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Investigation of *in vivo* radioprotective and *in vitro* antioxidant and antimicrobial activity of garlic (*Allium Sativum*)

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Abstract. – OBJECTIVE, In this study, we aimed to assess the *in vivo* antioxidant potential via evaluating radioprotective effects in kidney and liver tissues of rats and *in vitro* antimicrobial and radical scavenger activity of garlic extract.

MATERIALS AND METHODS, Thirty-two mature female Wistar rats were divided into four groups, each consisting of eight rats. Experimental groups were control group (1), GE group (2), irradiation group (3) and both GE and irradiation group (4). For the rats in two groups (group 3 and 4), irradiation was performed on a Cobalt-60 unit using a single fraction of 20 Gy. The GE was given to rats once a day during the month before irradiation and continued for five days after irradiation. The garlic cloves were peeled on crushed ice and 50 g of garlic was cut into small pieces and homogenized in 75 mL of 0.9% NaCl. The concentration of this garlic preparation was considered to be 500 mg/mL on the basis of weight of the starting material (0.5 g/mL). This extract was administered to rats by oral gavage.

RESULTS, Our findings suggest that the use of garlic extract could be useful for addressing the limited therapeutic gain due to the radiation sensitivity of normal tissues adjacent to the tumour which are exposed to radiation, by strengthening the antioxidant system. *In vitro* and *in vivo* experiments seem to yield similar conclusions.

CONCLUSIONS, It can be stated that garlic is may be recommended to be sufficiently included in the diets of radiotherapy patients considering its antioxidant and antimicrobial efficacy.

Key Words:

Irradiation, Garlic extract, Oxidative damage, Antimicrobial.

Introduction

In spite of the fact that radiotherapy is a common and effective tool for cancer treatment, the radiation sensitivity of normal tissues adjacent to the tumour which are unavoidably exposed to radiation, limits therapeutic gain[1]. Treatment with ionizing radiation produces reactive oxygen species (ROS) in the cells; these include the superoxide radical ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2). These reactive molecules initiate the oxidative degradation of the unsaturated fatty acids of membranes, nucleic acids and the oxidative damage of distinct enzymes through the alteration of their three-dimensional structures[2,3].

Garlic [*Allium (A.) sativum*] is one of the oldest medicinal plants used by different cultures. Its utilizations as a remedy for heart disease, tumors and headaches were documented in the Egyptian Codex Ebers, dating from 1550 BC[4,5]. As a member of *Allium* family, Garlic is rich source of phytonutrients, useful for the treatment or prevention of a number of diseases including cancer, coronary heart disease, hypercholesterolemia, type 2 diabetes, hypertension and infection diseases[6]. Medicinal properties of garlic have been attributed to its organosulfur compounds. The most important initial sulfur compound is alliin which is present in the intact garlic bulbs. When bulbs of garlic are damaged by crushing or cutting, alliin is transformed into allicin by aliinase. However, it was reported that garlic extract (GE) creates strong antioxidant effect by water-soluble organosulfur compounds,

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such as S-allylcysteine and S-allylmercaptocysteine, and inhibits the free radical-mediated damage of cellular biomolecules[7]. In addition, the oil soluble organosulfur compounds present in garlic, diallyl sulfide, diallyl disulfide, dipropyl sulphide, dipropyl disulfide and allyl methyl sulphide[8], have been shown to have free radical scavenging activity[9]. Furthermore, the antibacterial and antifungal activities against a variety of Gram-negative and Gram-positive were and continue to be extensively investigated. It was reported that the antibiotic activity of 1 mg of allicin has been equated to that of 15 IU of penicillin[10,11]. Recent investigations have also demonstrated an inhibitory effect by aqueous extracts on numerous bacterial and fungal species[12-14].

During the last 50 years, protective potential of natural compounds from pathogens and carcinogens aroused great interest and was achieved by various physical and chemical methods[10]. The aim of this investigation was to assess the *in vivo* antioxidant potential via evaluating radioprotective effects in kidney and liver tissues of rats and *in vitro* antimicrobial and radical scavenger activity of GE.

Materials and Methods

Experimental Design

The study was undertaken at the "Laboratory for Experimental Studies of Inonu University" in accordance with the guidelines established in the "Guide for the Care and Use of Laboratory Animals" following the approval of the design by the "Animal Ethics Committee of Inonu University". Thirty-two mature female Wistar rats (whose ages and weights are between three and four months, and 200 and 250 g, respectively) were divided into four groups, each consisting of eight rats. The rats in each group were kept in separate cages in rooms with controlled light and temperature and were fed with standard chow and water *ad libitum*. Experimental groups were control group (1), GE group (2), irradiation group (3) and both GE and irradiation group (4). For the rats in two groups (group 3 and 4) irradiation were performed on a Cobalt-60 unit using a single fraction of 20 Gy[3]. The GE was given to rats once a day during the month before irradiation and continued for five days after irradiation.

