International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.7, No.4, pp 606-615, 2014-2015

Dietary tomatine inhibits gene expression alterations and DNA damage induced by potassium bromate in male rats

Sabah A. A. Linjawi[1*] and Hanaa M. Roshdy[2]

[1]Department of Biology, College of Science, King Abdulaziz University, Jeddah, Saudi Arabia
[2]Cell Biology Department, National Research Center, 12622 Dokki, Giza, Egypt

Abstract: Tomatine provides defence against pathogenic fungi, bacteria, viruses and herbivores. Therefore, evaluation the potential protective effects of dietary tomatine against gene expression alterations, oxidative DNA damage and suppression of antioxidant enzyme induced by potassium bromate- (KBrO3-) mediated oxidative stress in rats was studied. The effects of tomatine on gene expression alterations and DNA damage induced by KBrO3 were evaluated by quantitative Real Time-PCR and DNA laddering assay in liver cells. The effects of tomatine on the activities of GPx in liver cells were determined in male rats treated with KBrO3. Endogenous antioxidant status, namely, the activities GPx and the levels of GST-mRNA were significantly decreased in the liver tissues of the KBrO3-treated rats, while the pretreatment of tomatine prevented the decreases of these parameters induced by KBrO3 treatment. Moreover, the pretreatment of tomatine was also able to prevent KBrO3-induced increases in the expression levels of Hsp70a and CYP450 genes as well as DAN fragmentation in the liver tissues of male rats. *Conclusion*: The current results suggested that tomatine might act as a dietary protective agent with antioxidant properties offering effective protection against gene expression changes, oxidative DNA damage in a concentration-dependent manner *in vivo*.

Keywords: Tomatine, KBrO3, Gene expression, DNA damage, rats.

Introduction

Cells that live in an oxygen-rich environment are inundated with various endogenous and exogenous sources of reactive oxygen species (ROS) [1]. The most important target for ROS in the carcinogenesis process is DNA [2, 3]. Irreparable DNA damage is involved in carcinogenesis, aging, and other degenerative diseases [1, 4]. However, enzymatic and nonenzymatic systems, which preserve the oxidant/antioxidant status, are disrupted during oxidative stress, a metabolic derangement due to an imbalance caused by excessive generation of ROS or a diminished capacity of the antioxidant defense system. Dietary factors and natural antioxidants that reduce the impact of ROS can protect DNA damage and thus reduce the risk of cancers [5, 6].

Potassium bromated (KBrO3) is commonly known to induce oxidative damage [7-9]. KBrO3 is a widely used food additive, a water disinfection by-product, and a known nephrotoxic agent. Cellular proliferation was enhanced in the kidney due to oxidative stress generated by KBrO3. It has also been reported that KBrO3 increased the levels of 8-hydroxydeoxyguanosine (8- OHdG), an oxidative DNA adduct, suggesting that it can indirectly induce DNA modifications by oxygen radicals that are involved in carcinogenesis [6, 10].

Plants have played significant roles in maintaining human health and improving the quality of human life for thousands of years [11, 12]. α−Tomatine is the major glycoalkaloid of tomato (*Solanum lycopersicum*

L., syn.: *Lycopersicon esculentum* Mill.). The molecule is composed of an aglycone core, tomatidine, (22*S*, 25*S*)-5α-spirosolan-3β-ol, which is attached with its 3-OH group to the saccharide moiety called lycotetraose. In plants, α−Tomatine may provide defence against pathogenic fungi, bacteria, viruses and herbivores [13]. This effect corresponds with the content of the agent and changes throughout the lifespan of the tomato plant; e.g. during tomato fruit maturation, the levels of α−Tomatine decrease considerably, which, among other factors, brings about a reduction in the bitter flavor [14].

In mammals, a wide variety of health-promoting properties of α−Tomatine have been reported, including the lowering of plasma concentrations of LDL and triacylglycerols [15] and cardiotonic [13] and antiviral activity [16]. α−Tomatine may also contribute to the stimulation of antigen-specific humoral and cellular immune response, including the augmentation of anticancer defence [17]. Friedman et al. [18] reported the chemopreventive effect of α−Tomatine against liver and stomach cancer in rainbow trout (*Oncorhynchus mykiss* Walb.). Lee et al. [19] showed the anti-proliferative effect of TOM ($c \sim 1$ μg/L) against human colon and liver cell lines, which was more powerful than that of some classical anticancer agents such as doxorubicin and camptothecin.

On investigating the mechanism of action, it was found that TOM affects important signaling pathways responsible for the regulation of cell proliferation, differentiation, and migration [20, 21]. Shih et al. [21] found TOM ($c \leq 2$ μmol/L) to suppress invasion and migration in a human lung adenocarcinoma cell line. It also inhibited the phosphorylation of Akt (protein kinase B), a serine-threonine kinase which regulates the function of many cellular proteins involved in metastasis and the proliferation of cancer cells [22], and the phosphorylation of extracellular signal-regulated kinase 1 and 2 (ERK 1,2). The ERK (extracellular regulated kinase) pathway often plays a role in oncogenic transformation and in the regulation of metalloproteinases and urokinase expression, i.e. of the enzymes which degrade the extracellular matrix and help cancer cells invade tissues and metastasize [23, 24]. Shih et al. [21] detected lower activities of metalloproteinases and urokinase in cells incubated with TOM. TOM also inhibited the activation of focal adhesion kinase, the phosphatidylinositol 3-kinase (PI3K)/Akt signal pathway, and NF-κB (nuclear factor kappa B) in cancer cells [25, 26]. In leukemic cells, TOM induced apoptosis, which was caspase-independent and associated with the down-regulation of survivin expression and the translocation of AIF (apoptosis inducing factor) into the nucleus [27]. In our recent study on MOLT-4 cells, too, TOM induced caspase-independent cell death associated with an increase in p53 and in the mitochondrial protein PUMA. The inhibition of proliferation by alpha-tomatine was linked with an increase in p21WAF1/ CIP1 level and activation of the checkpoint kinase 2 (Chk2) [28].

