

Full Length Research Paper

Comparison of *in vivo* chemoprotective and *in vitro* antimicrobial activity of different garlic (*Allium sativum*) preparations

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Medicinal properties of garlic have been attributed to its organosulfur compounds. Because of conversion of bioactive constituents to other compound during processing, various garlic preparations may have different bioactivities. In this study, we aimed to assess *in vivo* chemoprotective effects against 7,12- dimethylbenz(a)anthracene (DMBA) induced rat liver damage and *in vitro* antimicrobial activity of fresh (FGE) and aged garlic (AGE) and garlic tablet extracts (GTE). Fifty mature Sprague-Dawley female rats were divided into five groups as control (n = 10), 7,12-DMBA (n = 10), 7,12-DMBA + FGE (n = 10), 7,12-DMBA + AGE (n = 10) and 7,12-DMBA + GTE (n = 10). The rats in control group were treated with only corn oil, while other rats were treated with 7,12-DMBA 20 mg/kg b.w. i.p. Rats in extract groups received orally extracts of garlic with one day interval during 2 weeks before and after 7,12-DMBA injection. Catalase, superoxide dismutase and glutathione peroxidase activities, malondialdehyde and 8-hydroxy-deoxyguanosine levels as oxidative stress parameters in liver tissue, and *in vitro* antimicrobial activities of different garlic extracts were measured and statistical analysis were performed. As a result, all the extracts prevented the changes in the level of oxidative stress parameters significantly, demonstrated protective effect at the histological level, and AGE and FGE, especially, had an antimicrobial effect to a considerable level.

Key words: 7,12-DMBA, cancer, oxidative stress, superoxide dismutase, glutathione peroxidase, catalase, malondialdehyde, 8-hydroxy-deoxyguanosine.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) such as 7,12-dimethylbenz(a)anthracene (DMBA, Figure 1) have been shown to form free radicals and these compounds play a critical role in carcinogenesis (De Flora et al., 1996; Nigam and Shukla, 2007). This role is accompanied by direct interaction with DNA and the generation of reactive oxygen species (ROS), such as peroxides, hydroxyl and superoxide anion radicals, which induce cellular oxidative

damage through oxidation of biomolecules such as proteins and nucleo-bases, DNA strand breaks and lipid peroxidation (Srivastava et al., 2001). Under normal physiological conditions, any free radicals generated in sub-cellular compartments would subsequently be scavenged by antioxidant defence systems of the corresponding cells (Migdal and Serres, 2011). However, this protective mechanism can be broken easily by chemicals, such as PAH, which disrupt the prooxidant-antioxidant balance, leading to cellular anomalies. During daily routine life, people may be exposed to PAH. Sources of environmental PAH include industrial and domestic furnaces, tobacco smoke, gasoline, and diesel

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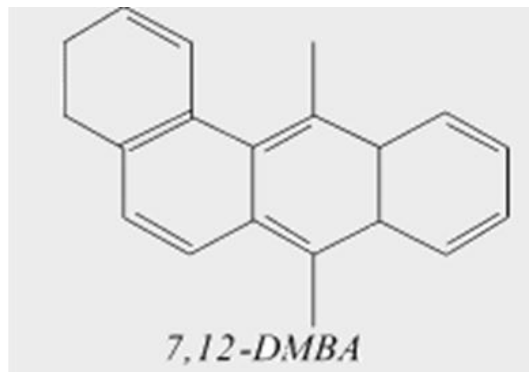


Figure 1. 7,12- dimethylbenz(a)anthracene (DMBA).

engines. They are also produced from animal fats in the process of broiling meats and fish (Cavalieri and Rogan, 1992).

Garlic (*Allium sativum*) is one of the oldest medicinal plants used by different cultures. Its utilization as a remedy for heart disease, tumors and headaches were documented in the Egyptian Codex Ebers, dating from 1550 BC (Morihara et al., 2002; Rahman, 2007). 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, diabetes type 2, hypertension and infectious diseases (Corzo-Martinez et al., 2007). Medicinal properties of garlic have been attributed to its organosulfur compounds. These bioactive components are converted/decomposed to other compounds during processing. Processing triggers the formation of a cascade of compounds that do not exist originally in garlic cloves; these compounds are the key to optimizing the benefits of garlic. Furthermore, because processing significantly affects the conversion of garlic's constituents, various processing techniques produce not only different forms but also different constituents in the preparations (Kasuga et al., 2001; Chen et al., 2004; Butt et al., 2009).

Because different garlic preparations may have different bioactivities and contain different garlic constituents, we aimed to investigate the chemoprotective effects of three garlic extracts (GEs), fresh garlic extract (FGE), aged garlic extract (AGE) and garlic tablet extract (GTE), against 7,12-DMBA- induced rat liver damage.

MATERIALS AND METHODS

Experimental design

The study was conducted 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". A total of 50 female Sprague-Dawley rats weighing 200 to 250 g were caged individually and maintained under controlled light (12:12 h light-dark cycle) and temperature (22 to 25°C) conditions with *ad libitum* access to lab chow pellets and tap water at all times during this study. Rats were divided randomly into five groups (10 rats per group): Control (1), DMBA (2), DMBA + FGE (3), DMBA + AGE (4) and DMBA + GTE (5).

