The protective role of Quercetin against Arsenic-induced genotoxicity in *Drosophila melanogaster*

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Abstract

This study aims to evaluate both genotoxic effect of Arsenic (As) and possible protective role of quercetin (Q) in *Drosophila melanogaster* Oregon R flies. Adult Drosophila flies were treated with with As (10, 50 and 100 ppb), quercetin (100 µm) and As+Q (10 ppb+100 µM Q, 50 ppb+100 µM Q and 100 ppb+ 100 µM Q) for 1 and 5 days. Relative expression levels of Mn-SOD (Mn-superoxide dismutase), catalase (CAT) and glutathione synthase (GS) genes significantly increased with 10 and 50 ppb As exposure especially for 5 days. However, the highest As concentration caused decreases in the expression levels of antioxidant enzyme responsible genes in same exposure time. Quercetin supplementation significantly reduced the expressions of Mn-SOD and CAT genes to about control levels. Rapid and significant alterations were observed expression levels of heat shock protein (HSP26 and HSP70) genes for both As and As+Q exposure at all treatment times. It was also determined that application of the As and As+Q affected the DNA profiles and genomic template stabilities of *D. melanogaster*. The most band changes were detected at As+Q treatment after first day of exposure. These results indicated that the As causes changes in the gene expressions and DNA polymorphisms and that quercetin supplement can alter responses of *D. melanogaster* to As.

Keywords: Arsenic genotoxicity; Quercetin; Oxidative Stress; Gene expression; heat shock protein; antioxidant enzymes; RAPD

1. Introduction

Arsenic (As) is one of the most toxic elements and enters the environment through both natural (volcanic, geothermal and presence in local bedrock) and anthropogenic (fossil burning, industrial effluents, agricultural wastes) activities (Sarkar 2002). Human exposure to As may occur via food, soil, air and water. Contamination of drinking water with As causes environmental and health problems both developed and developing countries (Smith et al., 2000). It has been reported that the exposure to As, sourced from drinking water, may cause cancer (skin, lung, bladder, kidney), metabolic diseases (diabetes mellitus, black food disease) and neurological diseases in human (Hei & Metka 2004; Banerjee et al. 2009; Sun et al. 2013). Because of these harmful effects of As, governments and different agencies i.e. the American Public Health Association (APHA), the World Health Organization (WHO), the Indian Standard Institution (ISI), the Central Pollution Control Board (CPCB) and the Indian Council of Medical Research (ICMR) enforce some limitations for As amounts in drinking water. The maximum permissible limit of As in drinking water lowered from 50 to 10 µg/l by WHO at

1993. According to "Regulation of Water Intended for Human Consumption" in the published in 2005, permissible limit of As was also changed by Turkish Government and 10 µg/l has been implemented since 2008 for maximum permissible limit of As.

The generation of reactive oxygen species (ROS) such as superoxide anion, singlet oxygen, hydroxyl radical and hydrogen peroxide (H_2O_2) is one of the effects of As toxicity (Banerjee et al. 2009). These free radicals are scavenged by enzymatic (such as superoxide dismutase, catalase, glutathione synthase and peroxidase) and non-enzymatic (glutathione, tocopherol, ascorbic acid) antioxidant systems (Hei et al. 2004). In addition to organisms' own antioxidant scavenging system, it was reported that the natural product compounds such as quercetin, curcumin, naringin and naringenin have help scavenging excess radicals (Sun et al. 2013). Quercetin is a plant pigment which is protect to cells from free radical damage. It was reported that quercetin have anti-inflammatory, antiviral, antitrombic, anti-ischemic, chemotherapeutic and chemoprotective effects (Gargouri et al. 2011). Heat shock proteins (HSPs) are one of the most abundant cellular proteins found under non-stress conditions and are expressed in response to various biological stresses, such as heat, oxidative, heavy metals, osmolarity, anoxia and viral infections (Singh et al. 2009; Desai et al. 2010; Singh et al. 2010). HSPs have cytoprotective role such as degradation or refolding of misfolded, damaged and non-native proteins. Arsenic induced HSP production was reported in different organisms and cells such as *Tetrahymena pyriformis*, mice, rat, chicken and Giunea pig tracheal cells (Haynes et al., 1996; Ito et al., 1997; Nriagu and Luda 2000; Del Razo et al., 2001; Liu et al., 2001). It was reported that As cause DNA breaks, chromosomal aberrations, sister-chromatid exchanges and micronucleus induction (Basu et al. 2001; Chen et al. 2005; Gebel 2001; Ghosh et al. 2006; Kumar et al. 2014). Therefore we selected Random Amplified Polymorphic DNA (RAPD) technique for determine genotoxic effect of As. This assay has been successfully used for the determination of genotoxic effects of different toxic agents such as heavy metals, pesticides and benxo(a)pyrene (Williams et al. 1990; Atienzar et al. 1999; Doganlar et al. 2014). In this study, we used *D. melanogaster* as a genetic model organism because of the high homology of many genes related with neurologic, metabolic and developmental diseases in human (Warrick et al. 1999; Demir et al. 2013). Additionally, the European Centre for the Validation of Alternative Methods recommends that *D. melanogaster* can be used for research and testing in order to reduce the use of laboratory animals (Festing et al.1998).

