre serisi üzerinde oksidan/antioksidan durumdaki değişmelerin ifadesiyle açıklanmasıdır.

Metot: Suda çözünür ekstreler sabit pH ve sıcaklıkta hazırlanmış ve 1 saatden 60 güne kadar olgunlaştırılmıştır. Toksisiteleri muamele edilmiş hücrelerin kontrole karşın yüzde büyüme inhibisyonu ve yüzde canlılıkları ile incelenmiştir. Ekstrelerin potansiyel antioksidan etkinliğini antioksidan savunma enzimleri, hücresel glutatyon ve süperoksit üretme kapasitesindeki değişimlerle belirlemek amacıyla ekstreye maruz kalmış ve kalmamış hücrelerden hazırlanan sitozolik çözeltiler analiz edilmişlerdir.

Bulgular ve Sonuç: Bu çalışmada etkin ekstre konsantrasyonu 132 µg/mL ve toksik konsantrasyon 1320 µg/mL olarak bulunmuştur. Hücrelerin 132 µg/mL ekstreye maruz kalması sonrasında hazırlanmış sitozolik çözeltilerin analiziyle enzim aktivitelerindeki artış katalaz için % 27.5, süperoksit dismutaz için % 160, ve glutatyon peroksidaz için % 21 olarak bulunmuştur. Dolayısıyla ekstrenin 132 µg/mL dozdaki antioksidan etkinliği, hücrelerde hidrojen peroksit birikimine bağlı antioksidan durumun düzenlenmesiyle bağdaştırılabilir.

Anahtar Kelimeler: Allium sativum, suda çözünür sarmısak ekstresi, akut promiyelösitik lösemi, antioksidan savunma.

Introduction

Changes in the level of reactive oxygen species (ROS) and the resulting oxidative damage is an important factor in the development of a wide range of tissue pathology including carcinogenesis, aging, neurodegenerative diseases, drug side-effects, as well as altered chemotherapeutic response (1-3). By definition, the oxidative damage is the change in the oxidant status of cells due to oxidative modification of biomolecules, including DNA, proteins, lipids and small cellular molecules that are essential for cellular machinery. The damage is, therefore, responsible for variety of diseases including inflammatory conditions, ischemia/reperfusion tissue injury, radiation toxicity, age-related degenerative conditions, and cancer (4-6). Reactive oxygen species are, in fact, the metabolic by products, and their existence is related with the biological processes to maintain cellular machinery. The ROS generation can be due to internal or external sources; however the outcome of such process is an internally occurring oxidative damage, if their abundance exceeds the maximum tolerated levels within the cells. On the other hand, cells have evolved to develop antioxidant defense system to regulate the oxidative modification of cellular molecules. This system is actually the group of enzymes (Figure 1) that has been evolved to dismutase superoxides to oxygen and hydrogen peroxide (SOD, superoxide dismutase); to destroy toxic and free hydrogen peroxides by reducing to their corresponding alcohols and water (GPx, glutathione peroxidase); to decompose the metabolic hydrogen peroxide by-product to gaseous oxygen and water (CAT, catalase); and also includes the small biomolecules with antioxidant potential (e.g., GSH, glutathione). In addition to cellular defense, the external sources are also available to provide full protection against oxidative damage. Some of these sources are the nutrients with antioxidant value, including garlic, providing important additional protection against oxidative damage. Garlic is the member of Liliaceae family and implicated in curative and preventive use for disease conditions since ancient times. Studies shown that on various disease models, including cancer, use of garlic derived compounds, extracts and other type of preparations have benefits for disease prevention and treatment (7). Garlic has been thought to bring about its anticarcinogenic effect through a number of mechanisms: free radical scavenging, activating the antioxidant defense enzymes (glutathione S-transferase, catalase), and activating DNA repair mechanisms are to name a few (8-11). Antioxidant value of the garlic has been verified in a study where garlic identified as the most antioxidant bearing dietary plant and shown that enriched in sulfur-containing compounds is the only reason for this property (12,13). Although the chemistry of garlic is quite complex, it is well known that plant contains organosulfur compounds (OSCs) as precursors of the bioactive products (14) that contributes for the plant's medicinal value. The bioactive products which normally do not exist in intact plant are released upon processing of garlic and its organosulfur content may vary with variations in the methods of processing (15,16). These compounds have shown to act in single or combined fashion to exert their effect on disease models (17). Several other studies with garlic extracts and preparations have shown that the raw garlic preparations and oil soluble extracts are highly toxic compared with the water soluble components (18-20). However, it has been shown that slow processing of the plant improves the product quality in terms of its efficacy, while reducing the toxicity (4,11,21-22). Studies to verify the therapeutic role of garlic oil extract and its components on human leukemia cell line have shown that oil soluble components, such as ajoene and diallyl disulfide, induces apoptosis (5) by virtue of inducing intracellular peroxide and ROS production, respectively (21,23-25). However, to our knowledge, there is no report available to identify the role of water soluble garlic extracts with aging on oxidant/antioxidant status of promyelocytic leukemia (PML) cell line HL60.

