Arch Biol Sci. 2017;69(3):439-448 https://doi.org/10.2298/ABS160825118A

Modulation of mRNA expression and activities of xenobiotic metabolizing enzymes, CYP1A1, CYP1A2, CYP2E1, GPx and GSTP1 by the *Salicornia freitagii* extract in HT-29 human colon cancer cells

Ahmet Altay[1,4,*], Deniz İrtem Kartal[2,4], Gökhan Sadi[3], Tülin Güray[4] and Ahmet Emre Yaprak[5]

[1] *Erzincan Univesity, Faculty of Science, Department of Chemistry*, 24100, Erzincan, Turkey
[2] *Yuzuncu Yıl University, Natural and Applied Sciences*, 65080, Van, Turkey
[3] *Karamanoglu Mehmetbey University, Department of Biology*, 70100, Karaman, Turkey
[4] *Middle East Technical University, Department of Biology*, 06800, Ankara, Turkey
[5] *Ankara University, Department of Biology*, 06800, Ankara, Turkey

***Corresponding author**: aaltay2013@gmail.com

Received: August 25, 2016; **Revised**: October 8, 2016; **Accepted**: October 13, 2016; **Published online**: November 3, 2016

Abstract: Phase I-II detoxification and antioxidant enzymes are responsible for the detoxification and elimination of activated carcinogens, acting as important biomarkers for chemoprevention. Among them, cytochrome P450s plays a prominent role in the metabolic activation of xenobiotics. The herb *Salicornia freitagii* (*SF*) (Amaranthaceae) is known for its anticancer, antioxidant, antidiabetic and antiinflammatory activities. In this study, we determined the bioactive phenolics in the *SF* methanol extract and investigated its antiproliferative potential in HT-29 human colon cancer cells. We also investigated the modulation of some phase I and II enzyme (CYP 1A1, 1A2, 2E1, GSTP1 and GPx) mRNA expression and enzymatic activities by the *SF* extract and its major bioactive phenolic compounds. LC/MS-MS analysis showed that the main phenolic compounds of the methanolic *SF* extract are vanillic acid (48 μg/100g) and p-coumaric acid (10.8 μg/100g). *SF* extract, vanillic acid and p-coumaric acid exhibited high antiproliferative activities in HT-29 cells, with IC_{50} values of 81.79μg/mL, 98.8 μM and 221.6 μM, respectively. The mRNA expression levels of CYP1A2 and CYP2E1 were decreased, while those of GSTP1 and GPx in HT-29 cells were increased after application of either the *SF* extract or vanillic acid. The *SF* extract by itself also increased the activities of GPx and GSTP1 enzymes 1.68- and 1.49-fold, respectively. Our data indicate that the *SF* extract and its major bioactive compound, vanillic acid, could exert a modulatory effect on the expression of enzymes that are involved in xenobiotic activation and detoxification pathways in the gastrointestinal tract. For this reason, *SF* can be considered as a natural source of chemopreventive agents.

Key words: glassworts; cytotoxicity; drug metabolism; colon cancer; phase I-II enzymes

INTRODUCTION

Plant phytochemicals have gained importance in recent years due to their diverse biological properties, including antioxidant, antimicrobial, antifungal and anticancer activities. Glassworts, a group of succulent halophytic plants, belong to the *Amaranthaceae* family. They are consumed as vegetables and used as medicinal plants around the world. They have been classified in two different genera as *Salicornia* and *Sarcocornia*. Many epidemiological studies have revealed a relation between health benefits and the consumption of glassworts [1-3]. Because they are rich sources of protein, vitamins, dietary fiber, essential fatty acids and minerals, they have become essential for human nutrition [4-7]. Glassworts have been reported to be rich sources of antioxidant compounds [8-11]. Therefore, the significance of glassworts is not only due to their nutritive values but also because they are an excellent source of bioactive compounds that possess potentially important therapeutic effects, such as anticancer, antioxidant, antihyperlipidemic, antidiabetic and antiinflammatory activities [12-15]. Glassworts are used for the treatment of intestinal ailments, nephropathy, cancer, asthma, arthritis, hepatitis, hypertension and hemorrhoids [16,17]. Colorectal cancer,

How to cite this article: Altay A, İrtem Kartal D, Sadi G, Güray T, Yaprak AE. Modulation of mRNA expression and activities of xenobiotic metabolizing enzymes, CYP1A1, CYP1A2, CYP2E1, GPx and GSTP1 by the *Salicornia freitagii* extract in HT-29 human colon cancer cells. Arc Biol Sci. 2017;69(3):.439-48.

which is the third most common cancer type worldwide and the fourth leading cause of cancer-related death, is diagnosed in over one million new cases per year [18]. As most gastrointestinal system cancers are highly linked to dietary habits, they are considered to be preventable [19].

By bioactivating a variety of xenobiotics, including drugs, food additives, industrial solvents and pollutants, to highly reactive and mutagenic metabolites, the cytochrome P450 (CYP) monooxygenase system assumes a key role in toxicity and carcinogenesis [20]. Although the main function of CYP enzymes is detoxification of xenobiotics, many CYP isoforms catalyze the metabolic activation of procarcinogens to their ultimate carcinogenic forms [21]. The induction of some CYP1A isozymes that metabolize a variety of polycyclic aromatic hydrocarbons (PAHs), is mediated by the aryl hydrocarbon receptor (AhR), which is involved in various biological processes [22]. Benzo[a]pyrene (BaP) plays a major role in lung cancer development. Conversion of inactive Benzo[*a*]pyrene (BaP) to the mutagenic metabolite BaP-7,8-diol-9,10-epoxide, and the subsequent formation of DNA adducts mainly with deoxyguanosine is an example of the catalytic activity of CYP1A1 [23]. CYP2E metabolizes many drugs, toxicants and carcinogens, such as acetaminophen, nitrosamines, phenol, benzene, 4-nitrophenol, carbon tetrachloride, chloroform, pyrazole and vinyl chloride [24,25]. The most prominent CYP isoforms expressed in colon tissue are CYP 1A1, 1A2, 2E1 and 3A4 [26].