The aims of the present study were to investigate the responses of *D. melanogaster* upon exposure to As either alone or in combination with quercetin with reference to changes in expressions of antioxidant enzyme (Mn-SOD, CAT and GS) and HSP (HSP26, HSP60, HSP70 and HSP83) genes and RAPD DNA profiles to evaluate toxic/genotoxic effect of As in permissible concentration in drinking water.

2. Material and methods

2.1. Drosophila stocks and treatment

Wild type *D. melanogaster* strain (Oregon R) were reared on standard Drosophila medium (cornmeal, agar, sugar, yeast, water and propionic acid) at 25±1 °C. All stocks were housed in the glass bottles. For all control and treatment groups, adult flies were used in the same developmental stage. Flies were transferred bottles including Drosophila medium prepared with distilled water (control), As (10 ppb, 50 ppb and 100 ppb), quercetin (100 µM) and As+Q (10 ppb As+100 µM Q, 50 ppb As+100 µM Q and 100 ppb As+100 µM Q) for 1 and 5 days. As treatment doses were selected based on permissible limits of As in drinking water by the US EPA (1993) and the Turkish Ministry of Health (2008).

2.2. Isolation of total RNA and cDNA synthesis

At the end of each treatment period, three flies were removed from the media and total RNA was isolated from each adult *D. melanogaster* specimen using the PureLink® RNA Mini Kit (Life Tecnologies, USA) according to the manufacturer's instructions. The extracted RNA concentrations were measured by the Qubit® Fluorometer (Life Tecnologies, USA). The concentration of total RNA was adjusted to 50 ng/µL for the synthesis of the first strand of cDNA using a High Capacity cDNA Reverse Transcription Kit (Life Tecnologies, USA). cDNA synthesis was performed using the thermal cycler Applied Biosystems® ProFlex™ PCR System (Step 1: 25°C, 10 min; Step 2: 37°C, 120 min; Step 3: 85°C, 5 min). The cDNA was stored -20°C for subsequent steps of the analysis.

2.3. Quantitative real-time PCR (qRT-PCR) analysis

Expression levels of the genes responsible for antioxidant enzymes (Mn-SOD, CAT and GS) and HSPs in response to treatment with the As and As+Q were analysed by qRT-PCR using the SYBR® Select Master Mix (Life Technologies, USA) on an ABI Step One Plus Real-Time PCR system (1 cycle of 2 min at 50°C and 10 min at 95°C followed by 40 cycles of denaturation at 95°C for 15 s, annealing and extension at 60°C for 1 min) with the primer pairs given in Table 1. Gene expressions were determined as the relative fold change compared the control and normalized with RP49 mRNA expressions. The comparative cycle threshold (ΔCt) method (User Bulletin 2, Applied Biosystems, CA) was performed to analyse the expressions levels of mRNAs. Additionally, differences in the degree of the relative fold change resulting from gene expressions under the effect of the As and As+Q were compared using analysis of variance (ANOVA) with Duncan's separation of means test using SPSS 18 software at a significance level of $P \leqslant 0.05$. Correlations between the relative expressions levels of antioxidant and HSP genes were analyzed by a bivariate correlation test with Pearson correlation coefficient and a two-tailed test of significance using SPSS 18 software at significance levels of $P \leqslant 0.01$ and 0.05.

Table 1. Primer sequences used in qRT-PCR

	qRT-PCR primers
HSP26	F 5' GCCCCGCAGCCCCATCTACGAG 3' R 5' GAGCACGCCATCCGACGACAGC 3'
HSP60	F 5' GTCGCGCCCCGTTAGCAC 3' R 5' CATCGCGTCCCACCTTCTTCAT 3'
HSP70	F 5' CGAGETCGACGCATTGTTTG 3' R 5' GAGTGGATCCGCCGACGAGTA 3'
HSP83	F 5' CCGGAGGCTCTTTCACAGTC 3' R 5' CTTCTCGCGCTCCTTCTCTAC 3'
Mn-SOD	F 5'-TCTGAAGAAGGCCATCGAGT-3' R 5'-GCAGATAGTAGGCGTGCTCC-3'
CAT	F 5'-TACGAGCAGGCCAAGAAGTT-3' R 5'-ACCTTGTACGGGCAGTTCAC-3'
GS	F 5'-TGGGACCAGCAAGTAAAACC-3' R 5'-TCGCGAATG TAGAACTCGTG-3'
RP49	F 5'-GCTAAGCTGTCGCACAAATG-3' R 5'-TGTGCACCAGGAACTTCTTG-3'