Garlic Extract

The garlic extract was prepared daily. Aqueous garlic extract was prepared from locally available garlic cloves. The garlic cloves were peeled on crushed ice and 50 g of garlic was cut into small pieces and homogenized in 75 mL of cold, sterile 0.9% NaCl in the presence of some crushed ice. The homogenization was carried out in a blender at high speed using six 2-min bursts for a total of 12 min. The homogenized mixture was filtered three times through cheesecloth. The filtrate was centrifuged at 2000 RCF for 15 min and the clear supernatant was made up to 100 mL with normal saline. The concentration of this garlic preparation was 500 mg/mL on the basis of weight of the starting material (0.5 g/mL). This extract was administered to rats by oral gavage.

Irradiation

Prior to irradiation, the rats received anesthesia using ketamine (Ketalar, Pfizer Drugs Company, Istanbul, Turkey) at a dose of 80 mg/kg and xylazine (Rompun, Bayer Turkish Chemistry Industry, Istanbul, Turkey) at a dose of 5 mg/kg administered using an intraperitoneal injection. The rats were immobilized in the prone position on a rough surface by way of taping from the head. The rats in Group 3 and Group 4 were immobilized in the prone position in a custom-designed acrylic restrainer and whole body irradiation was performed on a Cobalt-60 unit using a single fraction of 20 Gy. Following irradiation, the animals were closely observed until recovery from anesthesia.

Euthanasia

Prior to euthanasia, the rats received anesthesia using propofol (Propofol, Abbott Laboratories, Istanbul, Turkey) at a dose of 50 mg/kg administered using an intraperitoneal injection. Euthanasia was performed by way of transcardiac perfusion using 0.9% sodium chloride. Subsequently, the liver and kidney tissues were dissected for blinded biochemical evaluation and frozen on crushed dry ice.

Evaluation of Biochemical Parameters

Prior to the preparation of the tissue homogenates, the liver and kidney tissues were perfused with cold phosphate buffered saline (PBS) solution (50 mM, pH 7.4) in an attempt to prevent contamination with blood, dried and weighed. The temperature was kept at + 4°C throughout the preparation of the homogenates. The tissues were divided into two portions.

Measurement of Malondialdehyde Levels

One portion of the tissue was homogenized in PBS solution (with a weight to volume ratio of 1 to 5) for the measurement of enzyme activity and the other portion of the tissue was homogenized in 1.5% potassium chloride (KCl) for the measurement of malondialdehyde (MDA) level[15] with homogenizer (T 25 Ultra-Turrax, IKA Werke GmbH, Staufen, Germany). Half mL of the homogenate prepared with 1.5% KCl was mixed with 3 mL of 1% phosphoric acid (H_3PO_4). Following the addition of 1 mL of 0.67% thiobarbituric acid (TBA), the mixture was heated in boiling water for 45 minutes. The colored complex was extracted into *n*-butanol and the absorbance was measured at 532 nm on a spectrophotometer (UV-1601, Shimadzu Corporation, Kyoto, Japan) with tetramethoxypropane as the standard. The MDA level was expressed in nanomoles per milligram of protein (nmol/mg protein).

Measurements of Protein Levels and Enzyme Activities

Homogenate prepared with PBS was sonicated four times for 30 seconds at 20 seconds intervals using a sonicator (S-250A, Branson Ultrasonics Corporation, Danbury, CT, USA) and centrifuged at 20,000 relative centrifugal force for 15 minutes using a centrifuge (5417R, Eppendorf Aktiengesellschaft, Hamburg, Germany). The supernatant was separated and stored at -40°C until the measurement of the superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and 5'-nucleotidase (5'-ND) activities. The protein concentration in the supernatant was measured with bovine serum albumin as the standard[16].