Phase II drug-metabolizing enzymes convert xenobiotics or active chemical carcinogens to less toxic or inactive metabolites that are readily eliminated from the body [20]. Glutathione S-Transferases (GSTs) are phase II detoxifying enzymes that metabolize electrophilic substrates containing carbon, nitrogen and sulfur atoms by conjugation reaction with the endogenous tripeptide glutathione (GSH), which results in the formation of less reactive and more water soluble products [27]. Some drugs, including cisplatin and carmustine, are substrates of GSTP1 and GSTM1, respectively, and are excreted by conjugation with glutathione [28,29]. The enzyme glutathione peroxidase (GPx), catalyzes the reduction of hydrogen peroxides to water and alcohols at the expense of GSH [30]. It protects the organism from oxidative damage by reducing fatty acid hydroperoxides, H_2O_2, phospholipid hydroperoxides and cholesterol hydroperoxides [31]. It was reported that GPx knockout mice were highly susceptible to oxidative stressors [32]. Cardiovascular diseases and stroke incidences were found to be highly increased in the absence of GPx [33].

In this context, the inhibition of CYP1A1, CYP1A2 and CYP2E1 enzymes and the activation of GSTP1 and GPx enzymes could represent a promising cancer chemoprevention strategy. Therefore, in this study we aimed to determine the major phenolic compounds of *SF*, to examine their antiproliferative effects and to elucidate their effects on several phase I, phase II and antioxidant enzymes in HT-29 cells.

MATERIALS AND METHODS

Chemicals

Compounds for cell culture studies were McCoy's 5A medium with L-glutamine (Lonza, Belgium), sodium pyruvate (Lonza, Belgium), PBS (Lonza, Belgium), dimethyl sulfoxide (Appli Chem, Germany), Trypan blue (Biological Industries, Israel), RIPA buffer (Cell Signaling Technology, USA), nuclease-free water (Hyclone, USA), XTT Cell Proliferation kit (Biological Industries, Israel), cDNA Synthesis Kit (Thermo Scientific, USA), Fast Start Universal SYBR Green ROX (ROCHE, Switzerland). All primers were designed with NCBI BLAST and purchased from Iontek (İstanbul, Turkey). Standard phenolic compounds were purchased from Sigma-Aldrich (USA).

Plant material and extract preparation

Naturally growing samples of the glasswort *SF* were collected from Şereflikoçhisar, Ankara Province, Turkey, in 2014. Voucher specimens are kept in the Laboratory of Seed Plant Systematic, Department of Biology, Ankara University, Turkey. The aerial parts of the plants were dried at room temperature and protected from direct exposure to sunlight. About 30 g of dried leaves were mixed with 250 mL of methanol and shaken overnight. This process was performed three times. To obtain ultra-dry powders, the methanol extract was concentrated under vacuum in a rotary

evaporator (Heidolph, Germany) and then freeze-dried (Christ, Germany). The extracted powder was weighed and stored at -20°C in a brown bottle until further use.

Identification and quantification of phenolic compounds by LC-MS/MS

The methanol extract of *SF* was examined for its phenolic content by liquid chromatography (Agilent 1200 equipped with a micro degasser, autosampler and diode array detector (DAD), connected to an Agilent 6460 triple ion trap mass spectrometer via an electrospray interface). Separation was achieved on a Zorbax SB-C18 column (2.1x50 mm x 1.8 µm) at a flow rate of 0.3 mL/min at 35°C, using solvent A (0.05% formic acid, 5 mM ammonium formate) and solvent B (methanol) as a mobile phase. A gradient system was performed and running time was 13 min. The DAD recorded the spectra from 180 to 800 nm. Standards were prepared in the range of 0.031 to 10 ppm. The extract was dissolved in methanol and filtered (0.22 µm) to obtain a final concentration of 1mg/mL. The injection volume into the LC-MS/MS instrument was 5 µL. Agilent G3793AA MassHunter Optimizer software was used for data evaluation.

Cell culture

HT-29 human colon adenocarcinoma cells were obtained from ATCC (American Type Culture Collection, LGC Promochem, UK). Cancer cells were grown in McCoy's 5A medium containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (Pen Strep) solution. Cultures were incubated at 37°C with 5% CO_2 and 95% humidity. Cell culture studies were performed in a Metisafe Class II Safety Cabinet**.**

Cytotoxicity assay

Antiproliferative activity tests were performed by measuring the cellular metabolic activity by the colorimetric 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H tetrazolium-5-carboxanilide (XTT) assay. Cells were plated at a concentration of $1x10^5$ cells/mL in 96-well culture plates and allowed to adhere overnight at 37°C. After 24 h, the cells were treated for 48 h with different concentrations of extract (20-150 µg/mL), vanillic acid (50-200 µM) and p-coumaric acid (50-400 µM). After the incubation, the antiproliferative effects of the agents were evaluated using the XTT cell proliferation kit (Biological Industries, Israel). Absorbance was measured at 415 nm with a Multiscan GO microplate reader (Thermo Scientific, USA). Controls were treated with DMSO alone (0.2%). The results were expressed as IC_{50} (µg/mL) for the extract and IC_{50} (µM) for the phenolic compounds, and all are presented as means±SEM of three independent experiments, each performed in triplicate.

RNA isolation, cDNA synthesis and quantitative real time qPCR

HT-29 cells ($25x10^4$ cells/mL) were seeded in 6-well plates and incubated for 24 h in a CO_2 incubator at 37°C. The following day, the medium was refreshed and the cells were treated with *SF* extract and its main bioactive components, vanillic and p-coumaric acid, at the concentrations of the determined IC_{50} values and incubated for 48 h. After incubation, isolation of total RNA was performed according to the GeneJet RNA Purification Kit (Thermo Scientific USA). The isolated RNA was quantified by measuring the absorbance at 260 nm and its purity and quality were evaluated by measuring the 260/280 nm ratio. Reverse transcription of RNA to cDNA was performed using the RevertAid™ First Strand cDNA Synthesis Kit (Thermo Scientific USA) according to the manufacturer's protocol. The synthesized cDNA was stored at -80°C until further use.

The effects of *SF* extract, vanillic acid and p-coumaric acid on the expression of selected genes in HT-29 cells were studied by quantitative real-time PCR (qRT-PCR) using Corbett Rotor Gene 6000 (Corbett Life Science, Concorde, NSW, Australia). Ten µL of the final reaction mixture contained 1.6 µL cDNA (50-100 ng), 0.2 µL of 10 µM of the forward primer, 0.2 µL of 10 µM of the reverse primer, 5 µL of Maxima®SYBR Green qPCR Master Mix (Fermentas, Glen, Burnie, MD) and 3 µL of RNase-free distilled water. In order to check for genomic DNA contamination, NTC (no template control) was used during the reaction. As an internal standard, GAPDH was used. The qRT-PCR program consisted of the following cycling profile: ini-

Table 1. Primer pairs used for amplification, with annealing temperatures and sizes of the PCR products.