2.4. DNA Extraction and RAPD Procedures

Genomic DNA was isolated from *D. melanogaster* using a DNeasy ® Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The total genomic DNA was diluted with nuclease free water to a concentration of 25 ng/µl and this diluted DNA was used as template DNA for the PCR reaction. Standard 50 µl PCR reactions were performed using 2 µl (50±10 ng) of template DNA, AmpliTaq Gold PCR Master Mix (4326717, Applied Biosystems®), 1 µl of each primer and nuclease-free water (10977-015, Invitrogen™). DNA amplification was performed using the thermal cycler Applied Biosystems® ProFlex™ PCR System (40 cycles of 95 °C denaturation (30 s), 37 °C annealing (30 s), and 72 °C elongation (90 s) with an initial 94 °C denaturation (3 min) and a final 72 °C extension (30 min) with the primer pairs given in Table 2. After the amplification of DNA, 10 µl of product with 2 µl of loading dye (R0611, Fermentas) was loaded onto a 2% agarose gel with ethidium bromide in 2× TAE (Tris 1.6 M, acetic acid 0.8 M, EDTA 40 mM, Ambion® 10X TAE) buffer. The molecular weight standard Gene Ruler 100 bp plus DNA (Fermentas, SM0321) was used according to the manufacturer's instructions. The DNA bands were visualized with a UV transilluminator (ChemiDoc MP Imaging System, Bio-Rad) and the sizes of all of the bands were calculated with program software. The percentages of Polymorphism (P) and genomic template stabilities (GTS) were calculated by using RAPD data. P (%)=[(a+b) / n] x 100 GTS (%) = (1- [(a+b) / n]) x 100 where, a and b indicate appearance of new bands and disappearance of normal bands, respectively; n is the number of total bands in the control.

Table 2 Primer sequences used in RAPD

Primer code	Sequence
OPC 183260	5'-TGAGTGGGTG-3'
OPC193470	5'-GTTGCCAGCC-3'
OPN023470	5'-ACCAGGGGCA-3'
OPA033260	5'-AGTCAGCCAC-3'
OPA043260	5'-AATCGGGCTG-3'
OPA103260	5'-GTGATCGCAG-3'
OPC063260	5'-GAACGGACTC-3'

3. Results and discussions

Arsenic, passing through the cell membrane, cause a higher level of oxidative stress in Drosophila flies. The flies might react or adapt to such conditions by upregulating their own antioxidant genes. To test whether this was the situation, the expression levels of several antioxidant genes were investigated, including Mn-SOD, CAT and GS, by performing the sybergreen assay of quantitative RT-PCR. In the first day, significant relative expressions were observed in both Mn-SOD and CAT mRNA levels with 2.17-3.51 fold only at 10 ppb As treatment, respectively (Fig. 1). Compared to their respective controls, other concentrations caused a low impact on gene expressions. The Mn-SOD and CAT expressions after 5 days of treatment caused an approximately 1–2.5-fold increase compared to the first day especially at 10 and 50 ppb As treatments. Maximum relative expressions were detected at 50 ppb As exposure with 3.6 and 4.58 fold in Mn-SOD and CAT genes in long term applications. Exposure to the As at 10, 50, and 100 ppb caused 4.44, 1.01 and 1.79-fold increases in GS expressions in the flies after the first day of exposure, respectively (Fig. 1). Additionally significant increase of GS mRNA levels were observed at 10 and 50 ppb As exposure for 5 days treatment, compared with control. No significant differences between relative gene expressions of quercetin and water treated control group normalized with RP49 mRNA levels in antioxidant systems (except 1. day Mn-SOD and GS). However, quercetin supplementation significantly reduced the Mn-SOD and CAT gene expressions to about control levels in all exposure concentrations and treatment time. Significant decreasing in the overexpression of GS were observed 10 ppb exposure for 1 day and 50 ppb exposure for 5 days.