Therefore, evaluation the protective role of several concentrations of tomatine against KBrO3 induced alteration in the gene expression and DNA fragmentaion in male rats was studied.

Material and Methods

1- Drugs and Reagents

Tomatine and Potassium bromate ($KBrO_3$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Trizol was bought from Invitrogen (Carlsbad, CA, USA). The reverse transcription and PCR kits were obtained from Fermentas (Glen Burnie, MD, USA). SYBR Green Mix was purchased from Stratagene (La Jolla, CA, USA).

2- Experimental Animals

Eighty adult albino male rats (100-120 g, purchased from the Animal House Colony, Giza, Egypt) were maintained on standard laboratory diet (protein, 16.04%; fat, 3.63%; fiber, 4.1%; and metabolic energy, 0.012 MJ) and water *ad libitum* at the Animal House Laboratory, National Research Center, Dokki, Giza, Egypt. After an acclimation period of 1 week, animals were divided into groups (10 mice/ group) and housed individually in filter-top polycarbonate cages, housed in a temperature-controlled (23 ± 1°C) and artificially illuminated (12 h dark/light cycle) room free from any source of chemical contamination. All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee of National Research Center, Egypt.

3- Experimental Design

The male rats were randomly allocated in 10 groups (n = 10 per group) and treated for 8 week as follows: Group 1, control group: animals were treated intragastrically with solvent vehicle control (NaCl).

Groups 2-4: animals were fed on tomatine, where tomatine over a range of doses (500, 1000, and 2000 ppm, respectively) was added to the oil component of the diet and fed daily for 8 weeks [29] [Friedmanet al., 2007]. Group 5, animals were treated intraperitoneal (i.p.) injection of $KBrO_3$ at a dose of 125mg/kg bodyweight [30] [Ke et al., 2013]. Groups 6-8, animals were injected with $KBrO_3$ as in group 5 and then fed on tomatine as in groups 2-4, respectively.

4- Tissue Collection

At 24 h after the last injection, rats in each group were sacrificed by decapitation after anesthetized. The liver tissues were collected on ice bath in order to investigate the total RNA (for the determination Hsp70a, CYP450 and GST-mRNA) and GPx activity. Liver cells were collected to investigate the DNA (for determination DNA fragmentation levels).

5- Gene Expression Analysis

5.1 Isolation of Total RNA

TRIzol® Reagent (Invitrogen, Germany) was used to extract total RNA from liver tissues of male rats according to the manufacturer's instructions with minor modifications. Isolated total RNA was treated with one unit of RQ1 RNAse-free DNAse (Invitrogen, Germany) to digest DNA residues, re-suspended in DEPC-treated water and quantified photospectrometrically at 260 nm. Purity of total RNA was assessed by the 260/280 nm ratio which was between 1.8 and 2.1. Additionally, integrity was assured with ethidium bromide-stain analysis of 28S and 18S bands by formaldehyde-containing agarose gel electrophoresis [31]. Aliquots were used immediately for reverse transcription (RT), otherwise they were stored at -80°C.

5.2 Reverse Transcription (RT) Reaction

Complete Poly(A)$^+$ RNA isolated from liver tissues was reverse transcribed into cDNA in a total volume of 20 µl using RevertAid^{TM} First Strand cDNA Synthesis Kit (Fermentas, Germany). An amount of total RNA (5 µg) was used with a master mix. The master mix was consisted of 50 mM $MgCl_2$, 10x RT buffer (50 mM KCl; 10 mM Tris-HCl; pH 8.3), 10 mM of each dNTP, 50 µM oligo-dT primer, 20 IU ribonuclease inhibitor (50 kDa recombinant enzyme to inhibit RNase activity) and 50 IU MuLV reverse transcriptase. The mixture of each sample was centrifuged for 30 sec at 1000 g and transferred to the thermocycler. The RT reaction was carried out at 25°C for 10 min, followed by 1 h at 42°C, and finished with a denaturation step at 99°C for 5 min [32]. Afterwards the reaction tubes containing RT preparations were flash-cooled in an ice chamber until being used for cDNA amplification through quantitative Real Time- polymerase chain reaction (qRT-PCR).

5.3 Quantitative Real Time- PCR (qRT-PCR)

An iQ5-BIO-RAD Cycler (Cepheid. USA) was used to determine the male rats cDNA copy number. PCR reactions were set up in 25 µL reaction mixtures containing 12.5 µL 1× SYBR® Premix Ex TaqTM (TaKaRa, Biotech. Co. Ltd.), 0.5 µL 0.2 µM sense primer, 0.5 µL 0.2 µM antisense primer, 6.5 µL distilled water, and 5 µL of cDNA template. The reaction conditions consisted of denaturation at 95.0 °C for 3min, 30 cycles of denaturation at 95.0 °C for 15 sec, annealing at 60.0 °C for 30 sec and extension at 72.0 °C for 30 sec and then final step consisted of several cycles at 60.0°C for 10 sec with an increase of 0.5°C until 95°C. Each experiment included a distilled water control. The sequences of specific primer of the genes used [33, 34] and product sizes are listed in (Table 1). At the end of each qRT-PCR a melting curve analysis was performed at 95.0°C to check the quality of the used primers.