Gene	Sequences (5′– 3′)	Size of PCR pruduct	
		Temp. (°C)	(base pair)
GAPDH	F→ GAGCGAGATCCCTCCAAAAT	60	197
	R→ GGCTGTTGTCATACTTCTATGG		
CYP1A1	F→ TACCTCAGCAGCCACCTCCAAG	63	121
	R→ GGCCCTGATTACCCAGAATACC		
CYP1A2	F→ ATGCTCAGCCTCGTGAAGAAC	60	96
	R→ GTTAGGCAGGTAGCGAAGGAT		
CYP2E1	F→ AGCGCTGCTGGACTACAAGG	62	184
	R→ CCTCTGGATCCGGCTCTCAT		
GPX-4	F→ GAGGCAAGACCGAAGTAAACTAC	60	100
	R→ CCGAACTGGTTACACGGGAA		
GSTp1	F→ CCTACACCGTGGTCTATTTCCC	60	136
	R→ CAGGAGGCTTTGAGTGAGC		

tial melting at 95°C for 10 min; the amplification and quantification program was repeated 40 times; melting: 95°C for 20 s, annealing: 58-62°C (depending on the gene), as shown in Table 1, for 30 s; extension: 72°C for 20 s. In order to confirm the PCR product, melting curve analysis of the amplification product was carried out at the end of each amplification reaction.

Protein extraction

HT-29 cells ($25x10^4$ cell/ mL) were seeded in tissue culture plates for protein extraction. After 24 h, the cells were treated with the *SF* extract at a concentration corresponding to the IC_{50} (mg/mL) value and incubated for 48 h. Protein extraction was performed with 400 µL of 1X RIPA buffer containing1 mM phenylmethanesulfonylfluoride (PMSF). The cells were scraped and lysed by sonication for 5 min and centrifuged at 14000*x*g for 10 min. The collected supernatant was stored at -80°C until further use. The protein concentration was determined according to the Lowry method [34].

Measurement of enzyme activities

GST activity was determined by monitoring thioether formation between 1-chloro-2,4-dinitrobenzene (CDNB), which served as the substrate, and GSH as a cofactor, at 340 nm [35]. Briefly, to a single well of a 96-well plate, 162.5 µL dH_2O, 50 µL 500 mM phosphate buffer pH 7.4, 10 µL 25 mM GSH and 15 µL enzyme source (20-50 µg protein) were added. The reaction was started after the addition of 12.5 µL of 20 mM CDNB and measured for 5 min. GST activity was expressed as nmol/min *per* mg protein; the extinction coefficient was 6.29 mM^{-1} cm^{-1}.

GPx activity was measured according to [36], with some modifications. In a single well of a 96-well plate, 105 µL of 0.1 M Tris-HCl buffer (pH:8.0), 25 µL of 3 mM GSH, 25 µL of 0.5 unit/mL glutathione reductase (GR) and 20 µL of the cytosolic fraction were added and incubated for 3 min. The reaction was started after the addition of 25 µL H_2O_2. Reactions were measured for each well every 10 s for 4 min at 240 nm with a Multiskan™ GO spectrophotometer (Thermo Scientific, USA). GPx activity was expressed as nmol/min *per* mg protein; the extinction coefficient was 0.00373 mM^{-1} cm^{-1}.

Statistical analysis

Statistical analyses were performed by Student's t-test using the GraphPad Prism ver. 6 statistical software package for Windows. All results were expressed as means with their standard deviation (SD); $p<0.05$ was chosen as the minimum level for significance.

RESULTS

Characterization of *SF* extract by LC-MS/MS

Fig. 1A shows the chromatogram used to identify and quantify ten phenolic compounds, namely: vanillic acid, p-coumaric acid, caffeic acid, gallic acid, syringic acid, chlorogenic acid, rosmarinic acid, (-)-epicatechin (EC), epigallocatechin gallate (EGCG) and rutin trihydrate in the *SF* methanolic extract. LC-MS/MS analysis revealed that the *SF* methanol extract contained high concentrations of vanillic acid (48 µg/100 g extract) and p-coumaric acid (10.8 µg/100g extract), which were therefore by far the predominant phenolic compounds of all of the phenolics (Fig. 1B). All other phenolics were below the limit of quantification.

Cytotoxic activities

The cell-growth inhibitory effects of the *SF* methanol extract and its bioactive components on HT-29 human adenocarcinoma cell lines were investigated by

the XTT assay. This has not been examined previously. The findings from the present study clearly demonstrate that the extract, vanillic acid and p-coumaric acid can inhibit the proliferation of human cancer cells in a time- and concentration-dependent manner (Fig. 2). The XTT assays revealed the absence of any antiproliferative effects of the *SF* extract on HT-29 cells up to 24 h incubation. On the other hand, a 48-h treatment showed that proliferation of the cells decreased critically in the presence of the *SF* extract in the range of 25 to 200 μg/mL. At 200 μg/mL, the extract displayed maximum antiproliferative activity, observed as a significant decrease in cell viability. Additionally, vanillic acid and p-coumaric acid exhibited their cytotoxic activities against HT-29 cancer cells in the range of 25 to 500 μM. The IC_{50} values of *SF* extract, vanillic and p-coumaric acid were determined as 81.79 μg/mL, 98.8 μM and 221.6 μM, respectively.