Environmental stresses, such as volatile organic compounds, toxic metals, or agricultural chemicals (fertilizer, pesticides, etc.), cause excess production of ROS (Verma & Rana 2008; Silva 2014). Toxic ROS accumulation due to an inadequate antioxidant system can lead to genotoxic effects, such as DNA polymorphism and gene induction/repressions (Doganlar et al. 2014). Recent studies have shown that during stressful conditions, a differential regulation of genes encoding proteins involved in antioxidant enzymes (SOD, CAT and GS) and heat shock proteins exists (Singh et al. 2010; Tsuda et al. 2010). SOD, the first step of defense against ROS, catalyzes the dismutation of superoxide anions to H_2O_2. In the second step, CAT, glutathione peroxidase and peroxiredoxins catalyze the decomposition of H_2O_2 to water and oxygen (Chelikani et al. 2004). In this study, Mn-SOD and CAT exhibited cross talk and their gene expressions were significantly increased by different concentrations of As (Table 3). Quercetin treatment with As provide significant protection against the higher free radical damage. These data show that the induction of overexpression of Mn-SOD, CAT and GS genes refereed by As exposure, accelerated the change of superoxide radicals to harmless form and perhaps quercetin which is antioxidant flavonoids may directly scavenge free radicals in cells.

The expression analysis of HSP genes in *D. melanogaster* exposed to As and As+Quercetin groups for 1 and 5 days are shown in Fig. 2. While, no significant differences were observed in the relative expression of HSP26 at 10 and 100 ppb As concentrations, As+Quercetin treatment caused significant decreasing on the mRNA levels of this protein at the first day of exposure. However significant alternation in HSP26 expression was detected at all treatment concentration for 5 days. A significant increase in HSP60 mRNA levels (1.35 fold) was observed at only 10 ppb As treated groups after 1 day in comparison to their respective controls and hsp60 gene expressions level was significantly lower in the both As and As+Quercetin groups compared to control after 5 day. Compared with control, while

the quercetin supplementation cause significant reducing the relative expressions levels of HSP70 and HSP83 genes in control group, but the highest overexpression (HSP70:5.24 fold, HSP83:1.47) were determined at 100 ppb As+100 µM quercetin exposed groups after 1 day. Additionally, significant increase in HSP70 expression was observed at 10 ppb and 50 ppb of As, and 10 ppb of As+quercetin after 5 days treatment (Fig 2).