The SOD activity was measured in the supernatant[17]. For Solution A, 100 mL of PBS solution (50 mM, pH 7.4) including 0.1 mM of ethylenediaminetetraacetic acid (EDTA) and 2 μM of cytochrome *c* was mixed with 10 mL of 0.001 M sodium hydroxide (NaOH) including 5 μM of xanthine. For Solution B, 0.2 U/mL of xanthine oxidase was mixed with 0.1 mM of EDTA. Subsequently, 50 µL of the supernatant and 50 μL of Solution B were added to 2.9 mL of Solution A. The change in the absorbance was monitored at 550 nm. The change in the absorbance of a blank mixture including all of the ingredients except for the supernatant (substituted with 10 µL of ultrapure water) was also monitored. The SOD activity was expressed in the amount causing a 50% inhibition of the reduction of cytochrome *c* per milligram of protein (U/mg protein), with bovine copper-zinc SOD (Cu/Zn SOD) as the standard.

The CAT activity was measured in the supernatant[18]. The decomposition of the substrate H_2O_2 was monitored at 240 nm on a spectrophotometer. The CAT activity was expressed in micromoles of H_2O_2 decomposed per minute per milligram of protein (U/mg protein).

The GPx activity was measured in the supernatant[19]. One mL of PBS solution (50 mM, pH 7.4) was incubated at 37ºC for 5 minutes together with 5 mM of EDTA, 2 µM of nicotinamide adenine dinucleotide phosphate (NADPH), 20 µM of glutathione, 10 µM of sodium azide (NaN_3) and 23 mU of glutathione reductase. Subsequently, 20 µL of H_2O_2 solution (0.25 mM) and 10 µL of the supernatant were added to the mixture. The change in the absorbance was monitored at 340 nm for 3 minutes on a spectrophotometer. The change in the absorbance of a blank mixture including all of the ingredients except for the supernatant (substituted with 10 µL of ultrapure water) was also monitored. The GPx activity was expressed in micromoles of NADPH consumed per minute per milligram of protein (U/mg protein), using an appropriate molar absorptivity coefficient (6220 $M^{-1} cm^{-1}$).

The 5'-ND activity was measured in the supernatant[20] via Sigma Diagnostic Reagent (Procedure No: 265-UV). At this modified method, 5'-ND causes the hydrolysis of adenosine monophosphate and inorganic phosphorus. The auxiliary enzyme, adenosine deaminase deaminates adenosine, producing inosine and ammonium ion. In a coupled reaction catalyzed by L-glutamate dehydrogenase, the ammonium ion reacts with 2-oxoglutarate in the presence of reduced nicotinamide adenine dinucleotide (NADH) to form glutamate and oxidized nicotinamide adenine dinucleotide (NAD). The rate of NAD formation, which produces a decrease in absorbance at 340 nm, is directly proportional to the rate of adenosine formation and, hence, 5'-ND activity.

Measurements of *in vitro* antioxidant activities. Each assay was repeated two times and the arithmetic means were calculated and used.

Measurement of DPPH· and superoxide scavenger activity. (1) 50 μL of sample (five different concentrations of GE) solution was

added to 1.95 mL of 1,1-diphenyl-2-picrylhydrazyl $(DPPH)^{\cdot}$ solution (6×10^{-5} M in methanol). The decrease in the absorbance at 515 nm was determined using a Shimadzu 1601 spectrophotometer until the reaction reached the steady state in the dark[21]. The per cent radical scavenging activity (RSA) was expressed as the inhibition percentage and was calculated using the following formula:

$$\% \text{ RSA} = \frac{(A[DPPH^{\cdot}_0]-A[DPPH^{\cdot}_T])}{(A[DPPH^{\cdot}_0])) \times 100} \times 100$$

where $A[DPPH^{\cdot}_0]$ and $A[DPPH^{\cdot}_T]$ were the absorbance at time zero and at the time of steady state, respectively. The concentration of GE was plotted against per cent RSA (Figure 1), and the resulting exponential equation that corresponds to the situation where the concentration of GE to decrease the initial absorbance of DPPH by 50% (IC_{50}) was determined. Antiradical activity (A_{AR}) was defined $1/IC_{50}$. Trolox is known a powerful radical scavenger molecule and was used as standart antioxidant. (2) Inhibition of superoxide radicals is monitored by the cytochrome c method. Briefly, 3 mL system consists of 38 mM tris-HCl buffer pH 7.4, 16 μM xanthine, 10 μM cytochrome c and 0.02 U/mL of xanthine oxidase. The decrease in absorbance of cytochrome c was monitored at 550 nm in the presence and absence of GE (10-70 mg/mL). The aforementioned modified method of McCord and Fridovich[17] was used to measure SOD equivalent activity. In this method, one unit of SOD activity was defined as the amount of enzyme necessary to decrease the rate 50% of maximum inhibition of cyt c oxidation. When SOD replaced by an antioxidant such as GE, superoxide radicals were scavenged by radical scavenger components of GE and the expected result was the inhibition of the reduction of oxidized cyt c. The concentration of GE was plotted against per cent Inhibition, i.e. per cent superoxide scavenging activity of GE (Figure 2). The concentration of the GE at which the superoxide radical inhibited by 50% under the experimental conditions has been estimated. Superoxide scavenging activity of GE is assessed as SOD equivalent unit.