Rats in groups except for control group were injected intraperitoneally each with a single dose of 20 mg/kg body weight of 7,12-DMBA (Sigma Chemicals), was dissolved in corn oil and given in a volume of 0.5 ml (Saarinen et al., 2007).

Preparation of garlic extracts

Following the adaptation of rats to experimental conditions, GEs were given to rats with one day interval during two weeks before and after DMBA administration. FGE was prepared on the day of administration. 50 g of peeled garlic cloves were crushed in a mortar then homogenized in 100 ml of distilled water. Homogenate was filtered three times through cheesecloth. Filtrate was centrifuged at 2000 rcf for 15 min and clear supernatant was pooled. When preparing AGE, 50 g of peeled garlic cloves were crushed in a mortar, then homogenized in 100 ml of distilled water and kept in amber glass bottles at +4°C for six months. On the day of administration, the homogenate was filtered and centrifuged with the same procedure for FGE. GTE preparation was adjusted according to the amount of constituents of the tablet and raw garlic. Each 100 mg tablet contains 66 mg dried powder of garlic (equivalent to approximately 250 mg raw garlic), with glucose as sweetener and titanium dioxide as dye. Tablets were crushed in a mortar, homogenized with distilled water, filtered and centrifuged with same procedure for FGE. Concentration of all GEs was 500 mg/ml and extracts were administered to rats by oral gavage in a volume of 1 ml. The final dose of all GEs was 2 g/kg b.w. for each rats.

Biochemical assays

At the end of 4 weeks, rats in all groups were sacrificed by cervical dislocation under ether anaesthesia. Subsequently, the liver tissues were resected, washed in 0.9% NaCl and used for biochemical analyses and histological evaluation.

Preparation of tissue homogenates

Each liver tissue was divided into three portions. The first portion (0.5 g) was homogenized in 1.5% cold KCl solution using a homogenizer (T 25 Ultra-Turrax, IKA Werke GmbH, Staufen, Germany) to give a 10% homogenous suspension and used for the malondialdehyde (MDA) assay (Mihara and Uchiyama, 1978). The second portion of the tissue was used for DNA isolation in order to determine 8-hydroxy-deoxyguanosine (8-OH-dG) levels. The last portion was cut into a few small pieces, homogenized in phosphate buffer solution (PBS) (pH 7.4; with a weight to volume ratio of 1:5) and centrifuged at 13000 rcf for 15 min at 5°C using a centrifuge (5417R, Eppendorf Aktiengesellschaft, Hamburg, Germany). The supernatant was separated and stored at -80°C until the measurements of the superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities and protein concentration.

Measurement of protein concentration

The protein level in the supernatant was determined according to

the method of Lowry et al. (1951) using BSA as a standard. A UV-VIS spectrophotometer (Shimadzu 1601, Kyoto, Japan) with a PC was employed for all spectrophotometric assays.

Assay of SOD activity

The SOD activity in the supernatant was measured in accordance with the method of McCord and Fridovich, (1969). For Solution A, 100 ml of PBS (50 mM, pH 7.4) including 0.1 mM of ethylenediaminetetraacetic acid (EDTA) and 2 μ M of Cytochrome c was mixed with 10 ml of 0.001 M NaOH including 5 μ M of xanthine. For Solution B, 0.2 U/ml of xanthine oxidase was mixed with 0.1 mM of EDTA. Subsequently, 50 μ l of the supernatant and 50 μ l of Solution B was 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 other than the supernatant (substituted with 10 μ l 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), with bovine Cu/Zn SOD as the standard.

Assay of CAT activity

CAT activity was measured in supernatants by the method of Luck (1963). The decomposition of the substrate H₂O₂ was monitored spectrophotometrically at 240 nm. Specific activity was defined as micromole substrate decomposed per minute per milligram of protein. CAT levels were expressed as a micromol per milligram of protein (U/mg protein).

Assay of GPx activity

GPx activity was measured according to the method of Lawrence and Burk (1976). In brief, 1.0 ml of 50 nmol/L PBS solution (pH 7.4) including 5 mmol/L EDTA, 2 μ mol/L NADPH, 20 μ mol/L GSH, 10 μ mol/L NaN₃ and 23 mU of glutathione reductase was incubated at 37°C for 5 min. Then 20 μ l of 0.25 mmol/L H₂O₂ solution and 10 μ l of supernatant were added to the assay mixture. The change in absorbance at 340 nm was monitored for 1 min. A blank with all ingredients except for supernatants was also monitored. Specific activity was calculated as micromole NADPH consumed per minute per milligram of protein using an appropriate molar absorptivity coefficient (6220 M⁻¹ cm⁻¹). GPx levels were expressed as a micromole per milligram of protein (U/mg protein).

Measurement of MDA levels

The level of MDA in tissue homogenate was determined using the method of Mihara and Uchiyama, (1978). One-half ml of homogenate was mixed with 3 ml H₃PO₄ solution (1% v/v) followed by addition of 1 ml thiobarbituric acid solution (%0.67 w/v). Then, the mixture was heated in a (95°C) water bath for 45 min. The coloured reaction complex was extracted into n-butanol and absorption at 532 nm was measured using tetramethoxypropane as a standard. The levels of MDA were expressed as nanomole per milligram of protein (nmol/mg protein).