Table 1. Primers sequences used for RT-PCR

Gene	Primer sequence (5′–3′)[a]	Annealing Tm (°C)
Hsp70a	F: CGG GAG TTG TAG CGA TGA GA	60
	R: CTT CCT AAA TAG CAC TGA GCC ATA A	
CYP450	F: ATC AAG CAA GGG GAC GAG TT	55
	R: GCT CGC TGA CAA TCT TTT GC	

GST	CTG AAC TCA GGT AGT CCA GC-3'	60
	GGA GGT AGA AGT GCA CAA AG	
β-Actin	F: TGG GGC AGT ATG GCT TGT ATG	55
	R: CTC TGG CAC CCT AAT CAC CTC T	

[a] F: forward primer; R: reverse primer. Tm: temperature

5.4 Calculation of Gene Expression

First the amplification efficiency (Ef) was calculated from the slope of the standard curve using the following formulae [35]:

$Ef = 10^{-1/slope}$

Efficiency (%) = (Ef – 1) x 100

The relative quantification of the target to the reference was determined by using the ΔC_T method if E for the target (Hsp70a, CYP450 and GST) and the reference primers (β-Actin) are the same [35]:

$Ratio_{(reference/\ target\ gene)} = Ef^{C_T(reference)} - {}^{C_T(target)}$

6. DNA Fragmentation Analysis

Apoptotic DNA fragmentation was qualitatively analyzed by detecting the laddering pattern of nuclear DNA as described according to Lu *et al*. [36]. Briefly, liver tissues were homogenized, washed in PBS, and lysed in 0.5 ml of DNA extraction buffer (50 mM Tris–HCl, 10 mM EDTA. 0.5% Triton, and 100 µg/ml proteinase K, pH 8.0) for overnight at 37°C. The lysate was then incubated with 100 µg/ml DNase free RNase for 2h at 37°C, followed by three extractions of an equal volume of phenol/chloroform (1:1 v/v) and a subsequent re-extraction with chloroform by centrifuging at 15,000 rpm for 5 min at 4°C. The extracted DNA was precipitated in two volume of ice-cold 100% ethanol with 1/10 volume of 3 M sodium acetate, pH 5.2 at −20°C for 1 h, followed by centrifuging at 15,000 rpm for 15 min at 4°C. After washing with 70% ethanol, the DNA pellet was air-dried and dissolved in 10 mM Tris–HCl/1 mM EDTA, pH 8.0. The DNA was then electrophoresed on 1.5% agarose gel and stained with ethidium bromide in Tris-acetate/EDTA buffer (TAE) (pH 8.5, 2 mM EDTA, and 40 mM Tris–acetate). A 100-bp DNA ladder (Invitrogen, USA) was included as a molecular size marker and DNA fragments were visualized and photographed by exposing the gels to ultraviolet transillumination.

7. Determination of Gutathione Peroxidase (GPx) Activity

Glutathione peroxidase activity measurements were carried out by a procedure according to Miranda *et al*. [37] [40]. The reaction mixture consisted of 8 mM H_2O_2, 40 mM guaiacol, 50 mM sodium acetate buffer, pH 5.5, and a suitable amount of the enzyme preparation. The change in absorbance at 470 nm due to guaiacol oxidation was followed at 30 s intervals. One unit of GPx activity was defined as the amount of enzyme which increases the O.D. 1.0/min under standard assay conditions.

8. Statistical Analysis

All results were expressed as mean ± SE of the mean. Data were analyzed by one way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program, version 11 followed by least significant difference (LSD) to compare significance between groups. Difference was considered significant when $P < 0.05$.

Results

Effect of tomatine on the gene expression changes induced by KBrO3

Messenger RNA expression of stress related genes (Hsp70a and CYP450) and antioxidant enzyme gene (GST) was quantified by real-time RT-PCR. In groups of rats fed on tomatine fractions (tomatine500, tomatine1000 and tomatine 2000 ppm) Hsp70a and CYP450 and GST mRNA expressions elevated similar expression values to control group (Figure 1-3).

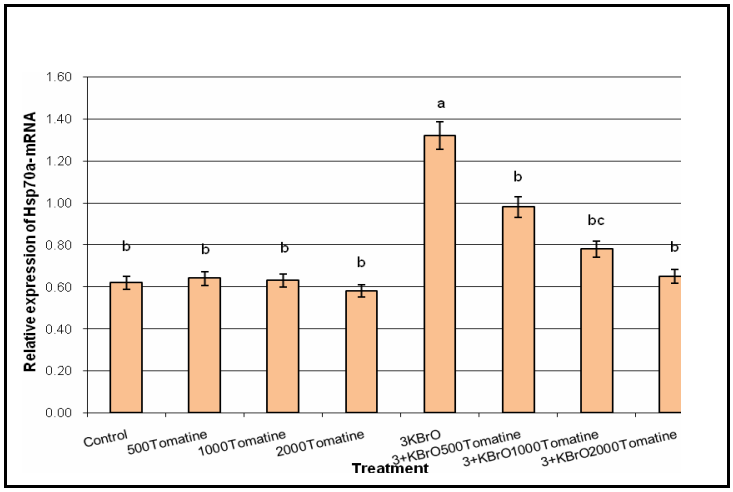

Figure 1: The alterations of Hsp70-mRNA in liver tissues of male rats after KBrO3 exposure and/or tomatine treatment. Data are presented as mean ± SEM. [a,b,c]Mean values within tissue with unlike superscript letters were significantly different (*P*<0.05, Scheffé-Test).