Measurement of total phenolic content (TPC). The Folin-Ciocalteau micro method was used[22]. Sixty microliters of the extract were diluted with deionised water to 4.8 mL, and 300 μL of Folin-Ciocalteau reagent were added before the mixture was shaken. After 8 min, 900 μL of 20% sodium carbonate were added and mixed. The solution was left at 40°C for 30 min before the absorbance at 765 nm was read. Gallic acid (0-50 μg) was used as standard, and the results were reported as mg gallic acid equivalent per g of sample.

Evaluation of Antimicrobial Activity

Antimicrobial activity of the garlic extract was assessed by using agar dilution procedure recommended by the Clinical and Laboratory Standards Instıtute[23,24]. Minimal inhibitory concentration (MIC) for garlic extract was investigated against standard bacterial strains; *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 were obtained from American Type Culture Collection (Rockville, MD, USA) and the fungal strains *Candida albicans* and *Candida tropicalis* ob-

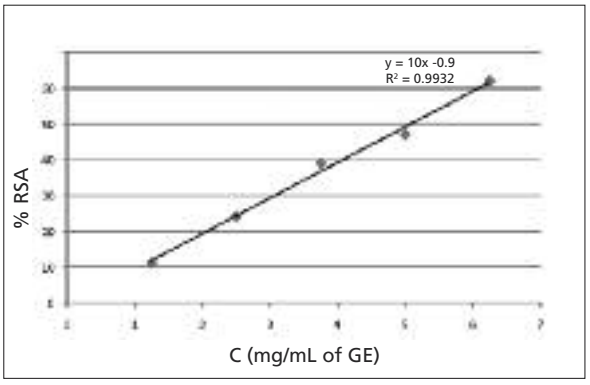

Figure 1. DPPH radical scavenging activity of garlic extracts at different concentrations.

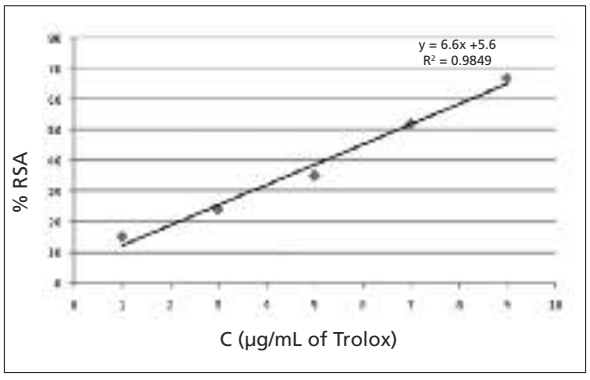

Figure 2. DPPH radical scavenging activity of Trolox at different concentrations.

tained from the Department of Microbiology, Faculty of Medicine, Ege University (Turkey). Bacterial strains were subcultured on Muller Hinton Broth (HiMedia Laboratories Pvt. Ltd. Mumbai-India) and fungal strains were also on RPMI 1640 Broth (Sigma-Aldrich Chemie, GmbH, Taufkirchen, Germany). Their turbidities matched that of a McFarland no. 0.5 turbidity standard. All of the dilutions were done with distilled water. The concentrations of the tested extracts were 1250, 625, 312.5, and 156.25 μg/mL. Ampicillin and ciprofloxacin were used as antibacterial standard drugs, while fluconazole were used as antifungal standard drugs whose minimum inhibitory concentration (MIC) values are provided. A loopful (0.01 mL) of the standardized inoculum of the bacteria and yeasts (10^6 CFUs/mL) was spread over the surface of agar plates. All the inoculated plates were incubated at 35°C and results were evaluated after 16-20 h of incubation for bacteria and 48 h for yeasts. The lowest concentration of the compounds that prevented visible growth was considered to be the MIC.