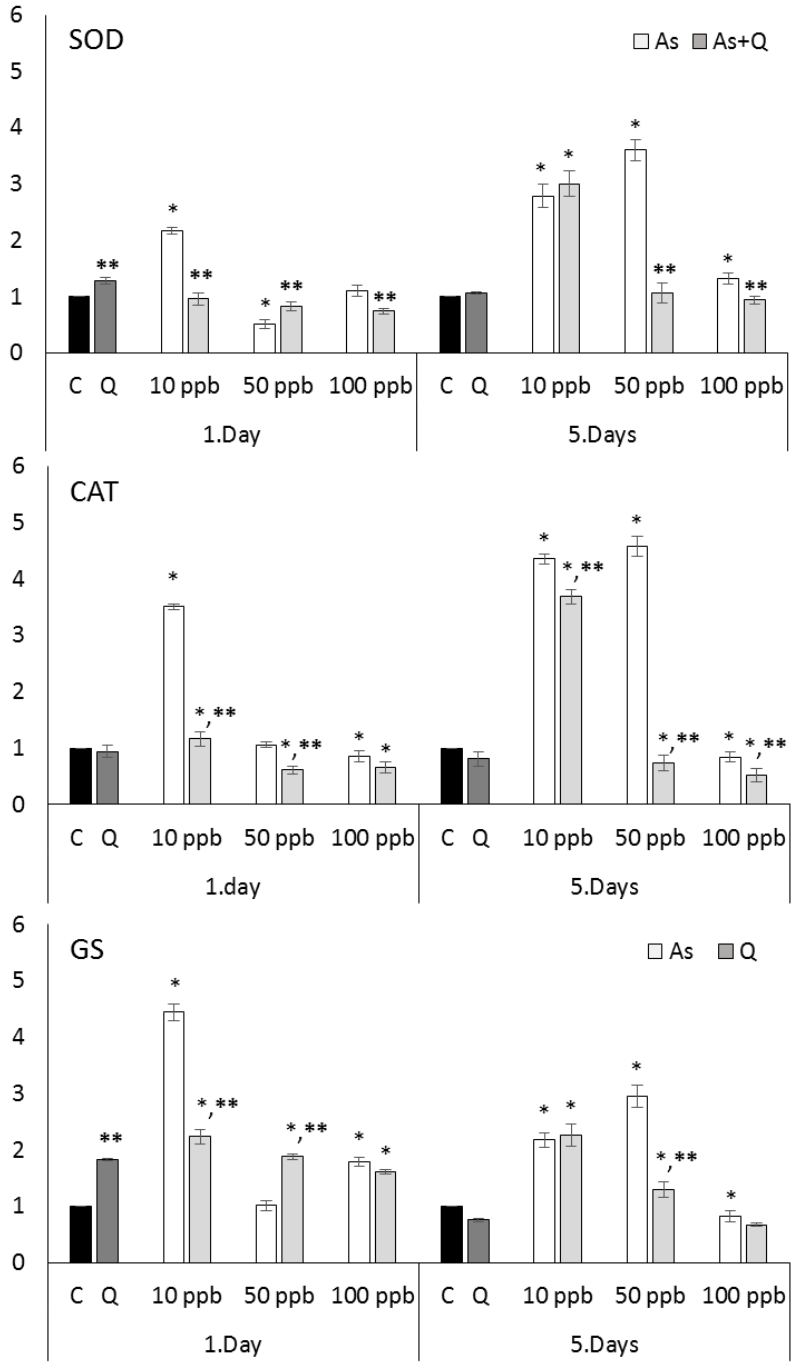

Figure 1. Quantitative real time PCR (qRT-PCR) analysis of catalase (CAT), glutathione synthetase (GS), Superoxide dismutase (Mn-SOD) in As and As+Quercetin exposed adult flies of D. melanogaster for 1 and 5 days. All data normalized with Rp 49 expression and given as relative to control. (C:Control, Q:Ouercetin)* indicate significantly differences values compared to their respective controls analysed by one-way ANOVA, Duncan test ($p \leq 0.05$), ** indicate significantly differences value between As and As+Quercetin exposure (student T test; $p \leq 0.05$)

