RESEARCH COMMUNICATION

Viscum album L. Extract and Quercetin Reduce Cyclophosphamide-Induced Cardiotoxicity, Urotoxicity and Genotoxicity in Mice

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Abstract

Possible protective effects of a methanolic extract of *Viscum album* (VA) and quercetin (QE) against cyclophosphamide (CP) induced cardiotoxicity, urotoxicity and genotoxicity in mice were evaluated. Mice were administered orally VA (250 mg/kg/day) and QE (50 mg/kg/day) for 10 days alone or in combination with CP. After the same doses of VA and QE given for 7 days, rats were intraperitoneally administered CP (40 mg/kg) on days 8 and 9 of the experiment. Cardiotoxic, urotoxic and genotoxic effects were examined in serum, heart, bladder and bone marrow. Significant decreases in the levels of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase), glutathione-S-transferases, reduced glutathione and mitotic index were observed. QE completely and VA partly ameliorated almost of all the examined parameters when given together with CP. Higher total nitrate/nitrite levels were observed in the myocardial tissue treated with QE and VA in combination with CP. In addition, the pre-treatment with VA and QE together with CP significantly decreased chromosome aberrations and aberrant cells compared to CP alone. Results from the current study suggest that QE and VA supplementation attenuates CP induced cardiotoxicity, urotoxicity and genotoxicity through a mechanism related to their ability to decrease oxidative stress and inflammation, and at least in part to its protective effects on the cardiovascular system. In addition, VA and QE may play a role in reducing cytogenotoxicity induced by anti-neoplastic drugs during cancer chemotherapy.

Keywords: *Viscum album* - quercetin - cyclophosphamide - oxidative stress - chromosomal aberrations

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Introduction

Cyclophosphamide (CP) is an alkylating agent and its tumor cell-killing activity is mainly due to its DNA alkylation. Phosphoramide mustard and acrolein are the two active metabolites of CP. CP metabolites can react with carboxyl (-C[O]OH), mercapto (-SH), amino ($-NH_2$), phosphate ($-PO_3H_2$) and hydroxyl (-OH) groups, and can form cross-links with DNA and proteins (Todorova et al., 2009). The precise mechanism by which CP causes toxicity is unknown; however numerous studies have shown that CP exposure can disrupt the redox balance of tissues, suggesting that biochemical and physiological disturbances may result from oxidative stress. Accordingly, the toxic effects of CP on cardiac tissues (Mythili et al., 2005; Senthilkumar et al., 2006; Todorova et al., 2009; Motawi et al., 2010; Nagi et al., 2010) and hemorrhagic cystitis (Bhatia et al., 2006; Linares-Fernández and Alfieri, 2007; Bhatia et al., 2008; Arafa, 2009; Motawi et al., 2010; Santos et al., 2010) were demonstrated in different animal models. Furthermore, in somatic cells, CP has been shown to produce gene mutations, DNA-strand breaks, chromosome aberrations (CA), micronuclei and sister chromatid exchanges in a variety of cultured cells (Bussing et al., 1995; Selvakumar et al., 2006).

European mistletoe (*Viscum album* L.) has been used in the traditional system of medicine for the treatment of various diseases, including cardiovascular illnesses, especially hypertension and cancer (Wagner et al., 1986). Generally represented compounds in *Viscum album* (VA) include the flavonoids (quercetin), terpenoids (beta-amyrin, resin acids, beta-sitosterol, stigmasterol and sterol A), amines and phenolic compounds (Newall et al., 1996).

Quercetin (QE) is a flavonoid present in many vegetables, fruits and beverages. Due to its anti-oxidant, anti-tumor and anti-inflammatory activity, QE has been studied extensively as a chemoprevention agent in several cancer models (Jeong et al., 2009). In addition quercetin minimized the toxic effects of CP on bladder by reducing inflammation (Pincemail et al., 1988) and oxidative stres (Ozcan et al., 2005).

The current study is the first to report on the anticytotoxic and antigenotoxic effects of a methanolic extract of VA or QE on CP in bone marrow cells *in vivo*.

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It has been shown previously that VA (Kuttan et al., 1990; Bussing et al., 1995; Kovacs, 2002) and QE (Farombi and Onyema, 2006; Attia, 2010; Gupta et al., 2010) possess antigenotoxic/antimutagenic activity and reduce the clastogenic effects of antitumor agents.

The clinical efficacy of CP is restricted due to its toxic effects in normal cells. Therefore, it is important to prevent the oxidative stress and DNA damage induced by CP in normal cells in clinical applications. In the present study, we investigated the mitigating effects of QE and VA on CP-induced oxidative stress and inflammation in the heart and bladder and chromosomal damage in the bone marrow cells of mice. The experimental end points included enzymatic superoxide dismutase (SOD),catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and nonenzymatic antioxidant glutathione (GSH) pro-oxidant enzymes; nitric oxide synthases (NOS), myeloperoxidase (MPO) and xanthine oxidase (XO) and also lipid peroxidation (LP) measurement for the determination of oxidative stress and inflammation. Genotoxicity end points included evaluation of mitotic index (MI) and chromosomal aberration (CA) in the bone marrow of mice.