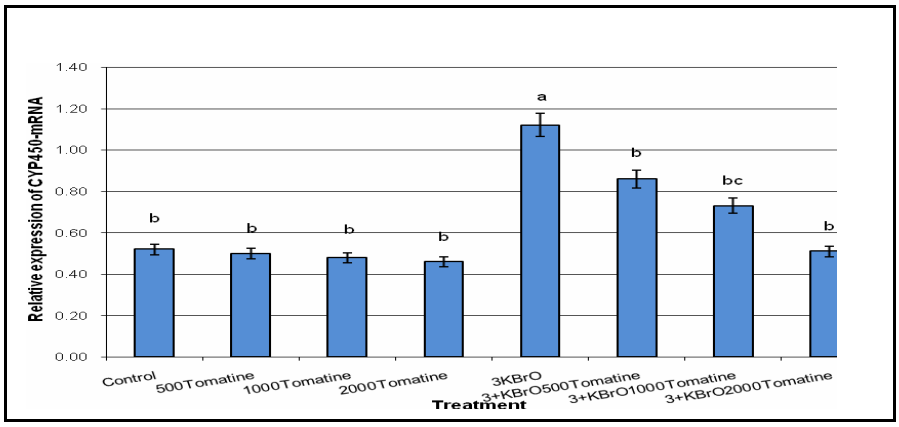

Figure 2: The alterations of CYP450-mRNA in liver tissues of male rats after KBrO3 exposure and/or tomatine treatment. Data are presented as mean ± SEM. [a,b,c]Mean values within tissue with unlike superscript letters were significantly different (*P*<0.05, Scheffé-Test).

However, KBrO3 exposure increased the mRNA expression values of Hsp70a and CYP450 to 209 and 233%% of control, respectively (Figure 1 and 2). In contrast, comparing with KBrO3 alone group, expression values of Hsp70a gene decreased by 74, 59 and 49% in tomatine500+KBrO3, tomatine1000+KBrO3, and tomatine 2000+KBrO3 groups, respectively (Fig. 1). In the same trend, expression values of CYP450 gene decreased by 77, 65 and 46% in tomatine500+KBrO3, tomatine1000+KBrO3, and tomatine 2000+KBrO3 groups, respectively compared with KBrO3 alone group (Fig. 2)

On the other hand, KBrO3 exposure decreased the mRNA expression value of GST to 29% of control group (Figure 3). However, expression values of GST mRNA increased by 212, 242 and 269% in tomatine500+KBrO3, tomatine1000+KBrO3, and tomatine2000+KBrO3 groups, respectively compared with KBrO3 alone group (Fig. 3). So, these results revealed that the best extract in reducing the negative effect of KBrO3 on the expression changes is tomatine2000.

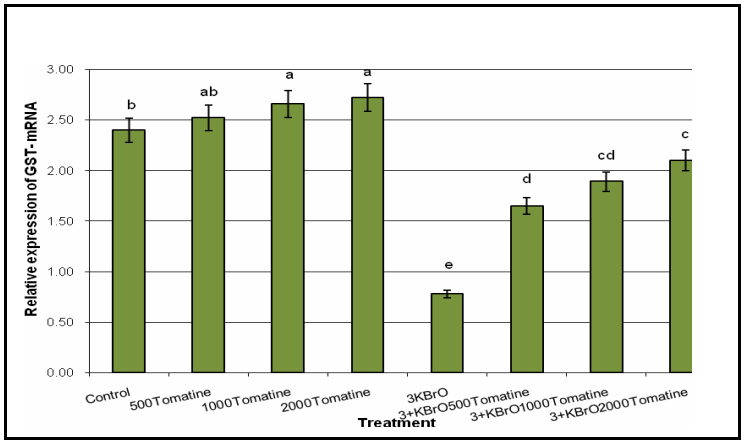

Figure 2: The alterations of GST-mRNA in liver tissues of male rats after KBrO3 exposure and/or tomatine treatment. Data are presented as mean ± SEM. [a,b,c]Mean values within tissue with unlike superscript letters were significantly different (*P*<0.05, Scheffé-Test).

Effects of tomatine on DNA fragmentation induced by KBrO3

Assessment of DNA fragmentation in liver tissues of male rats' genome following KBrO3and/or tomatine treatment as a surrogate for oxidative stress induced damage is summarized in Figure 4.

The results showed that the DNA fragmentation following tomatine fractions (500, 1000 and 2000 ppm) were relatively similar to that of the control group.

However, the DNA fragmentation increased in the form of several DNA bands following KBrO3 treatment in comparison to that of the control group (Fig. 4). In contrary, the DNA fragmentation decreased following tomatine fractions companied with KBrO3 (tomatine500+KBrO3, tomatine1000+KBrO3, tomatine2000+KBrO3) compared with KBrO3 alone (Fig. 4).