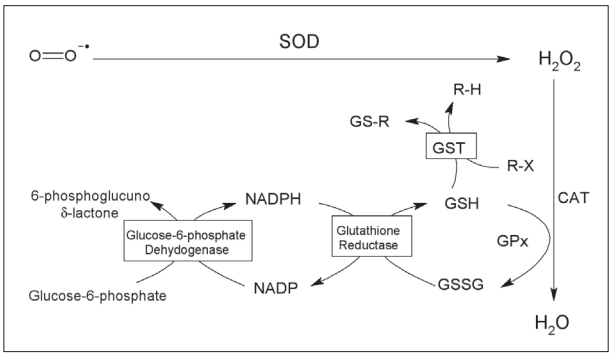

Figure 1. Antioxidant defense system and GSH-dependent enzymes to maintain the safe levels of cellular ROS.

In Turkey, *Allium longicuspis*, wild ancestors of modern garlic, and *Allium sativum*, the cultivated form, are both widely distributed throughout the country. Almost 12 % of marketed garlic in Turkey is provided from the Taşköprü province of Kastamonu, and hence the garlic clone cultivated in this region is called Kastamonu (Taşköprü) garlic (26,27) . This plant is a special form of *Allium sativum*, rich in water soluble solid content, contains 17.3 % crude protein, 0.34 % crude oil, 2.17 % crude fiber, 1.779 mg/kg dimethyl sulfite (DMS) and 0.14 % essential oil with capability for longer storage (26,28). Recent genetic research by Meryem Ipek and Philipp Simon (26) has revealed that Kastamonu (Taşköprü) garlic, is a well adapted form of *A. sativum* but a high quality plant due to environmental factors where the plant is cultivated. Here, we report the effects of less toxic, aged garlic (Kastamonu/ Taşköprü) extracts on HL60 cells by virtue of the alterations on the activities of antioxidant defense system enzymes, the capacity of superoxide production at the model cell line, and the level of cellular glutathione, *in vitro.* The reduced toxicity of water

soluble aged garlic extract was also reported by means of percent growth inhibition and percent viable cells after the treatment with respect to control.

Materials and Methods

Preparation of water soluble garlic extracts:

The garlic extracts used in this study was prepared from Kastamonu (Taşköprü) garlic clone purchased from local farmer. Briefly, the garlic bulbs peeled, washed, dried over paper towel and finely chopped into small cubes (3 mm). 300 grams of chopped material were kept at 25 °C for 30 minutes, and then transferred into 850 mL of 20 mM phosphate buffer (pH 6.5). This mixture (original sample) was kept at ambient temperature, stirred for several times for 1 hour to 72 hours. Appropriate amounts of buffer phase were aliquoted at 3 time points, namely 1 hr, 6 hr and 72 hr. After 72 hours, the original sample transferred to refrigerator (4 °C) for 15 days with stirring for every 5-8 hours. The solid material filtered off, and incubated for 15 days at dark, at ambient temperature (<25 °C). At the day 15, appropriate amounts of buffer phase were removed and labeled as *30 days aged extract*. The remaining buffer phase was further incubated for another 30 days under the same conditions and labeled as *60 days aged extract*. The aliquots taken at each time points were transferred to freezer and stored at -80 °C until they are used. The concentration of the extracts was determined as 0.3529 g/mL as the grams of dry weight of chopped material per mL of buffer used.