Measurement of 8-OH-dG levels

DNA isolated from rat liver in accordance with the method of Gupta (1984). 1 g of liver tissue was homogenized in 10 ml solution containing 1% SDS and 1 mM EDTA using a homogenizer, and the

homogenates incubated with proteinase K (500 μ g/ml) at 37°C for 30 min. 0.5 ml of 1 M Tris-HCl, pH 7.4 was added to homogenate, and the resulting solution was successively extracted with 1 volume each of phenol, and a 1:1 mixture of phenol:Sevag (Chloroform: Isoamyl alcohol 24:1, v:v). The phases separated by centrifugation, and the aqueous phases pooled. DNA was precipitated with 0.1 volume of ethanol at -20°C. After centrifugation and a 70% ethanol rinse, the DNA dissolved in 2 ml of a solution containing 1.5 mM NaCl, 150 μ M Na-citrate and 1 mM EDTA. The solution was incubated with RNase T₁ (50 units/ml) and RNase A (100 μ g/ml) at 37°C for 30 min. The DNA solution was extracted with Sevag and precipitated with NaCl and ethanol. Precipitated DNA was dissolved in 0.5 ml of 20 mM CH₃COONa, pH 7.4, and incubated with 63 mg of Nuclease P₁ at 37°C for 30 min. 50 μ l of 1 M Tris-HCl, pH 7.4, was added and the nucleotides incubated with 6.3 units of Alkaline phosphatase at 37°C for 60 min (Das and Engelmann, 1990).

HPLC analyses were conducted on an Agilent 1100 System (Agilent Technologies, Palo Alto, CA, USA) comprised a quaternary solvent delivery system, an on-line degasser, a manual injection, a column temperature controller and DAD detector. Separations were carried out using C18 column, 75 × 4.6 mm, 3 μ m particle size (ACE, Aberdeen, Scotland). Analyses were carried out isocratically using 0.1% (v:v) aqueous acetic acid containing 3% acetonitrile. The mobile phase was prepared daily and filtered through 0.45 μ m millipore membrane and degassed for 15 min in an ultrasonic bath prior to use. Mobile phase was delivered the system at a flow rate of 2 ml/min and the detector was set at 297 nm (Noblitt et al., 2007).

Histological examination

For light microscopic evaluation, liver samples were fixed in phosphate-buffered 10% formalin. Paraffin-embedded specimens were cut into 5 μ m thick sections, mounted on slides and stained with hematoxylin-eosin (H-E) and Masson's trichrome staining. The sections of liver were examined with a Leica DFC280 light microscope and analysed in Leica Q Win Plus V3 Image Analysis System (Leica Micros Imaging Solutions Ltd.; Cambridge, U.K). Assessment of tissue alterations in all area of sections for each specimen was conducted by an experienced histologist who was unaware of the groups. Hepatic damage was scored (0 to 3) by grading hepatocyte necrosis, intracellular vacuolization, vascular congestion, sinusoidal dilatation, inflammatory infiltration and fibrosis with a maximum score of 18 (Esrefoglu et al., 2008).

Measurement of antimicrobial activity of GEs

Antimicrobial activity of the GEs were assessed by using agar dilution procedure recommended by the Clinical and Laboratory Standards Institute (2003). Minimal inhibitory concentrations (MIC) for GEs were 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) and the fungal strains *Candida albicans* and *Candida tropicalis* obtained 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 μ g/ml. Ampicillin and ciprofloxacin were used as antibacterial standard drugs, while fluconazole were used as antifungal standard drugs whose MIC values are provided. A loopful

Table 1. Effects of DMBA and DMBA+GEs treatments on anti-oxidant parameters in rat liver tissues. Raw data given as mean \pm SE. The one-way ANOVA and LSD Post Hoc test techniques were performed to test the differences between groups, and $p < 0.05$ was considered statistically significant.

| Groups (n = 10) | CAT (U/mg prt) | SOD (U/mg prt) | GPx (U/mg prt) | MDA (nmol/mg prt) | 8-OH-dG (nmol/mgDNA) |
|-----------------|-------------------------------------|--------------------------------|------------------------------|---------------------------------|-----------------------------------|
| Control | 4324.16 \pm 754.28 | 2.70 \pm 0.50 | 1.11 \pm 0.19 | 15.47 \pm 1.50 | 28.15 \pm 3.11 |
| DMBA | 3556.48 \pm 433.76 ^a | 2.10 \pm 0.25 ^a | 0.83 \pm 0.15 ^a | 19.05 \pm 3.57 ^a | 40.08 \pm 10.41 ^a |
| DMBA + FGE | 3592.24 \pm 641.03 ^a | 2.41 \pm 0.48 | 1.05 \pm 0.15 ^b | 17.98 \pm 3.70 | 30.75 \pm 3.39 ^b |
| DMBA + AGE | 4191.24 \pm 681.56 ^b | 1.87 \pm 0.54 ^{a,c} | 0.98 \pm 0.19 | 15.17 \pm 3.44 ^b | 20.10 \pm 8.79 ^{a,b,c} |
| DMBA + GTE | 3406.07 \pm 702.72 ^{a,d} | 1.46 \pm 0.41 ^{a,c} | 0.93 \pm 0.12 ^a | 13.94 \pm 3.67 ^{b,c} | 15.78 \pm 6.07 ^{a,b,c} |

^a $p < 0.05$ when compared with control group; ^b $p < 0.05$ when compared with DMBA group; ^c $p < 0.05$ when compared with DMBA + FGE group; ^d $p < 0.05$ when compared with DMBA + AGE group; ^e $p < 0.05$ when compared with DMBA + GTE group.