The effects of the *SF* extract, vanillic acid and p-coumaric acid on different CYP isozyme and antioxidant enzyme mRNA expression in HT-29 cells

The presented data indicates that treatment of HT-29 cells with the methanolic extract of *SF* and its major bioactive phenolic compounds, vanillic acid and p-coumaric acid, caused remarkable changes in the CYP1A1, CYP1A2, CYP2E1, GPx and GSTP1 mRNA expression (Figs. 3 and 4). As can be seen in Fig. 3, after a 48-h incubation, CYP1A2 (Fig. 3B) and CYP2E1 (Fig. 3C) mRNA expression decreased 4.75- and 1.66-fold, respectively ($p<0.0001$), whereas CYP1A1 mRNA expression increased 1.3-fold as compared to the control ($p<0.005$) (Fig. 3A). Vanillic acid, the major phenolic content of the *SF* extract, lowered CYP1A1 (Fig. 3A), CYP1A2 (Fig. 3B) and CYP2E1 (Fig. 3C) mRNA expression 1.33-, 2.49- and

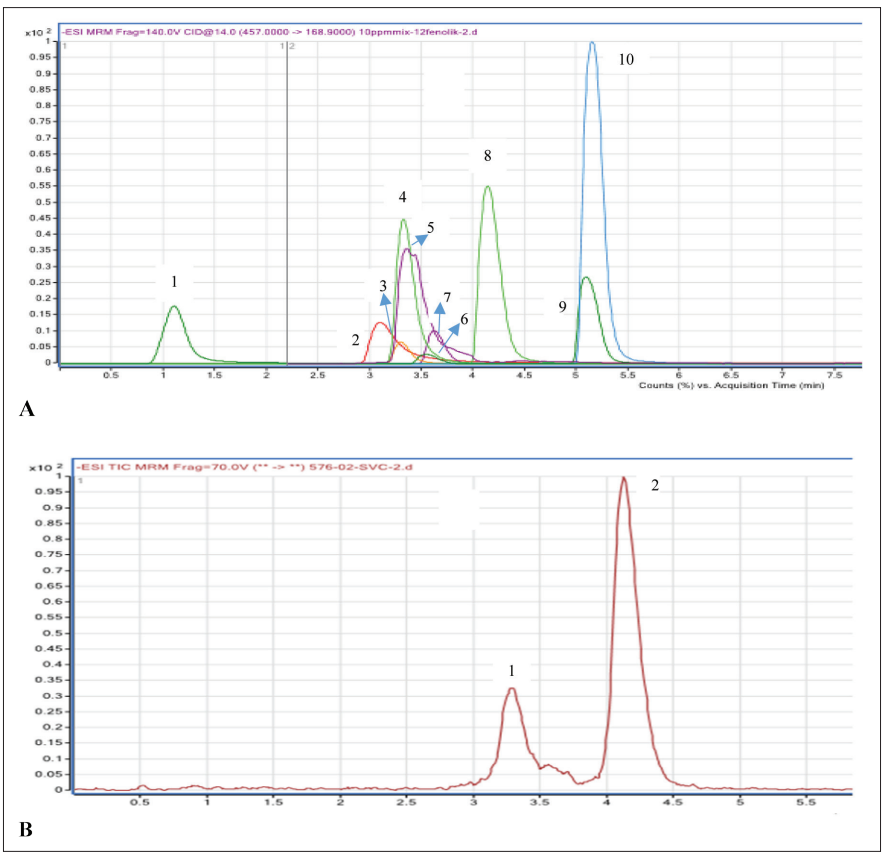

Fig. 1. LC-MS/MS chromatograms. **A** – standard polyphenols: 1 – gallic acid; 2 – chlorogenic acid; 3 – vanillic acid; 4 – caffeic acid; 5 – epigallocatechin gallate; 6 – epicatechin; 7 – syringic acid; 8 – p-coumaric acid; 9 – rosmarinic acid; 10 – rutin trihydrate. **B** – *SF* methanol extract: 1 – vanillic acid; 2 – p-coumaric acid.

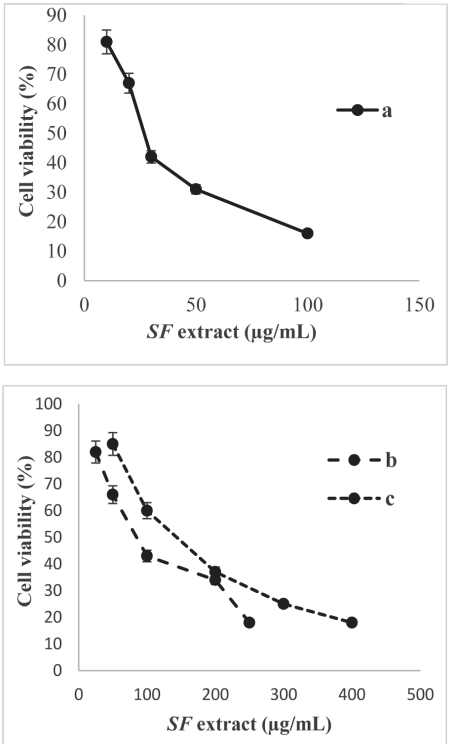

Fig. 2. Concentration-dependent cytotoxic effects against HT-29 cell lines after 48 h of treatment. *SF* extract (a); vanillic acid (b); p-coumaric acid (c). Each point represents the average of three independent measurements, each done in triplicate, with the means±SD.

1.62-fold, respectively (p<0.005). As for p-coumaric acid, it did not exhibit any remarkable effect on CYP1A, CYP1A2 and CYP2E1 mRNA expression compared to the control group (p>0.05) (Fig. 3A, B, C, respectively). Moreover, incubation of HT-29 cells with the *SF* extract for 48 h increased GPx (Fig. 4A) and GSTP1 (Fig. 4B) mRNA expression 1.57- and 1.3-fold, respectively (p<0.0005), compared to the control group. Vanillic acid increased GPx (Fig. 4A) and GST (Fig. 4B) mRNA expression 1.37- and 1.26-fold, respectively (p<0.005). On the other hand, p-coumaric acid did not exhibit any considerable effect on GPx mRNA expression (Fig. 4A), but increased GSTP1 mRNA expression (Fig. 4B) 1.25-fold in comparison to the control (p<0.005).

Effects of *SF* extract on enzyme activities of GPx and GSTP1 in HT-29 Cells

Fig. 5 shows the effects of the *SF* extract on GPx (Fig. 5A) and GSTP1 (Fig. 5B) enzyme activities in HT-29 cells. The *SF* extract at the concentration corresponding to IC_{50} increased cytosolic GPx activity 1.68-fold (p<0.005). The *SF* extract also increased the activity of cytosolic GSTP1 1.49-fold (p<0.005).