Statistical Analysis

For all enzymes and MDA levels; results were expressed as means ± standard deviation (SD). The mean MDA levels and the mean SOD, CAT, GPx and 5'ND activities were compared across groups in a pairwise manner by two-sample t test. We also conducted a pairwise median comparison via Mann-Whitney test for verification purposes. Results for these two tests were in the same direction in terms of statistical significance which was defined as the *p*-value being less than or equal to 0.05. Given the present context and the purposes of study, pairwise comparisons are more meaningful and appropriate than an overall comparison across groups from a substantive point of view. Statistical analyses were performed by "SPSS for Windows Version 16.0" (SPSS Inc, Chicago, IL, USA). Of note, for all *in vitro* measurements; all measurements were performed duplicate and arithmetic means were used.

Results

Kidney Tissue

There were no significant differences in CAT and 5'-ND enzyme activities among control group (1), garlic extract group (2), irradiation group (3) and both garlic extract and irradiation group (4), as evidenced by large p-values ($p > 0.05$). More specifically, 95% confidence intervals for four groups in CAT activities overlap, lending further support to insignificant differences. Same results apply to 5'-ND.

Increased SOD activity and MDA levels were observed in irradiation group (3) compared to the control group ($p < 0.05$). However, an inhibitory effect of garlic extract against increase in MDA was seen in group (4) (p value for comparing groups 3 and 4 is less than 0.05). Moreover, significantly decreased GPx enzyme activity was found in irradiation group (3) ($p < 0.05$). Interestingly, treatment with garlic extract in addition to radiation (group 4) did not lead to significant changes in comparison to radiation only group (3) ($p > 0.05$) for GPx. Of note, for all enzymes under consideration the addition of GE treatment in the presence of radiation did not yield substantial changes in enzyme activities.

Liver Tissue

There were no significant differences in SOD, CAT, 5'ND activities among control group (1), GE group (2), irradiation group (3) and both GE and irradiation group (4), as evidenced by large p-values ($p > 0.05$). More specifically, 95% confidence intervals for four groups in CAT activities are (2812.1 ± 567.8), (2632.8 ± 1399.2), (3052.6 ± 968.6), (2896.5 ± 598.6) for Group 1, 2, 3 and 4, respectively. These confidence intervals overlap, lending further support to nonsignificant differences. Same arguments apply to SOD and 5'-ND.

As far as MDA levels and GPx activity are concerned, changes with respect to groups are substantively parallel across liver and kidney results. MDA level went up significantly in irradiation group (Group 3) in comparison to the control group (Group 1). However, addition of garlic moved it back to the Control group levels. GPx levels decreased significantly for Group 3 in comparison to the Control group; addition of GE (Group 4) lead to an increase GPx activity, but this increase was statistically indiscernible between Group 3 and Group 4.

All relevant biochemical results of liver and kidney tissues were summarized in Table I.

Antimicrobial Activity

The antimicrobial activities were evaluated against *Staphylococcus aureus, Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aerugi-*

Table I. Liver and kidney MDA levels and CAT, SOD, GPx and 5'-ND activities for all groups (n = 8). Results were expressed as Mean ± SD.

	Liver groups				Kidney groups			
	1	2	3	4	1	2	3	4
CAT (U/mg protein)	2812.1 ± 289.7	2632.8 ± 713.9	3052.6 ± 494.2	2896.5 ± 305.4	845.3 ± 242.0	753.1 ± 131.2	1077.9 ± 196.2	1265.0 ± 301.5
SOD (U/mg protein)	1.559 ± 0.228	1.460 ± 0.286	1.664 ± 0.273	1.369 ± 0.143	0.814 ± 0.172^{c,d}	0.887 ± 0.122	1.101 ± 0.093^{a}	1.016 ± 0.061^{a}
MDA (nmol/mg protein)	15.21 ± 2.28^{c}	15.17 ± 3.28^{c}	22.15 ± 5.99^{a,b,d}	14.60 ± 3.35^{c}	57.56 ± 6.85^{c}	46.60 ± 5.72^{c,d}	95.11 ± 18.78^{a,b,d}	74.88 ± 10.75^{c,b}
5'-ND (U/mg protein)	0.0202 ± 0.0032	0.0188 ± 0.0023	0.0193 ± 0.0013	0.0189 ± 0.0023	0.011 ± 0.003	0.010 ± 0.003	0.010 ± 0.004	0.0014 ± 0.005
GPx (U/mg protein)	0.664 ± 0.252^{c,d}	0.633 ± 0.211^{c,d}	0.341 ± 0.126^{a,b}	0.379 ± 0.123^{a,b}	0.419 ± 0.121^{c}	0.373 ± 0.047	0.269 ± 0.057^{a}	0.399 ± 0.176

$^{a}p < 0.05$ when compared with group 1 (Control group); $^{b}p < 0.05$ when compared with group 2 (Garlic extract group); $^{c}p < 0.05$ when compared with group 3 (Irradiation group); $^{d}p < 0.05$ when compared with group 4 (Irradiation + garlic extract group).

nosa, *Candida albicans* and *Candida tropicalis* and the garlic extract were compared with ampicillin, ciprofloxacin, and fluconazole that are used to treat general bacterial and fungal infections. Especially the garlic extract has shown to have antibacterial activity to different extents depending on the type of infectious agent. The garlic extract turned out to be particularly more effective on *C. albicans* and *C. tropicalis*. The results of antimicrobial activity were summarized in Table II.