(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 to 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

Statistical analysis was carried out using the SPSS 16.0 statistical program (SPSS Inc., Chicago, IL, USA). The results were expressed as mean \pm standard deviation. The one-way ANOVA and LSD Post Hoc test techniques were performed to test the differences between groups, and $p < 0.05$ was considered statistically significant.

RESULTS

Biochemical evaluations

SOD, GPx, CAT activities and the levels of MDA and 8-OH-dG were measured in five experimental groups: Control (1), DMBA (2), DMBA + FGE (3), DMBA + AGE (4) and DMBA + GTE (5). The averaged raw data is shown in Table 1. Treatment with DMBA significantly decreased CAT, SOD and GPx activities by 18, 22 and 25%, respectively, and significantly increased MDA and 8-OH-dG levels by 23 and 42%, respectively, on average ($p < 0.05$ for all parameters).

Importantly, subsequent co-treatment with various GEs partially blocked these changes. Co-treatment with AGE inhibited the decrease of CAT activity and increase of MDA and 8-OH-dG levels ($p < 0.05$). Co-treatment with FGE inhibited the decrease of GPx activity and the increase of 8-OH-dG levels ($p < 0.05$). Also, co-treatment with GTE inhibited the increase of MDA and 8-OH-dG levels ($p < 0.05$). However, all three extracts could not prevent the reduction of SOD activity caused by DMBA.

Evaluation of antimicrobial activity

The antimicrobial activities were evaluated against *S.*

aureus, *E. faecalis*, *E. coli*, *P. aeruginosa*, *C. albicans* and *C. tropicalis* and the GEs were compared with ampicillin, ciprofloxacin, and fluconazole that are used to treat general bacterial and fungal infections. FGE and AGE have shown to have similar antibacterial activity and both of them were more effective than GTE. Especially, all GEs turned out to be particularly highly effective on *C. albicans* and *C. tropicalis*. The results of antimicrobial activity are summarized in Table 2.

Histological evaluations

Light microscopic observations

The livers of the control group presented normal histology (Figure 2A). The liver sections of the DMBA group showed histological alterations such as dark eosinophilic cytoplasm and heterochromatic nuclei (Figure 2B), vacuolated hepatocytes (Figure 2C), hepatic necrosis (Figure 2D), sinusoidal dilatation, vascular congestion and inflammatory cell infiltration especially in the periportal area (Figure 2E) in comparison to control group. In addition to portal fibrosis, bile duct proliferation in the liver parenchym (Figure 2F), thickening of the Glisson capsule, neoplastic tissue containing disorganized hepatocyte and vacuoles on the surface of Glisson capsule (Figure 2G) were determined.

The histological findings of DMBA + AGE (Figure 3A and B), DMBA + FGE (Figure 3C and D) and DMBA + GTE (Figure 3E and F) groups were similar and the histopathological evidence of hepatic damage was remarkably reduced. Hepatic damage scores and subscores of the groups are shown in Tables 3.

DISCUSSION

Garlic is a widely consumed medicinal plant with bioactive components are fully characterized. Garlic is generally consumed fresh, but in some cultures the

Table 2. Histological evaluation of hepatic damage was scored (0 to 3) by grading hepatocyte necrosis, intracellular vacuolization, vascular congestion, sinusoidal dilatation, inflammatory infiltration and fibrosis with a maximum score of 18. Raw data given as mean \pm SE. The One-way ANOVA and LSD Post Hoc test techniques were performed to test the differences between groups, and $p < 0.05$ was considered statistically significant.