HL60 cell culture maintenance, treatment and sample preparation

HL60 cell line was obtained from Foot-and-Mouth Disease Institute of Ministry of Agriculture & Rural Affairs of Turkey, Turkey (ATCC grade, HUKUK No: 96041201) and maintained in HEPES modified RPMI 1640 supplemented with 10 % Fetal Bovine Serum, 2 mM L-Glutamine (QIAGEN, Australia), and 100 units/mL Penicillin- Streptomycin (Sigma Chemical Company, St. Louis, MO, USA). HL60 cell line used in this study was undifferentiated and low-passaged line to prevent cell resistance against apoptosis induction observed with high-passaged lines. The cells were cultured, for 24 hours before treatment, in 25 and 150 cm^2 flasks at 37 °C in humidified air with 5 % CO_2 supplement. The treatment with single doses of the varying concentrations of extract was done at a concentration range between 3.5µg/ml and 3.2 mg/ml for GI_{50} analysis, and at final concentrations of 132 (GI_{50}) and 1320 µg/ml (GI_{max}) for enzymatic assays. After 24 hours of treatment at GI_{50} and GI_{max}, cells harvested at 900xg were homogenized in 50 mM phosphate buffer pH 7, containing 2 mM DTT, 1 mM EDTA (pH 7), 0.5 % NP40 and 2 mM PMSF. Upon centrifugation at + 4 °C, 9000 x g for 30 minutes, the supernatants of cell homogenates were collected and aliquoted in 0.250 ml volumes and stored at -80 °C until they are used.

Cell viability and growth inhibition (GI_{50} and GI_{max}) determination of HL60 cell line

After exposure to extracts, the percent viable cells were calculated by using the Equation 1. Here, the Trypanblue$^+$ and Trypanblue$^-$ stands for the number of dead and alive cells (29), respectively.

$$\%\ viability = \frac{Trypanblue^-}{\left(Trypanblue^+ + Trypanblue^-\right)} \times 100 \qquad \text{Equation 1}$$

The extract concentration that exerts 50 % growth inhibition (GI_{50}) and the concentration that exerts maximum growth inhibition (cell viability less than 30 %, GI_{max}) were reflecting the effective and toxic concentrations of extracts, respectively. GI_{50} and GI_{max} were determined by using *trypanblue dye exclusion method* with respect to control by using the formulation given below (29):

$$GI(\%\ \ control) = \left[1 - \left(\ \left(FC^+ - OC\right)/\ FC^- - OC\right)\right] \times 100$$

Here, GI (% control) is the percent growth inhibition of cells observed with respect to control, FC^+ is the number of alive cells after exposure of extracts for certain periods of time, FC^- is the number of alive cells of control group after exposure to vehicle (phosphate buffer) for certain periods of time, and OC is the mean of the number of cells in each flask, right before the cells treated with either extract or vehicle.

Enzyme activity measurements

Catalase: Catalase (CAT) activity was determined at 25 °C according to Aebi (30,31) by monitoring the decrease in absorbance of H_2O_2 at 240 nm and 1 unit of catalase activity defined as the enzyme activity decomposing 1µmole of H_2O_2 per min and expressed as Units per mg of total protein.

Superoxide Dismutase (SOD) activity: The Total (Cu-Zn and Mn) SOD activity was measured with slight modification of Geller and Winge's method (32,33) based on the inhibition of NBT reduction by xanthine/xanthine oxidase (superoxide generator) system. The SOD activity was expressed as units per mg of total proteins. One unit of SOD activity was defined as the amount of enzyme required to inhibit the reduction rate of NBT by 50 % under the specified conditions.

Glutathione Peroxidase: Glutathione Peroxidase activity was measured by the modified method of Paglia and Valentine (34) at 340 nm. The activity of the enzyme defined as the amount of enzyme that converts 1 µmol of NADPH per minute in 1 mL, and expressed as Units per mg of total protein.

Glutathione-S-Transferase: Glutathione-S-Transferase activity was determined by the method of Habig by monitoring the thioether formation at 340 nm using 1-chloro-2,4- dinitrobenzene (CDNB) as a substrate (35,36). Activity of the enzyme defined as the amount of enzyme producing 1 µmol of CDNB-GSH per minute and expressed as units per mg of total protein.

Xanthine Oxidase: Xanthine oxidase activity was assayed (37) with 2 mM xanthine as substrate. For the conversion of xanthine to uric acid, the initial velocities were monitored at 293 nm, and the activity was expressed as the micromoles of uric acid formed per minute per mg of total protein.

Lipid peroxidation (MDA): Malondialdehyde (MDA) levels were determined using the method (38,39) based on the reaction of MDA with thiobarbituric acid (TBA) at 95 °C. the absorbance of the organic phase was measured at 532 nm. Tetraethoxypropane (100 mM, TEP) was used as MDA standard. The values were presented as nmole MDA per mg of total protein.

Protein determination: Cytosolic protein concentration of HL60 cells was determined by Lowry Method (40) with bovine serum albumin (BSA) as the standard.