DPPH and Superoxide Scavenging Activity and Total Phenolic Content (TPC)

The IC_{50} value for the GE i.e. the concentration of which it scavenges 50% of the DPPH radical was found to be 4.9 mg/mL and A_{AR} value was calculated to be 0.204. The trolox equivalent value of GE was found as 187 mg mL^{-1} GE/mM trolox (Figures 1 and 2).

Figure 3 shows percentage scavenging of superoxide radical by the GE at different concentrations (10-70 mg/mL). From the figure, IC_{50}, value i.e. the concentration of the GE at which the superoxide radical inhibited by 50% under the experimental conditions has been estimated to be 36±1 mg/mL and this means that a concentration of approximately 36 mg/mL GE serves as 1 equivalent unit of SOD enzyme.

The amounts of total phenolic content was found 78 mg gallic acid equivalent per 100 g of fresh garlic.

Discussion

In recent years, many plant species have been investigated in many research works for medical

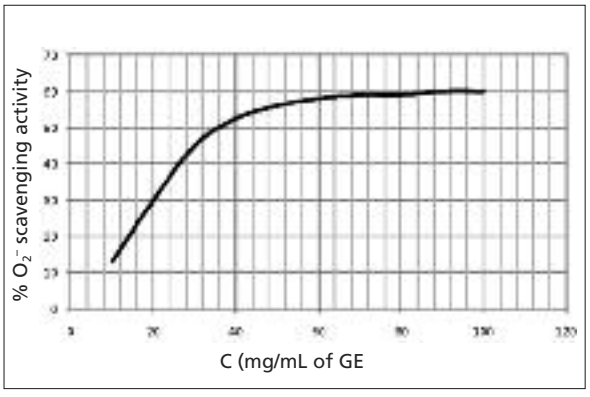

Figure 3. Superoxide radical scavenging activity of garlic extracts at different concentrations.

Table II. Antimicrobial activity of garlic extract was expressed with MIC. MIC values of standarts and garlic extract (μg/mL).

	E. coli	*S. aureus*	*E. faecalis*	*P. aeruginosa*	*C. albicans*	*C. tropicalis*
Garlic extract	625	625	625	1250	156.25	156.25
Ampicillin	3.12	3.12	1.56	–	–	–
Ciprofloxacin	1.56	0.39	0.78	3.12	–	–
Fluconazole	–	–	–	–	3.12	3.12

purposes and reported to have antioxidant, antimicrobial, anti-inflammatory and anticarcinogen effects. Among them, garlic as a food has a special importance since it is often a part of daily diet and is widely consumed in many societies. In this study we aimed to assess the garlic in a broad perspective and evaluated its antioxidant potential against oxidative stress created *in vivo* by radiation therapy and its *in vitro* antimicrobial and total antioxidant capacity. In this respect, the tissues of the two organs, namely liver and kidney that respond to oxidative stress the fastest have been included in the study. We preferred to use the radiotherapy technique to create *in vivo* oxidative stress. The reason is that the radiation therapy which is widely used in cancer treatment today and during the treatment process although the tumor tissue is targeted, oxidative damage is inevitable for the surrounding tissues exposed to radiation. Especially in whole-body radiotherapy for the treatment of leukemia, early and late phases of kidney and liver damage, is among frequently observed complications. Therefore, we thought that the evaluation of the possible damage in these two organs and the protective effect of implementation of garlic would be important.