| Groups (n = 8) | Necrosis | Inflammatory cell infiltration | Congestion | Fibrosis | Vacuolisation | Sinusoidal dilatation | Scores of hepatic damage |
|----------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|-----------------------------------|------------------------------------|------------------------------------|
| Control | 0.00 \pm 0.00 ^{b,c,e} | 0.00 \pm 0.00 ^{b,c,d,e} | 0.00 \pm 0.00 ^{b,c,d,e} | 0.00 \pm 0.00 ^{b,c,d,e} | 0.00 \pm 0.00 ^b | 0.00 \pm 0.00 ^{b,c,d,e} | 0.00 \pm 0.00 ^{b,c,d,e} |
| DMBA | 1.13 \pm 0.29 ^{a,c,d,e} | 1.25 \pm 0.16 ^{a,c,d,e} | 1.5 \pm 0.19 ^{a,c,d,e} | 0.63 \pm 0.18 ^{a,e} | 1.5 \pm 0.19 ^{a,c,d,e} | 1.5 \pm 0.19 ^{a,c,d,e} | 7.25 \pm 0.77 ^{a,c,d,e} |
| DMBA+FGE | 0.38 \pm 0.18 ^{a,b,d} | 1.00 \pm 0.00 ^{a,b,d,e} | 0.88 \pm 0.12 ^{a,b,e} | 0.63 \pm 0.18 ^{a,e} | 0.125 \pm 0.125 | 0.63 \pm 0.18 ^{a,b,d} | 3.62 \pm 0.32 ^{a,b,e} |
| DMBA+AGE | 0.00 \pm 0.00 ^{a,b,c,e} | 0.63 \pm 0.18 ^{a,b,c} | 1.00 \pm 0.00 ^{a,b,e} | 0.38 \pm 0.18 ^a | 0.00 \pm 0.00 | 1.00 \pm 0.00 ^{a,b,c} | 3.00 \pm 0.33 ^{a,b,e} |
| DMBA+GTE | 0.25 \pm 0.16 ^{a,b,d} | 0.63 \pm 0.18 ^{a,b,d} | 0.38 \pm 0.18 ^{a,b,c,d} | 0.25 \pm 0.16 ^{a,c} | 0.00 \pm 0.00 | 0.80 \pm 0.25 ^{a,b} | 2.00 \pm 0.38 ^{a,b,c,d} |

^a $p < 0.05$ when compared with Control group; ^b $p < 0.05$ when compared with DMBA group; ^c $p < 0.05$ when compared with DMBA + FGE group; ^d $p < 0.05$ when compared with DMBA + AGE group; ^e $p < 0.05$ when compared with DMBA + GTE group.

Table 3. Antimicrobial activity of GEs were expressed with MIC. MIC values of standarts and GEs ($\mu\text{g/ml}$).

| Variable | <i>E. coli</i> | <i>S. aureus</i> | <i>E. faecalis</i> | <i>P. aeruginosa</i> | <i>C. albicans</i> | <i>C. tropicalis</i> |
|---------------|----------------|------------------|--------------------|----------------------|--------------------|----------------------|
| FGE | 625 | 625 | 625 | 1250 | 156.25 | 156.25 |
| AGE | 625 | 625 | 312.5 | 625 | 156.25 | 156.25 |
| GTE | 825 | 825 | 825 | - | 156.25 | 312.5 |
| Ampicillin | 3.12 | 3.12 | 1.56 | - | - | - |
| Ciprofloxacin | 1.56 | 0.39 | 0.78 | 3.12 | - | - |
| Fluconazole | - | - | - | - | 3.12 | 3.12 |

consumption is in the form of aged garlic. Garlic contains at least 33 sulfur compounds, several enzymes, 17 amino acids, and minerals such as selenium. It contains a higher concentration of sulfur compounds than any other *Allium* species (Kemper, 2000).

Alliin that exists in fresh garlic in high concentration, turns into allicin that has strong bioactivity through allinase that gets activated by mechanical homogenization. In many studies, some evidence has been reported that AGE is more effective than FGE.

One may expect that AGE contains more allicin compared to FGE, and for this reason it may be

thought to be more beneficial, however, some studies demonstrate that allinase has a high turnover rate (Miron et al., 2002), and nearly all alliin turns to allicin in a very short time period in homogenized GE. At this point, it is possible that intense bioactivity of AGE may not be due to high concentration of allicin, the real reason may be other bioactive degradation products such as vinyldithiines and ajoenes that are generated out of allicin over time. On the other hand, garlic tablets manufactured by pharmaceutical industry are now on pharmacy shelves. Allicin is the active substance on the prospectus of these garlic tablets. In this study, we aimed to contrast the *in*

vitro antimicrobial and *in vivo* hepatoprotective effect of three different forms of GEs that have been consumed widely, in rats that are administered with 7,12-DMBA which is a strong carcinogen agent. In this regard, we measured SOD, GPx and CAT activities and MDA and 8-OH-dG levels, the antimicrobial activity of GEs, in addition we evaluated the histological changes of liver. By doing so, we had a chance to assess oxidative effects of the DMBA administration on antioxidant enzymes, lipids, nucleic acids and histologic parameters; and to compare protective potential of these GEs in liver, as well as antimicrobial activity. 7,12-DMBA that belongs to