Statistical analysis: In this study the experimental results obtained from three treatment groups, namely untreated control, effective dose group (GI_{50}) and toxic dose groups (GI_{max}) were compared statistically (A, B, C) by using three separate *t*-tests (comparing A with B, A with C, and B with C) and also one-way analysis of variance (ANOVA) to confirm the results (Table 1). The assumption made was P=0.05 and the data $p<0.05$ was assumed as statistically significant.

Results

Questioning the effect of extracts on HL60 cells, in a concentration range of 5-65 µg/ml, showed that 60 days old extract is the least toxic extract used in experiments in terms of improved cell viability (Figure 2). The growth inhibition exerted by extracts was in dose dependent manner where the lowest inhibition was observed upon treatment with 60 days aged extract (Figure 3).

Then, the effective and toxic doses of the extract, GI_{50} and GI_{max}, were determined after 24 hours of exposure to extract in a range of 5.5-1500 µg/ml (each in duplicate, data not shown) and the GI_{50} value was found as 130.78 ± 4.13µg/mL and GI_{max} as 1300 ± 148.82 µg/mL. To investigate the possible role of water soluble garlic extract on the oxidant/antioxidant status of HL60 cell line, cells were treated with the effective and toxic doses of extract, 132 µg/mL (GI_{50}) and 1320 µg/mL, respectively. The analysis of cellular homogenates as in the changes of the activities of antioxidant system enzymes, the capacity of superoxide production, and the level of cellular glutathione, is given in Table 1.

After 24 hours of treatment at GI_{50} (132 µg/mL), as given in Table 2, the activity increases were observed for CAT (27.5 %), SOD (160 %), GPx (27.5 %), and XO (23.4 %) with increase in cellular GSH level (246.9 %). Here, the decrease in the GST activity and the level of MDA was determined as 21.8 % and 29.8 %, respectively. For the treatment at GI_{max} (1320 µg/mL), increased activities were observed for CAT (12.3 %), SOD (75.9 %), XO (17.5 %), and GST (7 %), accompanied by the increase in cellular GSH level (37.9 %). Here, the decrease was observed for GPx activity and MDA was 7.0 % and 17.3 %, respectively.

The cellular antioxidant potential (AOP) was also measured according to an optimized protocol reported in the literature (41), for treatments at GI_{50} and GI_{max}, resulted in no significant change with respect to control (data not shown), supporting the results showing the slight change in MDA levels upon treatment with water soluble garlic extract.

Discussion

Human leukemia HL60 cells are sensitive to a wide variety of apoptotic stimuli including hydrogen peroxide (42), and hence it is the best model for experiments tailored to enlighten the possible anticancer efficacy of various substances, including natural products and natural product derived compounds. Based on our literature knowledge, fresh garlic extracts are highly toxic to cultured cells and this is attributed to the presence of oil soluble compounds as well as intermediate organosulfur compounds (OSCs) in preparations. This study was initially tailored to understand if water soluble extract of plant, so called water soluble garlic extract (here, in buffer), has any significant toxicity on a cancer model, HL60 cell line, and how the method of extraction and aging contributes to this toxicity. For this approach, cells

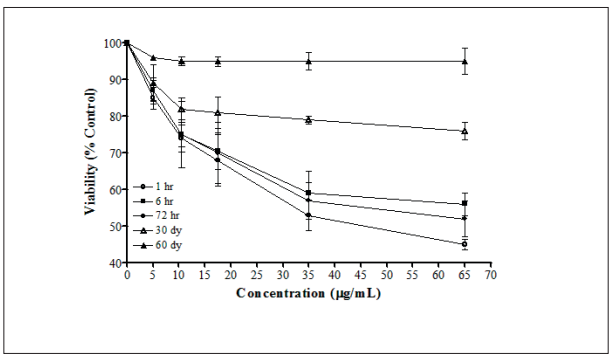

Figure 2. Viability (% Control) of HL60 cells upon 24 hr exposure to 1 hr to 60 days old water soluble garlic extracts (two independent experiments, each in duplicates, n=4-6).