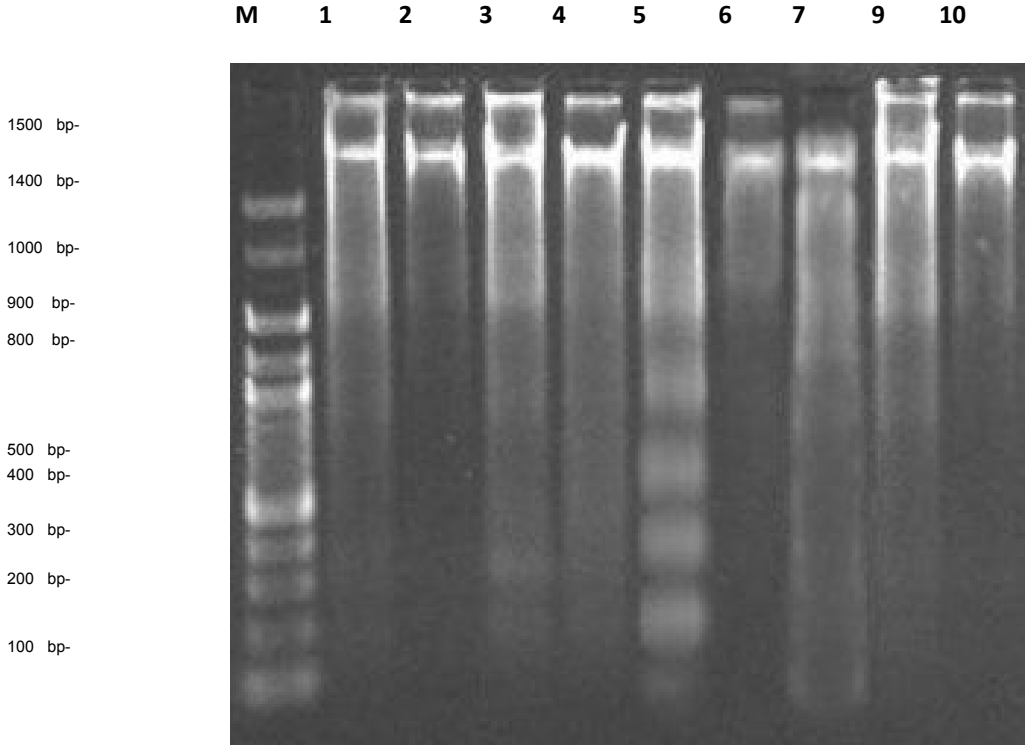

Figure 4: Comparison of DNA fragmentation profiles of different male rat genomic DNA treated with KBrO3 and/or tomatine. M: DNA marker. Lane 1 represents PCR products of untreated control samples; lanes 2-4 represent rats fed on tomatine; lane 5 represents rats treated with KBrO3; lanes 6-8 represent rats fed on tomatine plus KBrO3.

Effects of tomatine on GPx levels after KBrO3 Exposure

Table 2 shows the suppression effect of tomatine fractions on KBrO3-induced alteration in the antioxidant enzyme. Comparing with the control group, GPx activity levels were relatively similar with tomatine fractions (500, 1000 and 2000 ppm) treatment. However, the activity level of GPx decreased significantly compared with KBrO3 group. When compared with the KBrO3 alone group, GPx concentration elevated 1.7, 2.1 and 2.4-fold significantly in tomatine500+KBrO3, tomatine1000+KBrO3 and tomatine2000+KBrO3 groups, respectively (Table 2).

Table 2. The amount of glutathione peroxidase activity in rats exposed to KBrO3 and/or tomatine

Treatment	Glutathione peroxidase activity (U/mg tissues/min)
Control	6.2±0.06
Tomatine500	5.7±0.05
Tomatine1000	5.9±0.04
Tomatine2000	6.5±0.06
KBrO3	2.2±0.03**
Tomatine500+KBrO3	3.8±0.04*
Tomatine1000+KBrO3	4.6±0.05*
Tomatine2000+KBrO3	5.2±0.06

Data are presented as mean ± SEM. Values marked with an asterisk (*) are significantly different ($P < 0.05$), (**) are significantly different ($P < 0.01$).

Discussion

The current study showed increase of the expression alterations of stress related genes (Hsp70a and CYP450) and decrease of antioxidant enzyme gene (GST), DNA fragmentation and decreased activities of enzymatic antioxidant GPx in circulation, liver of KBrO3-treated rats. ROS generated in tissues are normally scavenged by enzymatic and non-enzymatic antioxidants [38, 39]. Antioxidant defense system protects the aerobic organism from the deleterious effects of reactive oxygen metabolites [40-42].

Glutathione is a crucial component of the antioxidant defense mechanism and it functions as a direct reactive free the highest content of GSH, which is transferred to kidney and liver by distinct GSH transport system [43, 44]. The decreased levels of GSH in circulation and tissues in KBrO3-treated rats may due to enhanced utilization during detoxification of KBrO3. GPx and CAT, which act as preventive antioxidants and SOD, a chain-breaking antioxidant, play important roles in protection against the deleterious effects of lipid peroxidation [45]. Decreases in the activities of SOD, CAT and GPx in plasma, liver and kidney of KBrO3-treated rats may due to the decreased synthesis of enzymes or oxidative inactivation of the enzyme proteins. In the present study, increased DNA fragmentation and expression alterations associated with decreased antioxidant status in KBrO3-treated rats could therefore give rise to insufficient antioxidant potential.