DISCUSSION

In recent years, traditional medicine such as herbal remedies or dietary supplements, have gained increasing popularity, encouraging investigators to examine the actions of phytochemicals on xenobiotic metabolism and their antioxidant, antitumor, anticarcinogenic and antimutagenic effects. The major phenolic compounds in *SF* extracts have been reported to be

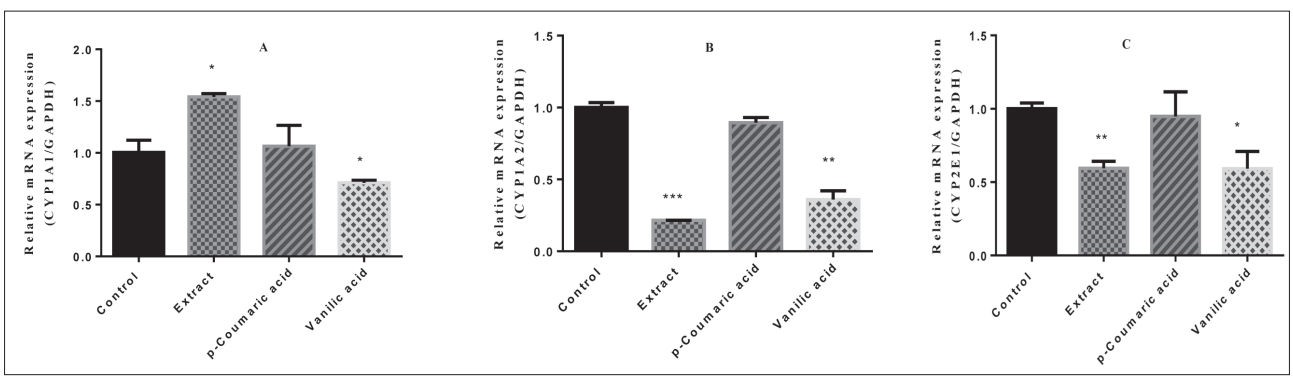

Fig. 3. Effects of the *SF* extract, vanillic and p-coumaric acid treatments for 48 h on CYP1A1 (**A**) (p<0.005), CYP1A2 (**B**) (p<0.0001) and CYP2E1 (**C**) (p<0.005) mRNA expression in HT-29 cells. Alterations in mRNA expression were analyzed by qRT-PCR. Results are presented as the mean from three independent experiments (n≥3) and are expressed as relative means±SD. Effects of agents on mRNA levels of the tested genes were normalized to housekeeping GAPDH mRNA. Fold-inhibition was calculated using the following formula: $2^{-\Delta\Delta Ct}$, where ΔΔCt=ΔCt(treated)−ΔCt(control); ΔCt(treated)=ΔCt(CYPs)−ΔCt(GAPDH); ΔCt(control)=ΔCt(CYPs)−ΔCt(GAPDH).

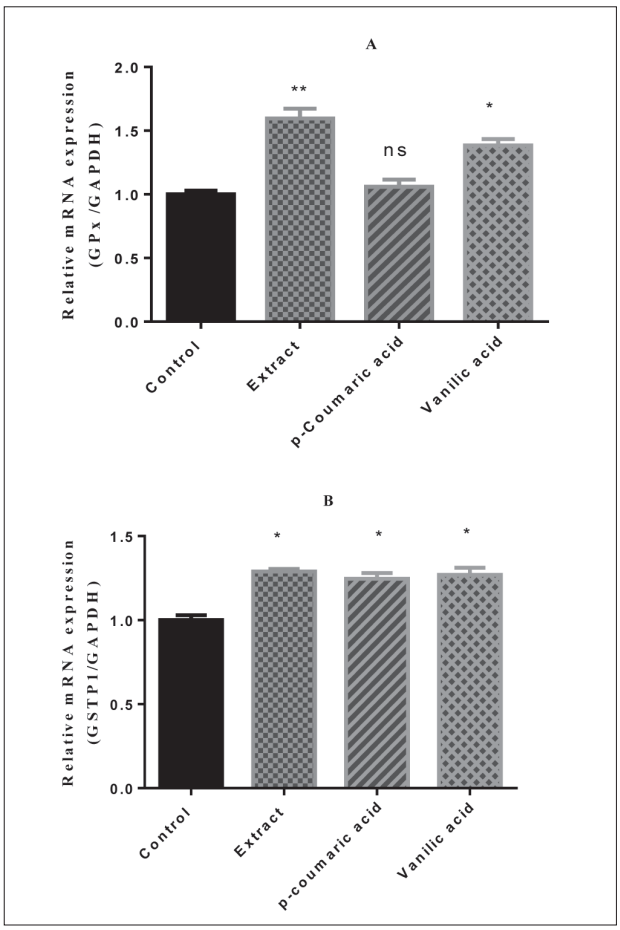

Fig. 4. Effects of *SF* extract, vanillic and p-coumaric acid treatments for 48 h on GPx ($p<0.0005$) (**A**) and GSTP1 ($p<0.0005$) (**B**) mRNA expression in HT-29 cells. Alterations in mRNA expression were analyzed by qRT-PCR. Results are presented as the mean from three independent experiments ($n\geq3$) and expressed as relative means±SD. Effects of agents on mRNA levels of the tested genes were normalized to housekeeping GAPDH mRNA. Fold-inhibition was calculated using the following formula: $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct=\Delta Ct(treated)-\Delta Ct(control)$; $\Delta Ct(treated)=\Delta Ct(CYPs)-\Delta Ct(GAPDH)$; $\Delta Ct(control)=\Delta Ct(CYPs)-\Delta Ct(GAPDH)$.

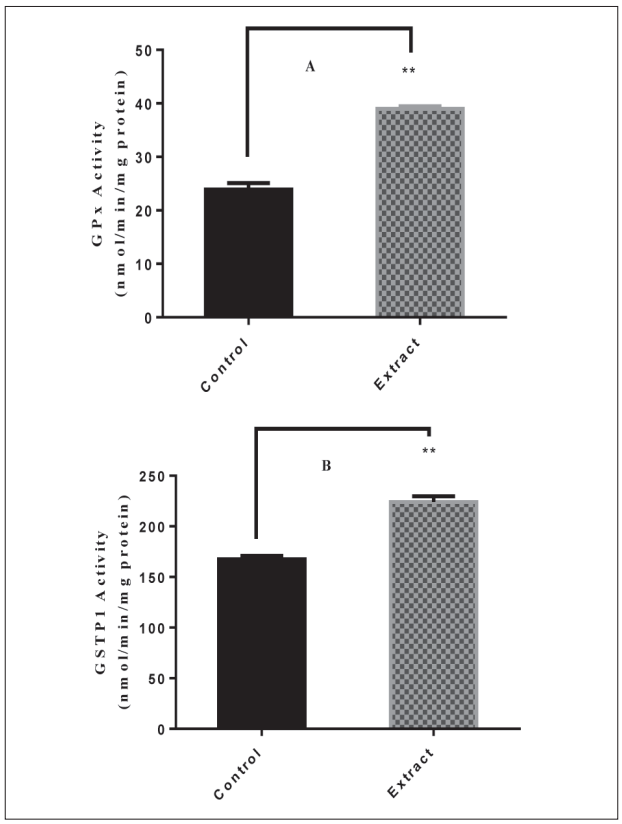

Fig. 5. Effects of *SF* extract on the activities of antioxidant enzymes in HT-29 cells. **A** – GPx; **B** – GSTP1. Enzyme activities were determined as described in the Materials and Methods section. Values are means±SD for triplicate determinations ($n\geq3$). Significantly different from the control by two-tailed Student's t-test – *$p<0.05$, ** $p<0.001$.