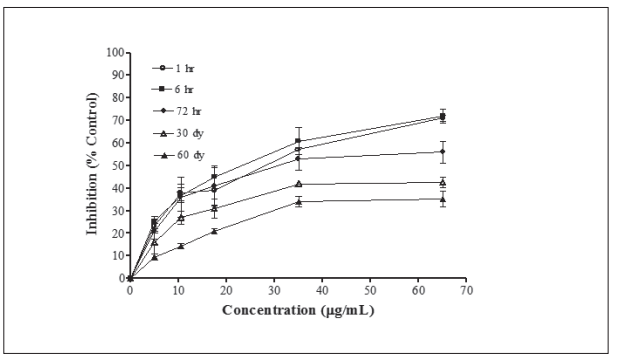

Figure 3. HL60 Growth Inhibition profile (% Control) upon 24 hr exposure to 1 hr to 60 days old water soluble garlic extracts (two independent experiments, each in duplicates, n=4-6).

Table 1. The effect of 60 days aged water soluble garlic extract on HL60 cell line*

	Water soluble Garlic Extract		
	0 μg/mL, Control (A)	132 μg/mL (B)	1320 μg/mL(C)
CAT (IU/mg Protein)	183.52 ± 5.09	234.02 ± 9.68	206.04 ± 13.17¥
SOD (U/mg Protein)	14.48 ± 2.40	37.71 ± 1.75	25.46 ± 0.99
GPX (mU/mg Protein)	55.05 ± 6.53	66.62 ± 4.56	51.18 ± 3.99
GST (U/mg Protein)	161.62 ± 6.43	126.26 ± 3.59	173.01 ± 5.36
MDA (nmol/mg Protein)	4.86 ± 0.39	3.41 ± 0.19	4.02 ± 0.75
XO (mU/mg Protein)	246.7 ± 6.39	304.40 ± 6.98	289.88 ± 21.95
GSH (nM/mg Protein)	198 ± 12.1	687 ± 10.8	273 ± 11.6

*Using three separate t-tests (comparing A-B, A-C, and B-C) and also ANOVA for confirmation revealed that, except B-C comparison for MDA and XO, and A-C comparison for GPx, all the parameters in the table is statistically significant, provided that $p<0.05$.

Table 2. The effect of 60 days aged water soluble garlic extract on HL60 cell line is shown in terms of percent changes in levels of enzymes and biomolecules, upon exposure to effective and toxic doses of extract.

	132 μg/mL (GI_{50})	1320 μg/mL (GI_{max})
CAT	↑ 27.5 %	↑ 12.3 %
SOD	↑ 160.0 %	↑ 75.9 %
GPX	↑ 21.0 %	↓ 7.0 %
GST	↓ 21.8 %	↑ 7.0 %
MDA	↓ 29.8 %	↓ 17.3 %
XO	↑ 23.4 %	↑ 17.5 %
GSH	↑ 246.9 %	↑ 37.9 %

treated for 24 hour with the extract aged from 1 hour to 60 days at varying concentrations revealed that the extended aging process of water soluble extract, leads to improved cell viability (Figure 2), which in turn reflected as reduced toxicity (Figure 3). Therefore, even for water soluble extract preparation, for reduced toxicity, the aging process is an important factor to eliminate the toxic intermediates present in the medium. To verify the antioxidant efficacy of a natural product *in vitro*, it is better to know the plants' antioxidant potential (AOP). In this sense, Cao (12) reported that, due to its capability to measure the sulfur bearing antioxidant molecules, Oxygen Radical Absorbance Capacity (ORAC) method is the efficient way to measure the AOP of garlic itself, rather than measuring the total concentration of electron-donating antioxidants in plant (13). Since measured AOP of plant is different than its exerted antioxidant effect measured in cell homogenate, ORAC method seems to provide more clues about how plant products enriched in OSCs act *in vitro*. Garlic, reported as the plant with highest AOP by this method, is known to have high organosulfur content, some of which are actual intermediates to biologically active water soluble compounds *in vitro*. Based on this information, a special form of *Allium sativum* (garlic) cultivated in Turkey, namely Taşköprü garlic, was chosen to investigate its possible oxidant/antioxidant potential on model cell line due to high water soluble solid content of plant enriched in organosulfur compounds. For this purpose, HL60 cells were treated with the effective (GI_{50}, 132 μg/mL) and toxic doses (GI_{max}, 1320 μg/mL) of extract prepared from the garlic. The observed alterations after treatment of HL60 cells with 60 days aged water soluble garlic extract at GI_{50} and GI_{max} concentrations with respect to untreated control cells are given in Table 1. With some exceptions, all the data given in the table was found statistically significant. For GPx, the data analyzing activity change at toxic dose with respect to control, and for MDA and XO, the data analyzing their dose dependent responses (from GI_{50} to GI_{max}) were not statistically significant. Overall analysis of the data in Table 1, in terms of percent changes (Table 2), reveals that except for GPX and GST (Table 2), all enzymes and biomolecules maintained their tendency of increase or decrease at both concentrations of extract used for treatment. For GPx, the moderate increase in the activity (21.0 %) at GI_{50} and the slight decrease (7.0 %) at GI_{max} was observed with respect to control. The opposite tendency was true for GST, for which the activity decrease at GI_{50} (21.8 %), and increase (7.0 %) at GI_{max} was observed. Considering that the reduced GST level is an indication of low or no resistance to exposed chemotherapeutics and other chemicals, including natural products, therefore, in our study, the observed 21.8 % decrease in GST activity at GI_{50} may indicate the reduced resistance to extract exposure. The significant increase in cellular GSH content at GI_{50} may be attributed to over production of GSH due to presence of water