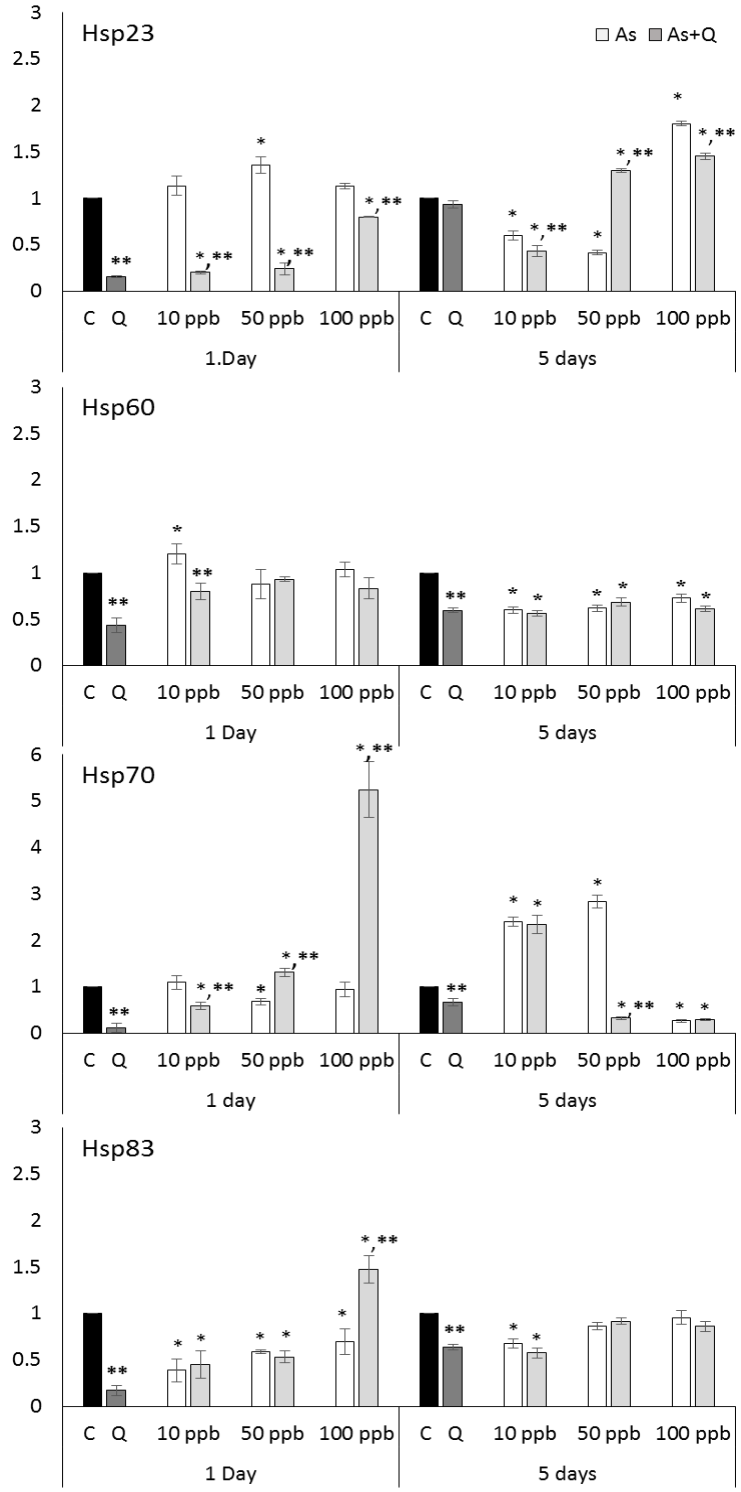

Figure 2. Quantitative real time PCR (qRT-PCR) analysis of Hsp26, Hsp60, Hsp70 and Hsp83 in As and As+Quercetin exposed adult flies of D. melanogaster for 1 and 5 days. All data normalized with Rp 49 expression and given as relative to control. * indicate significantly differences values compared to their respective controls analyzed by one-way ANOVA, Duncan test ($p \leq 0.05$), ** indicate significantly differences value between As and As+Quercetin exposure (student T test; $p \leq 0.05$)

Table 4 Changes in the RAPD profiles based on the appearance (A) and disappearance (D) of bands with specific molecular sizes (bp) using six primers in *D. melanogaster*

	Primers	C		**Quercetin**	10 As	50 As	100 As	10 As+Q	50 As+Q	100 As+Q
1st Day	OPC 183260	8	A	1324; 952; 553	233	233	553; 233	1564; 1012; 553; 233	1564; 1224; 850; 508	1564; 1224; 553;
			D	1050; 871; 519	251	1050; 251	251	1605; 1050; 519; 251	1605; 1250; 871; 519	1605; 1250; 871; 519
	OPC 193470	8	A	1024; 554; 184	596; 194	2034; 1217; 568; 194	730; 547	557; 184	1988; 1024; 551; 184	564; 184
			D	1056; 534; 189	1056; 534; 189	1056; 696; 534	696; 554	696; 534; 189	2034; 1056; 534; 189	534; 189
	OPN 023470	5	A	1019; 648; 329	506	325	533; 327	651; 331	1272; 794; 651; 533; 333	1038; 804; 658; 338
			D	633; 318	519	318	519; 318	633; 318	1240; 774; 633; 519; 318	1240; 774; 633; 318
	OPA 033260	6	A	-	3000; 1168; 1943; 675; 523	3000; 232	3000; 1173; 1033; 672; 525	3000; 234	3000; 1173; 1038; 672; 525	3000; 1043; 675; 527; 236
			D	431	-	-	634; 431	-	-	431
	OPA 043260	4	A	-	-	-	-	880; 368	880	880; 703; 375
			D	406	406	-	406	857; 359	857	857; 686; 406; 359
	OPA 103260	6	A	-	363	1070; 814; 555; 447; 367	370	447; 371	824; 450; 370	828; 563; 456; 376
			D	-	87; 541; 353	1044; 796; 541; 432; 353	796; 587; 541; 353	796; 541; 432; 353	796; 587; 541; 432; 353	1044; 796; 587; 541; 432; 353
	OPC 063260	6	A	1120; 366; 264	-	1120; 224	1120; 1000; 224	1120	1120; 464	1000; 464
			D	1092; 989; 400	400	1092; 989; 400	1092; 989; 400	1092; 989; 400	1092;989; 454; 400	1823; 1092; 989; 454; 400

Table 4 Changes in the RAPD profiles based on the appearance (A) and disappearance (D) of bands with specific molecular sizes (bp) using six primers in *D. melanogaster* (continued)

	Primers	C		Quercetin	10 As	50 As	100 As	10 As+Q	50 As+Q	100 As+Q
5th Day	OPC 183260	8	A	-	829; 230	833; 231	932; 843; 231	843; 220	846	-
			D	-	1016; 544; 502; 243	1016; 708; 502; 243	1556; 1016; 544; 243	1556; 1016; 708; 502; 243	1016; 502	1556; 502
	OPC 193470	8	A	-	819; 700	187	819	589; 182	225; 182	225; 167
			D	-	843; 688	174	843	2020; 1202; 1043; 518; 174	231; 174	2020; 1202; 1043; 231; 174
	OPN 023470	5	A	-	523	669	-	-	-	1051
			D	-	540	655;	-	655	-	-
	OPA 033260	7	A	1157	-	1146; 1038	1043	1053; 534	675	1184; 1073; 996
			D	235	-	-	440; 235	-	-	969; 440; 235
	OPA 043260	4	A	-	-	-	-	-	-	-
			D	-	419; 371	-	-	-	-	371
	OPA 103260	5	A	-	-	-	-	-	383	888; 464
			D	-	-	-	-	1076; 570; 449	449; 378	449
	OPC 063260	4	A	-	-	989; 374	296	1000	-	989; 374; 232
			D	-	1889; 1135	-	-	-	-	1889; 1135