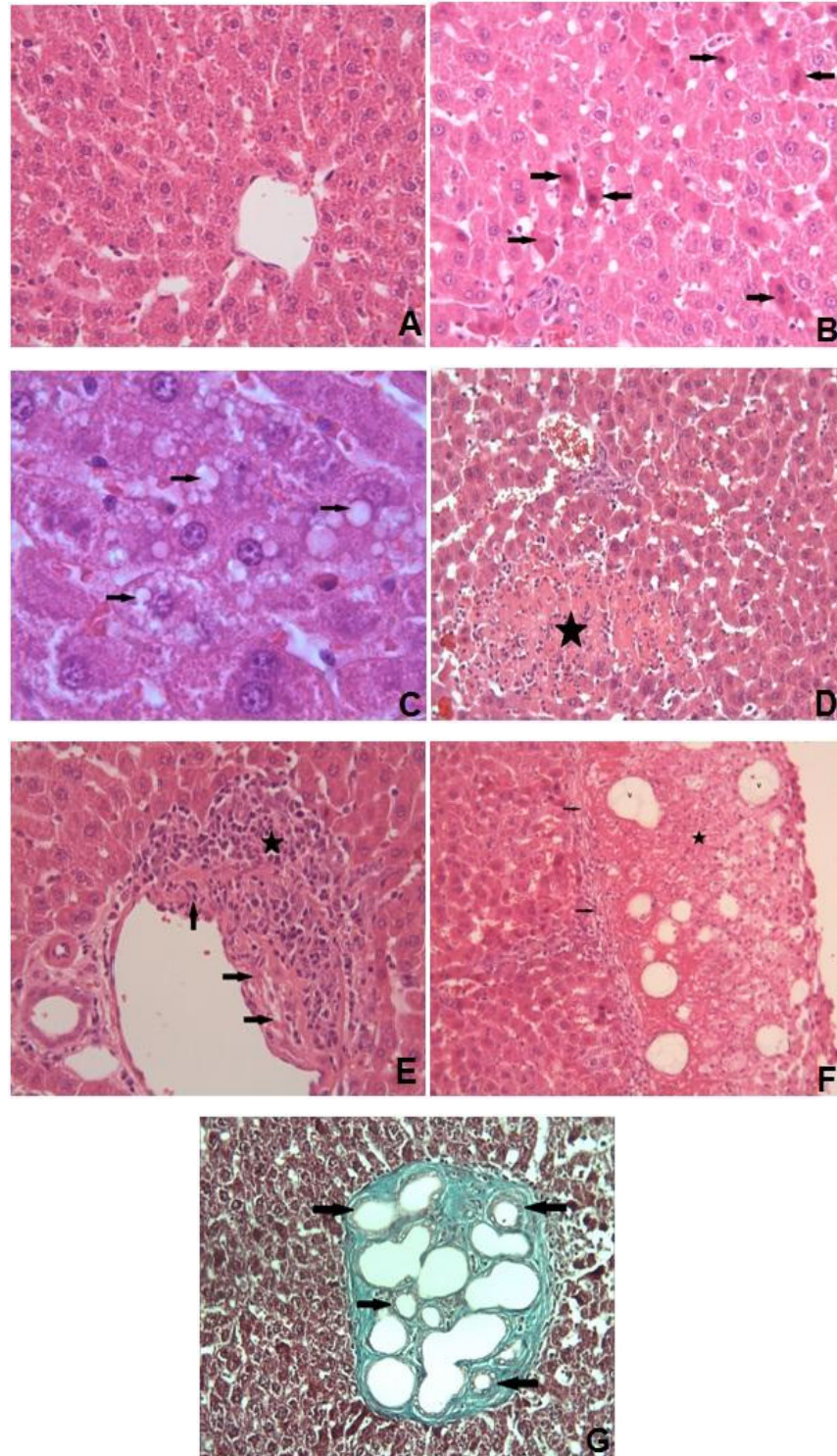


Figure 2. Histological alterations caused by DMBA administration. A; control liver section. Liver sections of the DMBA group B; Dark eosinophilic cytoplasm and heterochromatic nuclei in hepatocytes (➡), (H&E X40). C; Vacuolated hepatocytes (➡), (H&E X100). D; hepatocyte necrosis area and inflammatory cell infiltration (★), (H&E X20). E; Venous intimal hyperplasia and fibrosis (➡) and inflammatory cell infiltration (★) in portal area, (H&E X40). F; Thickening of the Glisson capsule (➡) and neoplastic tissue on the surface of Glisson capsule (★), (H&E X20). G; Bile duct proliferation in the liver parenchyma (➡), (Masson's trichrome X20).