soluble OSCs in extract, as well as the alterations in activities of GSH utilizing enzymes. This hypothesis was verified by the observation that increased extract concentration from GI_{50} to GI_{max} did only cause a moderate increase in the cellular GSH content (37.8 %) with slight increase in GST activity (7.1 %). The increase of GSH content upon exposure to garlic derived OSCs *in vitro* may also be supportive to conclude that water soluble garlic extract have the capacity to regulate the thiol-related cellular metabolism (43). In addition, the moderate increase observed for GPx and CAT activities seems to support this suggestion with significant increase in cellular GSH level (Table 2). The observed significant increase in SOD activity at GI_{50} (160 %, Table 2), may be attributed to the efficacy of soluble OSCs to modulate the SOD over expression reported in the literature with various isolated components of garlic (44). At GI_{max}, on the other hand, slightly increased SOD activity may be explained the toxicity of extract to prevent induction of SOD expression. (45-47). Moderate and slight increase in XO activity at GI_{50} and GI_{max}, respectively, supports the hypothesis that garlic components may induce ROS generation to some extent and again in a dose dependent manner (48). The observed low MDA levels may also supporting this hypothesis, due to already reported MDA instability (49) at higher doses of hydrogen peroxide. Garlic components were shown to inhibit the proliferation of variety of cancer cells in a dose-dependent manner and the antiproliferative effect may be attributed for the oxygen radical scavenging properties of OSCs of garlic, as well as its ability to regulate thiol-related cellular metabolism (10,44,48-51). The contradictions in the literature about these properties of OSCs may be directly related with the plant's chemical composition due to cultivation conditions and other environmental factors; the presence of antioxidant defense and thiol-utilizing enzymes without substantial activity to be mediated by OSCs in particular tissue and tumor type; and also the toxicity and the composition of extracts depending on the method used for processing. Overall, we may conclude that the antioxidant efficacy of the plant and its water soluble aged preparations may induce antiproliferative effect on leukemia cell line due to the capacity of extract to produce ROS by increasing the antioxidant defense enzymes (CAT, SOD) and hydrogen peroxide accumulation by regulating the cellular GSH levels, and mediating thiol utilizing enzymes (GST and GPx) in favor to accumulate GSH and hydrogen peroxide.

References

[1] Borek C. (1993) Molecular mechanisms in cancer induction and prevention. Environ Health Perspect. 101 Suppl 3: 237-45.

[2] Borek C. (2004) Dietary antioxidants and human cancer. Integr Cancer Ther. 3: 333-41.

[3] Yuncu M, Eralp A and Celik A. (2006) Effect of aged garlic extract against methotrexate-induced damage to the small intestine in rats. Phytother Res. 20: 504-10.

[4] Das T, Choudhury AR, Sharma A and Talukder G. (1996) Effects of crude garlic extract on mouse chromosomes in vivo. Food Chem Toxicol. 34: 43-7.

[5] Dirsch VM, Gerbes AL and Vollmar AM. (1998) Ajoene, a compound of garlic, induces apoptosis in human promyeloleukemic cells, accompanied by generation of reactive oxygen species and activation of nuclear factor kappaB. Mol Pharmacol. 53: 402-7.

[6] Dorant E, van den Brandt PA and Goldbohm RA. (1995) Allium vegetable consumption, garlic supplement intake, and female breast carcinoma incidence. Breast Cancer Res Treat. 33: 163-70.

[7] Agarwal KC. (1996) Therapeutic actions of garlic constituents. Med Res Rev. 16: 111-24.