This study demonstrated that tomatine protected against changes of the gene expression coincided with DNA damage and to varying degrees reversed the damages caused by oxidative stress via its antioxidant activities. These findings support the hypothesis that tomatine exerted a protective effect *in vivo*.

For our knowledge this is the first report on the anti-genotoxicity effects of tomatine *in vivo*. The results revealed that the expression of Hsp70a and CYP450 and GST mRNA in rats fed on several concentrations of tomatine fractions (500, 1000 and 2000 ppm) elevated similar expression values to control group. In addition, tomatine prevented the oxidative stress of KBrO3 induced increase in expression of stress related genes (Hsp70a and CYP450) and decrease of antioxidant enzyme gene (GST).

The antioxidant effects of tomatine may be due to different causes: firstly, flavonoid's high diffusion into the membranes [46] allowed it to scavenge oxyradicals at several sites throughout the lipid bilayer; secondly, its pentahydroxyflavone structure allowed it to chelate metal ions via the orthodihydroxy phenolic structure, thereby scavenging lipid alkoxyl and peroxyl radicals [47, 48]. In spite of the free radical scavenging activities, flavanoid glycosides and phenols in tomatine might be also involved in the indirect induction of

detoxifying genes [49], which might promote detoxification of KBrO3 and decrease their toxicity. *In vivo* studies have shown that flavanoid glycosides inhibited Fe^{2+}-induced lipid peroxidation in the rat liver [50]. It is suggested that the lipid peroxidative indices were probably attenuated by the chainbreaking action of flavanoid in the free radical process of the oxidation of membrane lipids.

Our results also showed that the liver was the major organ with the higher content of DNA damage after KBrO3 treatment, suggesting that the liver is the main target organ of KBrO3-induced DNA oxidation. DNA damage was significantly decreased after the treatment of tomatine, which may be attributed to the antioxidant property of phenols in tomatine, as phenols are known to bind DNA at sites that would normally react with the active metabolites of carcinogen during carcinogen-DNA binding, a crucial step for initiation of carcinogenesis [51–53]. Alternatively, when the phenols bind to DNA, their molecules might be positioned in such a way so as to effectively scavenge reactive intermediates that approach the critical sites on DNA, or phenols may directly interact with the ultimate reactive metabolites of carcinogen by donating their electrons and rendering it inactive [54]. Dok-Go et al. demonstrated phenolsact in many cell-free experimental systems to scavenge reactive oxygen radicals and reduce oxidativeDNA damage [55].

In conclusion, our data demonstrated that tomatine protected against KBrO3 toxicity by decreasing oxidant status resulting in decreasing of gene expression alterations and DNA damage and increasing the antioxidant status, indicating that tomatine possesses a spectrum of antioxidant and DNA-protective properties. However, further investigations are necessary to elucidate the precise mechanisms of protection of tomatine against KBrO3 toxicity, and the potential effects of tomatine against other carcinogens should be explored prior to evaluating as a chemopreventive agent against carcinogenesis.

References

1. Jeong JB, Hong SC, Jeong HJ. 3,4-Dihydroxybenzaldehyde purified from the barley seeds (*Hordeum vulgare*) inhibits oxidative DNA damage and apoptosis via its antioxidant activity. Phytomedicine 2009; 16(1): 85–94.
2. Lopaczynski W, Zeisel SH. Antioxidants, programmed cell death, and cancer. *Nutrition Research* 2001; 21 (1-2): 295–307.
3. Muthukumaran S, Sudheer AR, Menon VP, Nalini N. Protective effect of quercetin on nicotine-induced prooxidant and antioxidant imbalance and DNA damage in Wistar rats. *Toxicology* 2008; 243 (1-2): 207–215.
4. Bowerman B. Oxidative stress and cancer: a β-catenin convergence," *Science* 2005; 308 (5725): 1119–1120.
5. Khan N, Sultana S. Inhibition of potassium bromateinduced renal oxidative stress and hyperproliferative response by *Nymphaea alba* in Wistar rats. Journal of Enzyme Inhibition and Medicinal Chemistry *2005;* 20 (3): 275–283.
6. Ke Y, Duan X, Wenet F et al. Association of melamine exposure with urinary stone and oxidative DNA damage in infants. Archives of Toxicology 2010; 84 (4): 301–307, 2010.
7. McDorman KS, Pachkowski BF, Nakamura J, Wolf DC, Swenberg JA. Oxidative DNA damage from potassium bromate exposure in Long-Evans rats is not enhanced by a mixture of drinking water disinfection by-products. Chemico-Biological Interactions 2005; 152 (2-3): 107–117.
8. Raschke M, Rowland IR, Magee PJ, Pool-Zobel BL. Genistein protects prostate cells against hydrogen peroxide induced DNA damage and induces expression of genes involved in the defence against oxidative stress. *Carcinogenesis* 2006; 27 (11): 2322–2330.
9. Yin R, Zhang D, Song Y, Zhu BZ, Wang H. Potent DNA damage by polyhalogenated quinones and H2O2via a metal independent and Intercalation-enhanced oxidation mechanism. Scientific Reports 2013; 3: 1–6.
10. Maroz A, Anderson RF, Smith RAJ, Murphy MP. Reactivity of ubiquinone and ubiquinol with superoxide and the hydroperoxyl radical: implications for in vivo antioxidant activity. Free Radical Biology and Medicine 2009; 46 (1): 105–109.
11. Jeong JB, Seo EW, Jeong HJ. Effect of extracts from pine needle against oxidative DNA damage and apoptosis induced by hydroxyl radical via antioxidant activity. Food and Chemical Toxicology 2009; 47 (8): 2135–2141.