vanillic acid, p-coumaric acid, ferulic acid, caffeic acid, kaempferol and galangin [37]. Our *SF* methanol extract showed close similarities with the literature, displaying high amounts of vanillic and p-coumaric acid.

It has been reported that some CYP1A and CYP2E enzymes are expressed at high levels in extrahepatic tissues, such as the colon and intestine, and deemed to be responsible for the formation of unique extrahepatic metabolites and resulting tissue-specific consequences in cellular toxicity and organ pathology [26]. The expression of CYPs, phase II and antioxidant enzymes is controlled at the transcriptional and translational levels. The mRNA levels of CYP genes were shown to be affected by the expression of micro RNAs [38,39]. To examine aspects of the control mechanism of these enzymes after exposure to *SF* extract and its major phenolic compound vanillic acid at the transcriptional and translational levels, we examined changes in mRNA expression and enzyme activities.

CYP1A1and CYP1A2 are the principal members of the CYP1A family that are induced by PAHs and are also involved in the metabolic activation of PAHs and heterocyclic amines [40]. The metabolism of PAHs by members of the CYP1A family generates reactive products that irreversibly bind to protein and DNA, producing toxic and carcinogenic events [41]. It was reported that certain phenolic compounds/flavonoids have roles in reducing cancer susceptibility,

especially by preventing the induction of CYP1A isozymes [42,43]. In light of the role of CYP1A in the formation of reactive products, the observed inhibition of CYP1A mRNA expression by the *SF* extract and its major phenolic component vanillic acid could lead to the reduction or prevention of the onset of many diseases, including cancer. In some cases, the modulation of CYPs has been attributed to other minor bioactive phenolic compounds and/or their synergistic effects in the extract [44]. Our results demonstrated that the induction of CYP1A mRNA expression by the *SF* extract can be attributed to this event since vanillic acid, the major phenolic compound of the extract, suppressed CYP1A mRNA expression. Xenobiotic responsive elements (XRE) are located in the promoter regions of xenobiotic responsive genes encoding for some CYP isozymes. The expression of these genes can be regulated through the aryl hydrocarbon receptor (AhR), which is a cytosolic protein that can be activated by PAH. The activated AhR then translocates to the nucleus and dimerizes with the AhR nuclear translocator (ARNT) and finally interacts with XRE [45]. The induction of CYP1A isozymes by phenolic compounds occurs through various mechanisms, including direct stimulation of gene expression via specific receptors [46]. Some flavonoids induce CYPs by binding to AhR by mimicking 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) [47]. Although a variety of flavonoids are dietary ligands of the AhR, they can exert different effects on CYP expression [48]. Suppression of CYP isozymes by the *SF* extract and vanillic acid could be attributed to their binding to the AhR by blocking the binding of other AhR ligands, such as TCDD. In this context, the *SF* extract and vanillic acid behave as antagonists of AhR. The reason why p-coumaric acid did not have any considerable effect on CYP450 enzymes may be due to its differential structure (it does not optimally fit into the binding site on AhR) from vanillic acid.

Similarly, CYP2E1 has a wide range of exogenous substrates, such as industrial solvents, protoxins and procarcinogens, and some drugs such as acetaminophen, chlorzoxazone and methoxyflurane are metabolized by CYP2E1 [49]. Thus, inhibition of CYP2E1 can prevent the conversion of such procarcinogens to their carcinogenic forms such as acrylamide, nitrosamine, phenol and benzene. Consequently, in the present study, the inhibitory mechanisms of the *SF* extract and its major phenolic component vanillic acid on CYP2E1 might be regarded as a protective effect due to the potential suppression of tumor formation, induced by PAHs, drugs and other carcinogens.

Flavonoids in dietary foods were shown to be effective in the induction of many phase II enzymes, including GPx, GSTs and NQO1 [50,51]. The importance of GST in chemical carcinogen inactivation was reported in a study on mice deficient in transcription factor Nrf2, which is required for Phase II enzyme induction [52]. In our study, the *SF* extract and its major phenolic compounds, vanillic acid and p-coumaric acid, induced the GSTP1 mRNA expression. It seems that both phenolic compounds had a major impact on the modulation of GSTP1 enzyme. Therefore, the induction of GST by the vanillic acid- and p-coumaric acid-rich *SF* extract could help eliminate toxic metabolites from cells and would therefore have an important role in early defense against carcinogenesis.

GPx has been long been known to play a role in the first step of defense against reactive oxygen species (ROS). Increased levels of GPx activity could enhance the resistance against ROS. It was reported that GPx1-overexpressing mice were more resistant to paraquat-induced lethality than GPx1 knockout mice [53]. Furthermore, increased levels of GPx have been shown to have a protective role in cardiovascular diseases, as ROS cause significant changes in vascular tone and structure [54]. The *SF* extract and vanillic acid increased the expression of GPx mRNA, while p-coumaric acid did not. Therefore, the combined induction of GPx and GSTP1 by the *SF* extract and/or vanillic acid could represent a remarkable enhancement of the defense against the toxicity of various agents.

The examination of enzyme activities showed that the levels of GPx and GSTP1 activities were significantly increased by the *SF* extract. These results demonstrate that the modulation of the investigated enzymes by the *SF* extract occurred at gene and enzyme activity levels. Moreover, cross analyses of gene expression vs. enzyme activity revealed a strict correlation between the mRNA expression levels and corresponding enzyme activities in *SF*-treated and control groups.