The HSPs are conserved intracellular proteins that commonly known “heat shock” determined in a wide variety of cell belongs to several organisms examined to date (Lindquist & Craig, 1988). In cells, HSP inductions by toxic agents is events of early cytotoxicity and is a subsequent result of damages that affect cellular integrity (Ait-Aissa et al. 2000; Gupta et al. 2010). Heat shock proteins play a survival role against injurious conditions by binding and refolding damaged proteins, for this reason, they are suitable biomarkers to monitor the effect of abiotic and biotic stressors on various organisms. In this study we showed that As exposure at different concentration and treatment times caused changing in the expressions of HSP genes in *D. melanogaster*. Therefore, we thought that the exposed As concentrations caused toxic effects on *D. melanogaster*. According to our results, among the all other HSPs, HSP70 and HSP 26 were found to be more sensitive biomarker with changing expressions levels against As and quercetin exposures. In this study, positive correlation were found between relative expression levels of HSP70 and HSP26, in addition to these heat shock proteins correlated with SOD and CAT genes belongs to antioxidant systems (Table 3). Similar with our results, altered levels of HSPs have been measured in tissues of different organisms exposed to genotoxic agents, such as pesticide (Duffy et al. 1999; Gupta et al. 2007), volatile organic compounds (Singh et al. 2010), heavy metals (Duffy et al. 1999) and acetonitrile (Rossner et al. 2003).

Seven of the ten oligonucleotide primers generated consistently positive results and were used in the analyses of the RAPD profiles. While one primer has yielded no band and banding pattern of the other two primers showed identical bands compared with their respective controls. Using seven primers showed distinguishable band profiling the RAPD analysis showed bands ranging in molecular size from 167 (OPC 193470) to 3000 bp (OPA 033260); a total of 84 bands belonged to the control group, 159 new bands were formed, and 178 lost bands belonged to the treated group. The changes in the RAPD band profiles of these seven primers are given in Table 4 and Fig. 3. Compared with the control bands, band patterns (appearing, disappearing and intensity) showed significant changes especially first day of both single As and As+Q treatment. Based on RAPD profiles of seven primers we calculated both DNA polymorphism (%) and GTS (%) to determine DNA alterations. As seen in Fig. 3, GTS of control group showed no differences between treatment times. Only Quercetin treatment caused reducing of the GTS (%) after first day of treatment. It was showed that increasing treatment time caused returning GTS (%) to control levels. Compared to control and other As concentrations, 100 ppb As treatment caused maximum decrease of GTS in only As treated flies at the first day of treatment. However, As+Q treated *D. melanogaster* individuals exhibited relatively close the GTS values after one day of exposure (10As+Q: 20.71 %, 50As+Q: 13.33%, 100 As+Q: 12.50%). At the first day of exposure, GTS% values of As+Q treated flies were found to be 2.62 fold, 4.13 fold and 3.15 fold lower than 10 ppb, 50 ppb and 100 ppb As treated flies, respectively. At the fifth day of treatment, genomic template stabilities of both As and As+Q treated fruit flies exhibited an increasing trend compared with the first day. However, the increasing in the GTS% of As+Q treated *D. melanogaster* was more evident than only As treated files at the fifth day of exposure.

The changes in the genomic template stability can show not only DNA damage both also DNA repair and replication efficiency (Atienzar et al., 1999). In our study, we showed that As+Q exposure caused further decrease of GTS than only As treatment. We determined that quercetin have a protective role to overcome As toxicity in accordance with effect of quercetin on expressions of antioxidant and heat shock protein genes. When the changes in the gene expressions, RAPD band changes and GTS were considered together, we can say that the quercetin shows protection against As toxicity via changing molecular mechanism responsible for some physiological and/or biochemical changes. These changes in the molecular mechanisms may be caused alternations of RAPD banding patterns, which are directly correlated with GTS. RAPD band polymorphism may be due to the changes in the priming sites of the oligonucleotides caused by genome rearrangement (Doganlar et al. 2014). It was reported that the newly occurring bands, which can cause genomic template instability, can be explained as efficiency of DNA repair and replication mechanisms in cells (Duman et al. 2014). As seen in Table 4, we determined that As+Q exposure caused occurring more new bands than only As treatment at the first day of treatment. Therefore we thought that quercetin can protect *D. melanogaster* to As toxicity by altering some molecular mechanisms.

In conclusion, it was determined that all concentrations of an As treated at drinking water levels permitted by several countries, particularly in the 10 ppb, and under 5 days exposures cause oxidative stress in Drosophila flies. Additionally, expressions of Mn-SOD, CAT, GS belong to antioxidant

systems, HSP70 and HSP26 belong to heat shock protein family genes were determined to be acceptable biomarkers of oxidative stress in fruit fly tissues exposed to As. Our results demonstrated that there is strong interactions between quercetin and cell protective mechanisms such as antioxidant defense enzymes and HSPs proteins. For this reason, we thought that the quercetin is able to successful flavonoids to protect cell damage sources from oxidative stress.