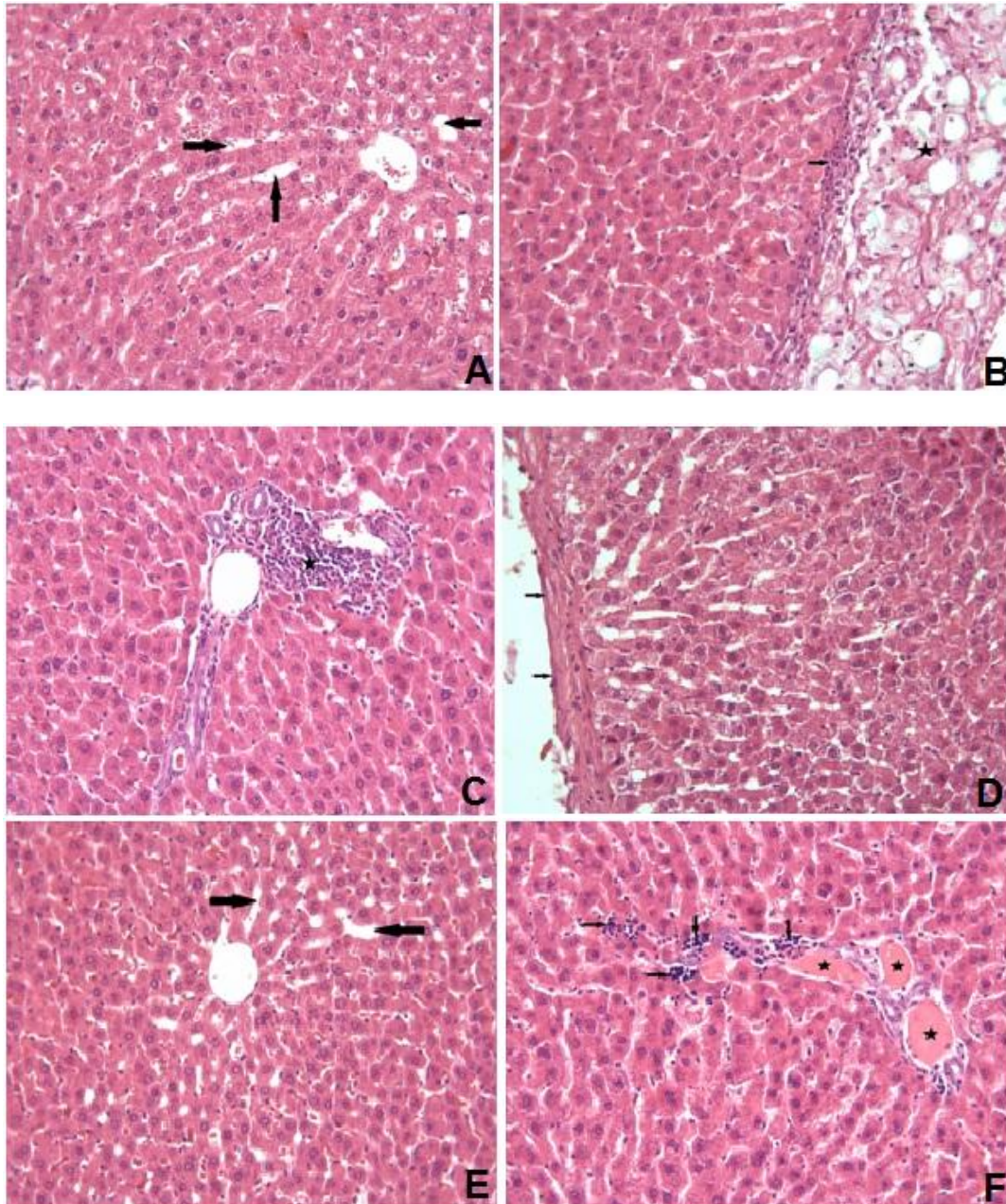


Figure 3. Garlic extracts protects liver from DMBA-induced injury. A and B; rats treated with DMBA and followed by FGE administration. A; Liver section is nearly normal except mild sinusoidal dilatation (➡), (H&E X20). B; Thickening of the Glisson capsule (➡) and neoplastic tissue (★) on the surface of Glisson capsule, (H&E X20). C and D; rats treated with DMBA and followed by AGE administration. C; Mild inflammatory cell infiltration in portal area (★), (H&E X20). D; Thickening of the Glisson capsule (➡) and slight sinusoidal dilatation, (H&E X20). E and F; rats treated with DMBA and followed by GTE administration. E; Liver is normal in histological appearance except minimal sinusoidal dilatation (➡), F; Vascular congestion (★) and slight inflammatory cell infiltration (➡), (H&E X20).

PAH family, possesses mutagenic effects due to the fact that its metabolites as well as itself could directly interact with DNA; and also owing to oxidative stress which is generated by the free oxygen radicals formed during

metabolization through cyclooxygenases. A commonly accepted way of evaluating the magnitude of oxidative stress via indirect parameters since it is challenging to take a direct measurement of free radicals *in vivo*

conditions as free radicals are known to be overly reactive and their half life is very short. MDA is a biomarker which is often used in the evaluation of lipid peroxidation. We found increased MDA levels in the DMBA group compared to the control group ($p < 0.05$). Out of three GEs that have been administered in conjunction with 7,12-DMBA, and AGE and GTE significantly inhibited the increase of MDA levels ($p < 0.05$).

We found decreased GPx activity due to injection of 7,12-DMBA ($p < 0.05$). During the detoxification of increased H_2O_2 by GPx, the consumption of reduced glutathione (GSH) also increases and thus sufficient GSH may not be supplied for the detoxification of excessive H_2O_2 generated by SOD activity and free radical dependent metabolism of DMBA. During oxidative stress, the ratio of GSH/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, GSSG converted by glutathione reductase through NADPH should be provided as GSH to the site (Cotgreave and Gerdes, 1998). 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. Free radical dependent metabolism of 7,12-DMBA causes overproduction of H_2O_2 (Georgellis, 1987). GPx is the dominant antioxidant defensive enzyme participating in getting rid of H_2O_2 in the cell. During the detoxification of increased H_2O_2 by GPx, the consumption of GSH also increases and thus sufficient GSH may not be supplied for the detoxification of excessive H_2O_2 generated during 7,12-DMBA metabolism. In our study, the decrease of GPx activity was inhibited by FGE ($p < 0.05$). We have speculated that the FGE partially restores the oxidative damage of hepatic GPx and inhibits the decrease of GSH. Jeong et al. (2011) reported that AGE reduces cigarette smoke extract-induced cell death but increasing cellular glutathione levels. However, we found AGE and GTE did not prevent the reduction of GPx.