Effect of Garlic Administration on Irradiated Rat Liver Tissue

The liver is remarkably vulnerable to injury induced by irradiation as a result of the ROS. In this work, a slight but insignificantly increased SOD activity was assessed in irradiation group compared to the control group, and this increase was inhibited by GE. The increase of SOD activity may be a response of antioxidant system to high superoxide concentration produced via irradiation. It is known that free radicals at a low level of intensity might enhance the antioxidant defense system via stimulation of gene expression of antioxidant enzymes, while an oxidant stress at a high level of intensity might exceed the capacity of the antioxidant defense system because of oxidant damage especially at three dimensional structures of antioxidant enzymes active sites. At this point, it is possible that superoxide anion radicals were scavenged by radical scavenger compounds of GE, and remaning superoxide radicals could weakly affect the hepatic SOD activity via upregulation. In addition, the increased SOD activity results in overproduction of H_2O_2 that is a product of dismutation reaction. GPx is the dominant antioxidant defensive enzyme participating in getting rid of H_2O_2 in the cell. We found decreased GPx activity levels in irradiation group in comparison to the control group. During the detoxification of increased H_2O_2 by GPx, the consumption of reduced glutathione (GSH) also increases and thus sufficient reduced GSH may not be supplied for the detoxification of excessive H_2O_2 generated by SOD activity and irradiation. During oxidative stress, the ratio of reduced/oxidized glutathione (GSSG) and as a result of which that of reduced/oxidized nicotinamide adenine dinucleotide phosphate (NADPH/NADP) has been reported to have changed since, on one hand, GSH is rapidly consumed by GPx, on the other hand, oxidized GSH generated by glutathione reductase through NADPH should be provided as reduced GSH to the site[25]. Because of insufficient GSH and presence of high concentration of H_2O_2 for a long time, this mechanism may not occur fast enough. Ultimately, the activity of GPx may have decreased. In our report, the decrease of GPx enzyme activity was slightly inhibited by GE. We have speculated that the GE partially restores the oxidative damage of hepatic GPx and inhibits the decrease of reduced GSH. However, our findings showed that the tissue CAT activity levels were unchanged with radiation. In general, the most striking changes via free radical attacks are seen in GPx among the antioxidant enzymes. The probable reason is that the first defense against the peroxides comes from the GPx, and increasing oxidative stress in the course of time, CAT may have joined in this defense[26]. It has been shown that chronic garlic intake did not change the activity of CAT but increased GPx in the liver tissue[27]. The ability of GE to increase GPx activi-

ty is important in radioprotection and UV suppression of certain forms of immunity in reducing the risk of radiation and chemically induced cancer[28], and in preventing the range of ROS-induced DNA, lipid and protein damage implicated in the disease and aging processes[29]. Furthermore, researchers reported that garlic increases cellular reduced GSH levels in normal liver and mammary tissues[30].

5'-Nucleotidase (5'-ND) catalyses the hydrolysis of phosphate esterified at carbon 5'of the ribose and deoxyribose portions of nucleotide molecules. It has been reported that tissue 5'-ND activity was triggered by oxidative stress especially superoxide anion radicals in rats[31], increased liver nucleotidase activity in rats exposed irradiation[32], and increased liver 5'-ND activity levels in chemical carcinogene dimethylbenz(a)anthracene-induced female rats[33]. According to these results, we expected increased 5'-ND activity because of accelerated catabolism of nucleic acids as a result of oxidative base damages via irradiation in irradiation group and induction of enzyme by oxidative stress but we have not observed this trend.

Additionally, we found increased MDA levels in the irradiation group compared to the control group. We found that MDA levels are dramatically higher in irradiaton group (3) than in control group (1) and irradiation plus GE group (4). Clearly, GE protected the liver tissue against lipid peroxidation. It was recently shown that administration of exogenous antioxidants prior to irradiation limited the increase of MDA levels by irradiation in rat liver[34]. Moreover, it has been demonstrated that the elevation of MDA level was dramatically inhibited by garlic in rat liver induced by ethanol[35]. We can state that GE inhibits lipid peroxidation produced by oxidative stress via irradiation.

Effect of Garlic Administration on Irradiated Rat Kidney Tissue

From the standpoint of serious or fatal damage, the kidneys are probably the most radiosensitive of the abdominal organs[36]. Kunkler et al.[37] established that a total dose of 23 Gy carried a high risk of renal injury. More recently a total dose of 20 Gy has been proposed[38]. The recognition of this, radiotherapeutic practices may result in a radiation-induced renal damage in patients, defined as radiation nephropathy. In this research, the significant increase was observed in irradiation group compared to the control group for SOD activity and this increase was slightly inhibited by GE. It is possible that produced superoxide radicals could affect the renal SOD activity via upregulation. Increased SOD activity results in overproduction of H_2O_2 that is a product of dismutation reaction. We found decreased GPx activity levels in irradiation group in comparison to the control group. The decrease of GPx enzyme activity was slightly inhibited by GE. We have speculated that the garlic extract partially restores the oxidative damage of renal GPx by ROS, and inhibits the decrease of reduced GSH. On a related note, we found unchanged CAT activity in kidney tissue with irradiation.