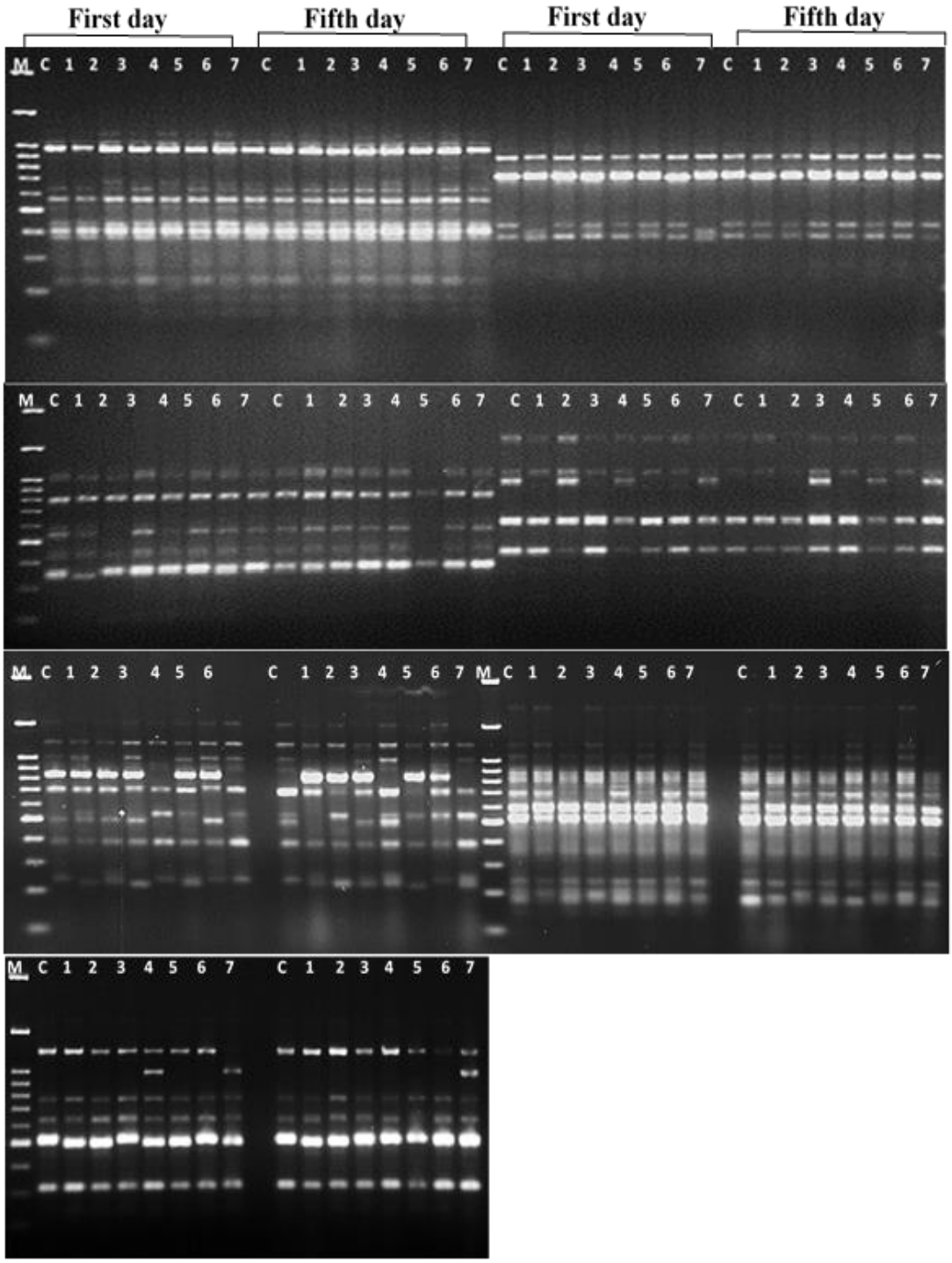

Figure 3 The RAPD profiles of genomic DNAs extracted D. melanogaster adults exposed to Arsenic (M marker, C control, 1=10 ppb As, 2=50 ppb As, 3=100 ppb As, 4= Q, 5=10 ppb As+Q, 6=50 ppb As+Q, 7=100 ppb As+Q) Primers, OPA 033260, OPA 043260, OPA 103260, OPC 063260, OPC 183260, OPC 193470, OPN 023470.

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