Another enzyme that also protects the organism against peroxidative damage is CAT. Results of this study show that depending DMBA injection, CAT activity decreased by 18% whereas GPx activity decreased by 24%. The probable reason is that the first defence against the peroxides comes from the GPx, and increasing oxidative stress in the course of time, CAT may have joined in this defence (Dalton et al, 1999). In the literature, FGE and AGE exhibit direct antioxidant effects and enhance the serum levels of two antioxidant enzymes, CAT and GPx (Ide and Lau, 1997; Yamasaki and Lau, 1997; Torok et al., 1994). On the other hand, the decrease in CAT activity was prevented by only AGE in this study.

No GEs were able to prevent the decrease, caused by

DMBA injection, in the activity of antioxidant enzyme SOD which is responsible for the dismutation of superoxide radicals. At this point, the results of our study suggest that GEs are more effective against peroxidant agents.

Oxidative injury may produce unrepaired DNA damage and result in the accumulation of mutations. DNA mutation is a critical step in carcinogenesis, and elevated levels of oxidative DNA lesions such as 8-OH-dG have been noted in various tumors, strongly implicating such damage in the etiology of cancer (Prasad et al., 2008). In our study, we have determined a 42% increased 8-OH-dG level in DMBA group compared to the control group. This result indicates that ROS caused by DMBA lead to a serious oxidative damage.

In many studies related with cancer, the increase in levels of 8-OH-dG was inhibited and the extent to which agents used in the anticarcinogenic effects of agents used in this way were evaluated. Garlic and its active components, as well, are natural anticarcinogenic used for this purpose. In a study, in human peripheral blood lymphocytes, a water extract of raw garlic and S-allylcysteine (SAC) significantly inhibited adduct formation induced by benzo[a]pyrene (BaP); in addition, ROS-induced 8-OH-dG in DNA was reduced in the presence of SAC (Hageman et al., 1997). In addition, in hamsters, oral administration of GEs three times a week for 14 weeks significantly inhibited 7,12-DMBA-induced buccal pouch carcinogenesis, reduced lipid peroxidation and enhanced the levels of GSH, GPx, and glutathione S-transferase (GST) (Balasenthil et al., 1999). Similarly, oral administration of garlic supplements inhibited the development of BaP-induced neoplasia and induced increased GST activity in the forestomach (Sparnins et al., 1988). Also, topical application of diallyl sulfide and diallyl disulfide significantly inhibited skin papilloma formation induced by 7,12-DMBA and 12-O-tetradecanoylphorbol-13-acetate, and significantly increased the rate of survival in the murine model (Dwivedi et al., 1992). Results of our study indicate that the increase in 8-OH-dG levels was significantly reversed by each three used GEs.

In addition to the protective effects of garlic against cancer, it is also considered that garlic can provide support to treatment strategies such as chemotherapy and radiotherapy applied during cancer treatment. It is known that radiation therapy and chemotherapy as cancer treatment weakens the immune system. Therefore, infection is one of the serious risks during treatment process. In this context, we investigated the antimicrobial activities of different GEs. According to our findings, all GEs are particularly more effective on *C.albicans* and *C.tropicalis* comparatively. Furthermore, we observed considerable effect of FGE and AGE on *E.coli*, *S.aureus*, *P. aeruginosa* and *E.faecalis* bacteria. One recent chemical characterization of garlics' sulphur compounds suggests that these compounds are the main

active antimicrobial agents. Whitmore and Naidu (2000) 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 (Griffiths et al., 2002). 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 (Miron et al., 2000). Ajoene, a garlic-derived sulfur-containing compound, demonstrated antimicrobial activity against gram-positive bacteria, such as *Bacillus cereus*, *Bacillus subtilis*, *Mycobacterium smegmatis*, *Streptomyces griseus*, *S. aureus* and *Lactobacillus plantarum* and against gram-negative bacteria, such as *E. coli*, *Klebsiella pneumoniae*, and *Xanthomonas maltophilia*; ajoene also inhibited yeast growth at concentrations below 20 µg/ml (Naganawa et al., 1996; Ankri and Mirelman, 1998). Allicin exerted antibacterial activity against *Salmonella typhimurium*, primarily by interfering with RNA synthesis (Feldberg et al., 1988).

As a result, when the effectiveness of three different GEs which were used as preventive against hepatic damage after 7,12-DMBA injection, a strong carcinogen, is compared; therefore, we can say that all the extracts prevent the changes in the level of antioxidant parameters significantly, demonstrate histological level of protection, and AGE and FGE, especially, have an antimicrobial effect to a considerable level. However, FGE and GTE are effective on two of the antioxidant parameters while AGE is effective on three of the parameters, and it also has come to the fore by displaying antimicrobial activity in lower concentrations. At this point, we can argue that the most effective form of GEs is AGE.

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