12. Singh BN, Singh BR, Singh RL et al. Oxidative DNAdamage protective activity, antioxidant and anti-quorum sensing potentials of *Moringa oleifera*. Food and Chemical Toxicology 2009; 47 (6) 1109–1116.
13. Friedman M. Tomato glycoalkaloids: Role in the plant and in the diet. J Agric Food Chem 2002; 50:5751-80.
14. Schmidt B, Ribnicky DM, Poulev A, Logendra S, Cefalu WT, Raskin I. A natural history of botanical therapeutics. Metabolism 2008; 57:S3-9.
15. Friedman M, Fitch TE, Yokoyama WE. Lowering of plasma LDL cholesterol in hamsters by the tomato glycoalkaloid tomatine. Food Chem Toxicol 2000; 38:549-53.
16. Thorne HV, Clarke GF, Skuce R. The inactivation of herpes simplex virus by some Solanaceae glycoalkaloids Antiviral Res 1985; 5:335-43.
17. Morrow WJW, Yang YW, Sheikh NA. Immunobiology of the tomatine adjuvant. Vaccine 2004; 22:2380-4.
18. Friedman M, McQuistan T, Hendricks JD, Pereira C, Bailey GS. Protective effect of dietary tomatine against dibenzo[a,l]pyrene (DBP)-induced liver and stomach tumors in rainbow trout. Mol Nutr Food Res 2007; 51:1485-91.
19. Lee KR, Kozukue N, Han JS, Park JH, Chang EY, Baek EJ, Chang JS, Friedman M. Glycoalkaloids and metabolites inhibit the growth of human colon (HT29) and liver (HepG2) cancer cells. J Agric Food Chem 2004; 19:2832-9.
20. Chiu FL, Lin JK. Tomatidine inhibits iNOS and COX-2 through suppression of NF-kappaB and JNK pathways in LPS-stimulated mouse macrophages. FEBS Lett 2008; 582:2407-12.
21. Shih YW, Shieh J M, Wu PF, Lee YC, Chen YZ, Chiang TA. α-Tomatine inactivates PI3K/Akt and ERK signaling pathways in human lung adenocarcinoma A549 cells: effect on metastasis. Food Chem Toxicol 2009; 47:1985-95.
22. Liao DW, Wang L, Zhang XG, Liu MQ. Expression and significance of PTEN/PI3K signal transduction-related proteins in non-small cell lung cancer Chin J Canc 2006; 25:1238-42.
23. Itoh Y, Nagase H. Matrix metalloproteinases in cancer. Essays Biochem 2002; 38,21-36.
24. Duffy MJ, Duggan C. The urokinase plasminogen activator system: A rich source of tumor markers for the individualised management of patients with cancer. Clin Biochem 2004;37:541-8.
25. Lee ST, Wong PF, Cheah SC, Mustafa MR Alpha-tomatine induces apoptosis and inhibits nuclear factor-kappa B activation on human prostatic adenocarcinoma PC-3 cells. PLoS ONE 2011; 6:e18915.
26. Shieh JM, Cheng TH, Shi MD, Wu PF, Chen Y, Ko SC, Shih YW. α-Tomatine suppresses invasion and migration of human non-small cell lung cancer NCI-H460 cells through inactivating FAK/PI3K/Akt signaling pathway and reducing binding activity of NF-κB. Cell Biochem Biophys 2011; 60:297-310.
27. Chao MW, Chen CH, Chang YL, Teng CM, Pan SL. α-Tomatine- Mediated Anti-Cancer Activity In Vitro and In Vivo through Cell Cycle-and Caspase-Independent Pathways. PLoS ONE 2012; 9:e44093.
28. Kúdelová J, Seifrtová M, Suchá L, Tomšík P, Havelek R, Řezáčová M. Alpha-tomatine activates cell cycle checkpoints in the absence of DNA damage in human leukemic MOLT-4 cells. J Appl Biomed 2013; 11:93-103.
29. Friedman M, McQuistan T, Hendricks JD, Pereira C Bailey GS. Protective effect of dietary tomatine against dibenzo[a,l]pyrene (DBP)-induced liver and stomach tumors in rainbow trout. Mol. Nutr. Food Res 2007; 51, 1485 – 1491
30. Ke Y, Xu X, Wu S, Huang J, Geng Y, Misra H, Li Y. Protective Effects of Extracts from *Fructus rhodomyrti* against Oxidative DNA Damage *In Vitro* and *In Vivo*. Oxidative Medicine and Cellular Longevity, 2013, ID 507407, 8 pages, http://dx.doi.org/10.1155/2013/507407
31. Ali F Kh., El-Shafai SA, Samhan FA, Khalil WKB. Effect of water pollution on expression of immune response genes of Solea aegyptiaca in Lake Qarun. African Journal of Biotechnology 2008; 7 (10): 1418-1425.
32. Mahrous KF, Khalil WKB, Mahmoud MA. Assessment of toxicity and clastogenicity of sterigmatocystin in Egyptian Nile tilapia. African Journal of Biotechnology 2006; 5 (12): 1180-1189.
33. Persson M, Rönnbäck L. Microglial self-defence mediated through GLT-1 and glutathione. Amino Acids 2012; 42(1): 207–219.
34. Boulland JL, Osen KK, Levy LM, Danbolt NC, Edwards RH, Storm-Mathisen J, Chaudhry FA. Cell specific expression of the glutamine transporter SN1 suggests differences in dependence on the glutamine cycle. Eur J Neurosci 2002; 15(10): 1615-1631.
35. Dolińska M, Zablocka B, Sonnewald U, Albrecht J. Glutamine uptake and expression of mRNA's of glutamine transporting proteins in mouse cerebellar and cerebral cortical astrocytes and neurons. Neurochem Int 2004; 44(2): 75-81.