CONCLUSIONS

The current state of our knowledge indicates that the selective induction of carcinogen-detoxifying enzymes and suppression of enzymes responsible for xenobiotic activation could be a useful approach in chemoprevention and carcinogenesis inhibition. The present study demonstrated that the *SF* methanol extract and its major bioactive compound, vanillic acid, exert modulatory effects on the expression of the enzymes involved in xenobiotic activation and detoxification pathways. However, necessary precautions should be taken before this plant is consumed in replacement treatments because of its possible interactions with drugs and dietary foods.

Acknowledgments: The authors express their gratitude to Associate Professor Dr. Gülçin Celep (Gazi University) who kindly collected and provided the samples for the study. This work was financially supported by Nezahat Gokyigit Botanical Garden (Research Project No. 60.05.02) in Turkey and METU (Project No. BAP-08-11-DPT-2011K121010) in Turkey.

Conflict of interest disclosure: The authors declare that there are no conflicts of interest.

REFERENCE

1. Mudie P, Greer S, Brakel J, Dickson J, Schinkel C, Peterson-Welsh R. Forensic palynology and ethnobotany of Salicornia species (Chenopodiaceae) in northwest Canada and Alaska. Can J Bot. 2005;83(1):111-23.
2. Chevalier A. Les Salicornes et leur emploi dans l'alimentation: Etude historique, botanique, économique. Rev Bot appliqué d'Agriculture Colon. 1922;2(16):697-785.
3. Geslin M, Verbist J. Flavonoides de Salicornia europaea. J Nat Prod. 1985;48(1):111-3.
4. Anwar F, Bhanger M, Nasir M, Ismail S. Analytical characterization of Salicornia bigelovii seed oil cultivated in Pakistan. J Agric Food Chem. 2002;50(15):4210-4.
5. Lu D, Zhang M, Wang S, Cai J, Zhou X, Zhu C. Nutritional characterization and changes in quality of Salicornia bigelovii Torr. during storage. LWT. Food Sci Technol. 2010;43(3):519-24.
6. Austenfeld F. Nutrient reserves of Salicornia europaea seeds. Physiol Plant. 1986;68(1):446-50.
7. Guil J, Torija M, Giménez J, Rodríguez I. Identification of fatty acids in edible wild plants by gas chromatography. J Chromatogr A. 1996;719(3):229-35.
8. Chung YC, Chun HK, Yang JY, Kim JY, Han EH, Kho YH, Joeng HG. Tungtungmadic acid, a novel antioxidant, from Salicornia herbacea. Arch Pharmacal Res. 2005;28(10):1122-6.
9. Borkowski B, Drost K. Alkaloide aus Salicornia herbacea L. Pharmazie. 1965;20(38):390-3.
10. Chiji H, Aiba T, M I. Isolation and identification of two 2,3-unsubstituted chromones from glasswort (Salicornia europaea L.). Agric Biol Chem. 1978;4(1):159-65.
11. Arakawa Y, Chiji H, Izawa M. Structural elucidation of two new chromones isolated from Glasswort (Salicornia europaea L.). Agric Biol Chem. 1983;47(1):2029-33.
12. Kong CS, Kim YA, Kim MM, Park JS, Kim JA, Kim SK, Lee BJ, Nam TJ, Seo Y. Flavonoid glycosides isolated from Salicornia herbacea inhibit matrix metalloproteinase in HT1080 cells. Toxicol Vitr. 2008;22(7):1742-8.
13. Kang S, Kim D, Lee BH, Kim MR, Hong J, Chiang M. Antioxidant properties and cytotoxic effects of fractions from glasswort (Salicornia herbacea) seed extracts on human intestinal cells. Food Sci Biotechnol. 2011;20(1):115-22.
14. Bang MA, Kim HA, Cho YJ. Hypoglycemic and antioxidant effect of dietary hamcho powder in streptozotocin-induced diabetic rats. J Korean Soc Food Sci Nutr. 2002;31:840-6.
15. Lee YS, Lee SH, Kim BK, Oguchi K, H SK. Inhibitory effects of isorhamnetin-3-O-β-D-glucoside from Salicornia herbacea on rat lens aldose reductase and sorbitol accumulation in streptozotocin-induced diabetic rat tissues. Biol Pharm Bull. 2005;28(5):916-8.
16. Rhee M, Park H. Salicornia herbacea: botanical, chemical and pharmacological review of halophyte marsh plant. J Med Plants. 2009;3(8):548-55.
17. Han SK, Kim SM, Pyo BS. Antioxidative effect of glasswort (Salicornia herbacea L.) on the lipid oxidation of pork. Korean J Food Sci Anim Resour. 2003;23(38):46-9.
18. Brenner H, Kloor M, Pox CP. Colorectal cancer. Lancet Oncol. 2014;383(9):1490-502.
19. Anand P, Kunnumakara AB, Sundaram C, Kuzhuvelil B, Harikumar KB, Tharakan ST, Lai OS, Sung B, Bharat B, Aggarwal BB. Cancer is a preventable disease that requires major lifestyle changes. Pharm Res. 2008;25(9):9661-9.
20. Lee SB, Cha KH, Selenge D, Solongo A, Nho CW. The Chemopreventive Effect of Taxifolin Is Exerted through ARE-Dependent Gene Regulation. Bioll. Pharm Bull. 2007;30(6):1074-9.
21. Guengerich FP, Shimada T. Oxidation of toxic and carcinogenicchemicals by human cytochrome P-450 enzymes. Chem Res Toxicol. 1991;4(4):391-407.
22. Androutsopoulos V, Tsatsakis A, Spandidos D. Cytochrome P450 CYP1A1: wider roles in cancer progression and prevention. BMC Cancer. 2009;9(187).
23. Hecht S. Tobacco smoke carcinogens and lung cancer. J Nat Cancer Institue. 1999;91(14):1194-210.
24. Arinc E, Adali O, Gencler-Ozkan A. Induction of N-nitrosodimethylamine metabolism in liver and lung by in vivo pyridine treatments of rabbits. Arch Toxicol. 2000;74(6):329-34.
25. Arinc E, Arslan S, Bozcaarmutlu A, Adali O. Effects of diabetes on rabbit kidney and lung CYP2E1 and CYP2B4 expression and drug metabolism and potentiation of carcinogenic activity of N-nitrosodimethylamine in kidney and lung. Food Chem Toxicol Toxicol. 2007;45(1):107-18.
26. Ding X, Kaminsky LS. Human Extrahepatıc Cytochromes P450: Function in Xenobiotic Metabolism and Tissue-Selec-