[8] Lin, JG Chen GW, Su CC, Hung CF, Yang CC, Lee JH and Chung JG. (2002) Effects of garlic components diallyl sulfide and diallyl disulfide on arylamine N-acetyltransferase activity and 2-aminofluorene-DNA adducts in human promyelocytic leukemia cells. Am J Chin Med. 30: 315-25.

[9] Liu J, Lin RI and Milner JA. (1992) Inhibition of 7,12-dimethylb enz[a]anthracene-induced mammary tumors and DNA adducts by garlic powder. Carcinogenesis. 13: 1847-51.

[10] Siegers CP, Robke A and Pentz R. (1999) Effects of garlic preparations on superoxide production by phorbol ester activated granulocytes. Phytomedicine. 6: 13-6.

[11] Borek C. (2001) Antioxidant health effects of aged garlic extract. J Nutr. 131: 1010S-5S.

[12] Cao G, Sofic E and Prior RL. (1996) Antioxidant Capacity of Tea and Common Vegetables. J. Agric. Food Chem. 44: 3426-31.

[13] Halvorsen BL, Holte K, Myhrstad MC, Barikmo I, Hvattum E, Remberg SF, Wold AB, Haffner K, Baugerod H, Andersen LF, Moskaug O, Jacobs DR, Jr. and Blomhoff R. (2002) A systematic screening of total antioxidants in dietary plants. J Nutr. 132: 461-71.

[14] Lawson LD and Hughes BG. (1992) Characterization of the formation of allicin and other thiosulfinates from garlic. Planta Med. 58: 345-50.

[15] Fenwick GR and Hanley AB. (1985) The genus Allium. Part 2. Crit Rev Food Sci Nutr. 22: 273-377.

[16] Fenwick GR and Hanley AB. (1985) The genus Allium--Part 1. Crit Rev Food Sci Nutr. 22: 199-271.

[17] Amagase H, Schaffer EM and Milner JA. (1996) Dietary components modify the ability of garlic to suppress 7,12-dimethylb enz(a)anthracene-induced mammary DNA adducts. J Nutr. 126: 817-24.

[18] Fukushima S, Takada N, Hori T and Wanibuchi H. (1997) Cancer prevention by organosulfur compounds from garlic and onion. J Cell Biochem Suppl. 27: 100-5.

[19] Rivlin RS. (2006) Is garlic alternative medicine? J Nutr. 136: 713S-15S.

[20] Amagase H, Petesch BL, Matsuura H, Kasuga S and Itakura Y. (2001) Intake of garlic and its bioactive components. J Nutr. 131: 955S-62S.

[21] Parish RA, McIntire S and Heimbach DM. (1987) Garlic burns: a naturopathic remedy gone awry. Pediatr Emerg Care. 3: 258-60.

[22] Lawson LD and Gardner CD. (2005) Composition, stability, and bioavailability of garlic products used in a clinical trial. J Agric Food Chem. 53: 6254-61.

[23] Kodera Y, Suzuki A, Imada O, Kasuga S, Sumioka I, Kanezawa A, Taru N, Fujikawa M, Nagae S, Masamoto K, Maeshige K and Ono K. (2002) Physical, chemical, and biological properties of s-allylcysteine, an amino acid derived from garlic. J Agric Food Chem. 50: 622-32.

[24] Banerjee SK, Maulik M, Manchanda SC, Dinda AK, Das TK and Maulik SK. (2001) Garlic-induced alteration in rat liver and kidney morphology and associated changes in endogenous antioxidant status. Food Chem Toxicol. 39: 793-7.

[25] Kwon KB, Yoo SJ, Ryu DG, Yang JY, Rho HW, Kim JS, Park JW, Kim HR and Park BH. (2002) Induction of apoptosis by diallyl disulfide through activation of caspase-3 in human leukemia HL-60 cells. Biochem Pharmacol. 63: 41-7.

[26] Ipek M, Ipek A and Simon PW. (2006) Sequence homology of polymorphic AFLP markers in garlic (Allium sativum L.). Genome. 49: 1246-55.

[27] Taskopru-Belediyesi. (2007) www.taskoprubelediyesi. com, Taskopru, Kastamonu, Turkey.

[28] Hacıseferoğulları H, Özcan M, Demir F and Çalışır S. (2005) Some nutritional and technological properties of garlic (Allium sativum L.) Journal of Food Engineering 68: 463-69.

[29] Milner JA. (1996) Garlic: its anticarcinogenic and antitumorigenic properties. Nutr Rev. 54: S82-6

[30] Aebi, H. (1984) Catalase in vitro. Methods Enzymol. 105: 121-6.