36. Lu T, Xu Y, Mericle MT, Mellgren RL. Participation of the conventional calpains in apoptosis. Biochimica et Biophysica Acta 2002; 1590: 16-26.
37. Miranda MV, Fernandez Lahore HM, Cascone O. Horseradish peroxidase extraction and purification by aqueous two-phase partition. Appl Biochem Biotechnol 1995; 53: 147−154.
38. Sener G, Sehirli AO, Y Ipci et al. Taurine treatment protects against chronic nicotine-induced oxidative changes. Fundamental and Clinical Pharmacology 2005; 19 (2): 155–164.
39. Zhang M, Swarts SG, Yin L et al. Antioxidant properties of quercetin," *Advances in ExperimentalMedicine and Biology* 2011; 701: 283–289.
40. Lee M, Kwon BM, Suk K, McGeer E, McGeer PL. Effects of obovatol on GSH depleted glia-mediated neurotoxicity and oxidative damage," *Journal of Neuroimmune Pharmacology 2012;* (7): 173–186.
41. Romao PR, Tovar J, Fonseca SG et al. Glutathione and the redox control system trypanothione/trypanothione reductase are involved in the protection of Leishmania spp. against nitrosothiol-induced cytotoxicity. *Brazilian Journal of Medical and Biological Research* 2006; 39 (3): 355–363.
42. Luchese C, Stangherlin EC, Gay BM, Nogueira CW. Antioxidant effect of diphenyl diselenide on oxidative damage induced by smoke in rats: involvement of glutathione," *Ecotoxicology and Environmental Safety* 2009; 72 (1): 248–254.
43. Husain K, Scott BR, Reddy SK, Somani SM. Chronic ethanol and nicotine interaction on rat tissue antioxidant defense system. Alcohol 2001; 25 (2): 89–97.
44. Liang Q, Sheng Y, Jiang P et al. The gender-dependent difference of liver GSH antioxidant system in mice and its influence on isoline-induced liver injury. *Toxicology 2011;* 280 (1-2): 61–69.
45. Fischer TW, Kleszczynski K, Hardkop LH, Kruse N, Zillikens D. Melatonin enhances antioxidative enzyme gene expression (CAT, GPx, SOD), prevents their UVR-induced depletion, and protects against the formation of DNA damage (8-hydroxy-2-deoxyguanosine) in *ex vivo* human skin. *Journal of Pineal Research* 2012; 54: 303–312.
46. Moridani MY, Pourahmad J, Bui H, Siraki A, Obrien PJ. Dietary flavonoid iron complexes as cytoprotective superoxide radical scavengers. *Free Radical Biology and Medicine 2003;* 34 (2): 243–253.
47. Cao G, Sofic E, Prior RL. Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. *Free Radical Biology and Medicine 1997;* 22 (5): 749–760.
48. Lien EJ, Ren S, Bui HH, Wang R. Quantitative structure-activity relationship analysis of phenolic antioxidants," *Free Radical Biology and Medicine* 1999; 26 (3-4): 285–294.
49. Kim MR, Lee JY, Lee HH et al. Antioxidative effects of quercetin-glycosides isolated from the flower buds of *Tussilago farfara* L. *Food and Chemical Toxicology* 2006; 44 (8) 1299–1307.
50. Cai Q, Rahn RO, Zhang R. Dietary flavonoids, quercetin, luteolin and genistein, reduce oxidativeDNAdamage and lipid peroxidation and quench free radicals. *Cancer Letters* 1997; 119 (1) 99–107.
51. Bhattacharya RK, Firozi PF. Effect of plant flavonoids on microsome catalyzed reactions of aflatoxin B1 leading to activation and DNA adduct formation. *Cancer Letters 1988*; 39 (1): 85–91.
52. Dixit R, Gold B. Inhibition of N-methyl-N-nitrosoureainduced mutagenicity and DNA methylation by ellagic acid," *Proceedings of the National Academy of Sciences of the United States of America* 1986; 83 (21): 8039–8043.
53. Khanduja KL, Majid S. Ellagic acid inhibitsDNAbinding of benzo(a)pyrene activated by different modes," *Journal of Clinical Biochemistry and Nutrition* 1993; 15: 1–9.
54. Wood AW, Huang MT, Chang RL et al. Inhibition of the mutagenicity of bay-region diol epoxides of polycyclic aromatic hydrocarbons by naturally occurring plant phenols: exceptional activity of ellagic acid. *Proceedings of the National Academy of Sciences of the United States of America* 1982; 79 (18): 5513–5517.
55. Dok-Go H, Lee KH, Kimet HJ al. Neuroprotective effects of antioxidative flavonoids, quercetin, (+)-dihydroquercetin and quercetin 3-methyl ether, isolated from*Opuntia ficusindica* var. saboten. *Brain Research 2003;* 965 (1-2): 130–136.

*****