tive Chemical Toxicity in the Respiratory and Gastrointestinal Tracts. Annu Rev Pharmacol Toxicol. 2002;43(1):149-73.
27. Bousova I, Skalova L. Inhibition and induction of glutathione S-transferases by flavonoids: possible pharmacological and toxicological consequences. Drug Metab Rev. 2012;44(4):267-86.
28. Ban N. Transfection of glutathione S-transferase (GST)-π antisense complementary DNA increases the sensitivity of a colon cancer cell line to adriamycin, cisplatin, melphalan, and etoposide. Cancer Res. 1996;56(15):3577-82.
29. Smith M, Evans C, Doane-Setzer P. Denitrosation of 1, 3-bis (2-chloroethyl)-1-nitrosourea by class mu glutathione transferases and its role in cellular resistance in rat brain tumor cells. Cancer Res. 1989;49(10):2621-5.
30. Ursini F. Diversity of glutathione peroxidases. Methods Enzym. 1995;252(4):38-53.
31. Imai H, Narashima K, Arai M. Suppression of leukotriene formation in RBL-2H3 cells that overexpressed phospholipid hydroperoxide glutathione peroxidase. J Biol Chem. 1998;273(4):1990-7.
32. Haan JB, Bladier C, Griffiths P, Kelner M, Ross D, Cheung NS, Bronson RT, Silvestro MJ, Wield S, Zheng SS, Beart PS, Hertzog PJ, Kola I. Mice with a Homozygous Null Mutation for the Most Abundant Glutathione Peroxidase, Gpx1, Show Increased Susceptibility to the Oxidative Stress-inducing Agents Paraquat and Hydrogen Peroxide. J Biol Chem. 1998;273(35):22528-36.
33. Freedman JE, Loscalzo J, Benoit SE. Decreased platelet inhibition by nitric oxide in two brothers with a history of arterial thrombosis. J Clin Invest. 1996;97(4):979-87.
34. Lowry O, Rosebrough NJ, Farr AL, Randall RJ. Protein Measurement with the Folin Phenol Reagent. J Biol Chem. 1951;193(1):265-75.
35. Habig WH, Pabst MJ, Jakoby WB. Glutathione Stransferase: the first enzymatic step in mercapturic acid formation. J Biol Chem. 1974;249(22):7130-39.
36. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Lab Clin Med. 1967;70(1):158-69.
37. Bertin RL, Gonzaga VG, Borges GSC, Azevedo MS, Maltez HF, Heller M. Nutrient composition and, identification/quantification of major phenolic compounds in Sarcocornia ambigua (Amaranthaceae) using HPLC-ESI-MS/MS. Food Res Int. 2014;55(1):404-11.
38. Choi YM, An S, Lee EM. CYP1A1 is a target of miR-892amediated post-transcriptional repression. Int J Oncol. 2012;41(1):331-36.
39. Mohri T, Nakajima M, Fukami T. Human CYP2E1 is regulated by miR-378. Biochem Pharmacol. 2010;79(7):1045-52.
40. McManus ME, Burgess WM, Veronese ME. Metabolism of 2 acetylaminofluorene and benzo (A) pyrene and activation of foodderived heterocyclic amine mutagens by human cytochromes P-450. Cancer Res. 1990;50(11):3367-76.
41. Miller EC, Miller JA. Mechanisms of chemical carcinogenesis: Nature of proximate carcinogens and interactions with macromolecules. Pharmacol Rev. 1966;18(1):805-38.
42. Arinç E, Yilmaz D, Bozcaarmutlu A. Mechanism of inhibition of CYP1A1 and glutathione S-transferase activities in fish liver by quercetin, resveratrol, naringenin, hesperidin, and rutin. Nutr Cancer. 2015;67(1):137-44.
43. Karakurt S, Semiz A, Celik G, Gencler-Ozkan AM, Sen A, Adali O. Epilobium hirsutum alters xenobiotic metabolizing CYP1A1, CYP2E1, NQO1 and GPx activities, mRNA and protein levels in rats. Pharm Biol. 2013;51(5):650-8.
44. Suganuma M, Okabe S, Kai Y, Sueoka N, Sueoka E, Fujiki H. Synergistic effects of (−)-epigallocatechin gallate with (−)-epicatechin, sulindac, or tamoxifen on cancer-preventiveactivity in the human lung cancer cellline PC-9. Cancer Res. 1999;59:(1):44-7.
45. Kronenberg S, Esser C, Carlberg C. An aryl hydrocarbon receptor conformation acts as the functional core of nuclear dioxin signaling. Nucleic Acids Res. 2000;28(12):2286-91.
46. Lin J, Lu A. Inhibition and induction of cytochrome P450 and the clinical implications. Clin Pharmacokinet. 1998;35(5):361-90.
47. Kohn MC, Walker NJ, Kim AH, Portier CJ. Physiological modeling of a proposed mechanism of enzyme induction by TCDD. Toxicology. 2001;162(3): 193-208.
48. Ciolino H, Daschner P, Yeh G. Dietary flavonols quercetin and kaempferol are ligands of the aryl hydrocarbon receptor that affect CYP1A1 transcription differentially. J Biochem. 1999;340(3):715-22.
49. Gonzalez FJ. Role of cytochromes P450 in chemical toxicity and oxidative stress: studies with CYP2E1. Mutat Res. 2005;56(1):101-10.
50. Karakurt S, Semiz A, Celik G, Gencler-Ozkan A, Sen A, Adali O. Contribution of ellagic acid on the antioxidant potential of medicinal plant Epilobium hirsutum. Nutr Cancer. 2016;68(1):173-83.
51. Karakurt S, Adali O. Tannic Acid Inhibits Proliferation, Migration, Invasion of Prostate Cancer and Modulates Drug Metabolizing and Antioxidant Enzymes. Anticancer Agents Med Chem. 2016;16(6):781-9.
52. Na HY, Surh YJ. Modulation of Nrf2-mediated antioxidant and detoxifying enzyme induction by the green tea polyphenol EGCG. Food Chem Toxicol. 2008;46(4):1271-8.
53. Cheng WH, Ho YS, Valentine BA. Cellular glutathione peroxidase is the mediator of body selenium to protect against paraquat lethality in transgenic mice. J Nutr. 1998;128(7):1070-6.
54. Battin EE, Brumaghim JL. Antioxidant activity of sulfur and selenium: A review of reactive oxygen species scavenging, glutathione peroxidase, and metal-binding antioxidant mechanisms. Cell Biochem Biophys. 2009;55(1):1-23.