[31] Aebi H, Wyss SR, Scherz B and Skvaril F. (1974) Heterogeneity of erythrocyte catalase II. Isolation and characterization of normal and variant erythrocyte catalase and their subunits. Eur J Biochem. 48: 137-45.

[32] Geller BL and Winge DR. (1984) Subcellular distribution of superoxide dismutases in rat liver. Methods Enzymol. 105: 105-14.

[33] Durak I, Canbolat O, Kavutcu M, Ozturk HS and Yurtarslani Z. (1996) Activities of total, cytoplasmic, and mitochondrial superoxide dismutase enzymes in sera and pleural fluids from patients with lung cancer. J Clin Lab Anal. 10: 17-20.

[34] Paglia DE and Valentine WN. (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 70: 158-69.

[35] Habig WH, Pabst MJ and Jakoby WB. (1974) Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem. 249: 7130-9.

[36] Pabst MJ, Habig WH and Jakoby WB. (1974) Glutathione S-transferase A. A novel kinetic mechanism in which the major reaction pathway depends on substrate concentration. J Biol Chem. 249: 7140-7.

[37] Hashimoto S. (1974) A new spectrophotometric assay method of xanthine oxidase in crude tissue homogenate. Anal Biochem. 62: 426-35.

[38] Ohkawa S, Fukatsu K, Miki S, Hashimoto T, Sakamoto J, Doi T, Nagai Y and Aono T. (1997) 5-aminocoumarans: dual inhibitors of lipid peroxidation and dopamine release with protective effects against central nervous system trauma and ischemia. J Med Chem. 40: 559-73.

[39] Dahle LK, Hill EG and Holman RT. (1962) The thiobarbituric acid reaction and the autoxidations of polyunsaturated fatty acid methyl esters. Arch Biochem Biophys. 98: 253-61.

[40] Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. (1951) Protein measurement with the Folin phenol reagent. J Biol Chem. 193: 265-75.

[41] Biri H, Cimen MY, Kacmaz M, Buyukkocak S, Sen I, Ozturk HS, Bozkirli I and Durak I. (1998) Antioxidant potential of cancerous human kidney tissues. Cancer Biochem Biophys. 16: 265-72.

[42] Mansat V, Laurent G, Levade T, Bettaieb A and Jaffrezou JP. (1997) The protein kinase C activators phorbol esters and phosphatidylserine inhibit neutral sphingomyelinase activation, ceramide generation, and apoptosis triggered by daunorubicin. Cancer Res. 57: 5300-4.

[43] Pinto JT, Qiao C, Xing J, Rivlin RS, Protomastro ML, Weissler ML, Tao Y, Thaler H and Heston WD. (1997) Effects of garlic thioallyl derivatives on growth, glutathione concentration, and polyamine formation of human prostate carcinoma cells in culture. Am J Clin Nutr. 66: 398-405.

[44] Shukla Y and Kalra N. (2007) Cancer chemoprevention with garlic and its constituents. Cancer Lett. 247: 167-81

[45] Janssen AM, Bosman CB, Sier CF, Griffioen G, Kubben FJ, Lamers CB, van Krieken JH, van de Velde CJ and Verspaget HW. (1998) Superoxide dismutases in relation to the overall survival of colorectal cancer patients. Br J Cancer. 78: 1051-7.

[46] Macmillan-Crow LA and Cruthirds DL. (2001) Invited review: manganese superoxide dismutase in disease. Free Radic Res. 34: 325-36.

[47] Bianchi MS, Bianchi NO and Bolzan AD. (1992) Superoxide dismutase activity and superoxide dismutase-1 gene methylation in normal and tumoral human breast tissues. Cancer Genet Cytogenet. 59: 26-9.

[48] Das A, Banik NL and Ray SK. (2007) Garlic compounds generate reactive oxygen species leading to activation of stress kinases and cysteine proteases for apoptosis in human glioblastoma T98G and U87MG cells. Cancer. 110: 1083-95.

[49] Kostka P and Kwan C-Y. (1989) Instability of malondialdehyde in the presence of H2O2: Implications for the thiobarbituric acid test. Lipids. 24: 545-49.

[50] Seki T, Tsuji K, Hayato Y, Moritomo T and Ariga T. (2000) Garlic and onion oils inhibit proliferation and induce differentiation of HL-60 cells. Cancer Lett. 160: 29-35.

[51] Milner JA. (2006) Preclinical perspectives on garlic and cancer. J Nutr. 136: 827S-31S.