It has been reported that increased kidney nucleotidase activity in rats exposed irradiation[39]. According to these results, we expected increased 5'-ND activity because of accelerated catabolism of nucleic acids as a result of oxidative base damages via irradiation in irradiation group and induction of enzyme by oxidative stress, but we have not observed this trend.

Our findings clearly show that kidney malondialdehyde levels are dramatically higher in irradiaiton group (3) than in control group (1) and irradiation plus GE group (4) the inhibitory effect of GE is evident. Furthermore, Erol et al.[40] evaluated melatonin and vitamin E as protectants against the oxidant injury induced by irradiation via measurement of MDA levels in the rat brain and the mean MDA levels that underwent irradiation were significantly increased, similar to our results. It has also been reported the increased MDA levels in plasma, erythrocyte, brain, heart, lung, kidney, spleen, liver, thymus and bone marrow tissues of rats exposed wholebody gamma irradiation[41].

Radical Scavenging Activity of GE

In this study, water was used as extracting solvent since about 97% of garlic components are water-soluble[42], and several previous studies also confirmed that water is a perfect solvent for measuring antioxidant capacity and total phenolic content (TPC) of GE[43,44]. *In vitro* antioxidant capacity and TPC of aqueous garlic extract have been reported by several researchers but results are difficult to compare because of the differences in sample sources, sample preparations, methodology details, standards used, among others. Jastrzebski et al.[45] reported that 69% inhibition as RSA and 49.3 mg gallic acid equivalent as TPC per 100 g of garlic (fresh weight).

Wangcharoen et al[44] reported 0.16 mg vitamin C equivalent per g of sample (fresh weight), and TPC = 0.41 mg gallic acid equivalent per g of garlic (fresh weight). Nuutila et al[46] found DPPH = 62.1, 60.9, and 43% inhibition, and TPC = 75, 115, and 95 mg gallic acid equivalent per kg of freeze-dried samples of Finnish organic garlic, Finnish garlic, and Hungarian garlic, respectively. We found that via DPPH measurement, IC_{50} value was 4.9 mg/mL, A_{AR} value was 0.204 and trolox equivalent was 187 $mg.mL^{-1}$ GE/mM trolox. However, TPC value was found to be 78 mg gallic acid equivalent per 100 g of fresh garlic. In addition, when superoxide radical scavenging activity of GE was assessed we calculated 36 mg/mL GE serves as 1 equivalent unit of SOD enzyme. Superoxide radicals are primarily scavenged by SOD enzyme in the cell. Nevertheless, there may be cases in which SOD is inhibited or is not produced enough to work efficiently, or the amount of superoxide radicals produced in the cell is more than the amount that is scavenged by SOD. In recent years, it is suggested that exogenous antioxidants such as garlic may be protective against free radical damage when the antioxidant system is weakened.

Antimicrobial Activity of Garlic

It is known that radiation therapy and chemotherapy as cancer treatment weakens the immune system. Therefore, the infection is one of the serious risks during treatment process. In this context, we investigated the antimicrobial activity of garlic extract. According to our findings, garlic extract particularly more effective on *Candida albicans* and *Candida tropicalis* comparatively. Furthermore, we observed considerable effect of garlic extract on *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis*. One recent chemical characterisation of garlics' sulphur compounds suggests that these compounds are the main active antimicrobial agents[47]. Whitemore et al[10] reported that the antibiotic activity of 1 mg of allicin, which is a (+)-S-methyl-l-cysteine sulfoxide, has been equated to that of 15 IU of penicillin. However, some proteins, saponins and phenolic compounds can also contribute to this activity[48]. Interestingly, garlic weakly inhibits beneficial intestinal microflora, but it is more effective against potentially harmful enterobacteria, probably due to a greater sensitivity of enterobacteria to allicin[49].

Conclusions

We investigated the antioxidant and antimicrobial activity of garlic, which has been widely consumed in many societies. Our findings suggest that the use of garlic extract could be useful for addressing the limited therapeutic gain due to the radiation sensitivity of normal tissues adjacent to the tumour which are exposed to radiation, by strengthening the antioxidant system. *In vitro* and *in vivo* experiments seem to yield similar conclusions. It can be stated that garlic is recommended to be suffiently included in the diets of radiotherapy patients considering its antioxidant and antimicrobial efficacy. More comprehensive researches regarding the dose and duration are needed to support this premise.

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