Materials and Methods

Drugs and chemicals

Cyclophosphamide was purchased from ASTA Drug Co.(Turkey), and Quercetin and all other chemicals and reagents were purchased from Sigma-Aldrich Scientific International. Inc. (Hampton. NH. USA).

Collection of plant materials and preparation of Viscum album methanolic extract

Leaves of *V. album* L. ssp. album were harvested in March from the host plant. *Pyrus communis* L ssp. communis (common pear) in the center of Çorum, a city in the central Black Sea region of Turkey. The dried samples were finely chopped in a blender and the soxhlet was extracted exhaustively with 80% methanol. The extract was concentrated under reduced pressure in a rotary evaporator to yield a gum-like brown extract and then refrigerated at 4 ºC until use. A portion of the extract was reconstituted in 0.9 % NaCl before the experiment.

Preliminary dose-finding study

Duration of pretreatment and test doses of VA were decided on the basis of preliminary studies. Three different doses of VA (250. 500 or 1000 mg/kg/day for 10 days) were given to mice (n = 4) orally. The levels of the liver injury markers alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured to determine the optimal dose of VA. ALT and AST values significantly ($p< 0.05$) increased at 1000 mg/kg. The dose of VA (250 mg/kg/day) was selected for the main experiment on the basis of these data.

Animals and treatment

All animal handling procedures and the study design were approved by the Medical Research Ethics Committee of Ondokuz Mayis University in Samsun, Turkey (34/HADYEK, 27th Dec 2007). Six groups of adult and male Swiss albino mice (eight mice/group) each weighing 30-45 g were used. They were housed in cages under laboratory conditions (natural day/night cycle, 21±1°C, standard food rations and tap water freely available) during the experiment. For the control group (C) a total of 1 mL/kg of 0.9% NaCl solution was administered orally by intragastric intubation at the same time every day for 10 days until the mice were euthanized. Methanolic extract of VA (250 mg/kg/day) and QE (50 mg/kg/day) were administered orally by gastric gavage for 10 days in the VA and QE groups, respectively. Cyclophosphamide was administered (40 mg/kg/day, i.p) on days 8 and 9 of the experiment in the CP group. VA and QE were given in combination with CP, as per the CP+VA and CP+QE groups, respectively.

Sample collection and preparation

Immediately after being removed from each animal, the bladder and heart were rinsed thoroughly in cold phosphate-buffered saline (PBS). The atria and ventricles of the heart were opened up to remove the residual blood. The heart tissue (approx. 0.15 g) and bladder tissue (approx. 0.03 g) were pulverized in PBS (0.1 M, pH 7.4) chilled with liquid nitrogen. Two hundred microliters of 50mM PBS at pH 6 and containing 0.5% hexadecyltrimethylammonium were added to an equal volume of heart or bladder homogenate to negate the peroxidase activity of hemoglobin and myoglobin and to solubilize membrane-bound MPO. The rest of the heart and bladder homogenates were centrifuged at 5000 x g for 30 min at 4˚C and supernatants were used for LP and GSH assays. The other part of the heart homogenates were separated and centrifuged again at 12,000 x g for 30 min at 4˚C and supernatants were used for determination of enzyme activities and total NOx level in the heart.

Measurement of serum lactate dehydrogenase

The level of lactate dehydrogenase activity in the serum was determined in a COBAS Integra 800 automated analyzer (Roche Diagnostics, Basel, Switzerland) by using commercial kits supplied by Roche Diagnostic (Mannheim, Germany).

Determination of enzyme activity in the heart

CAT activity in the heart was measured spectrophotometrically at 240 nm by calculating the rate of degradation of H_2O_2 (Aebi, 1984). Superoxide dismutase (EC 1.15.1.1) activity was measured by employing the method of Winterbourn et al. (1975). The rate of inhibition of NBT reduction by superoxide generated by the photoreduction of riboflavin was determined by measuring the absorbance at 560 nm with a T7 spectrophotometer. Glutathione peroxidase (EC 1.11.1.9) activity was measured by the coupled assay method, as described by Lawrance and Burk (1976). The activity of glutathione S-transferase was determined spectrophotometrically at 25°C. with 1-chloro-2.4-dinitrobenzene as the general substrate (Habig et al., 1974). The spectrophotometric assay of XO based on the production of uric acid at 295 nm (extinction coefficient 1.1.104 mM^{-1} cm^{-1}) at 25 °C (Parks

et al., 1988). Myeloperoxidase (EC 1.11.1.7) activity in the heart and bladder tissue was determined by the method of Bradley et al. (1982).

Measurement of reduced glutathione, lipid peroxidation and total NOx (NO_2 + NO_3) level

Tissue levels of GSH were determined by the method described by Moron et al. (1979). It reacts with 5.5 0-dithiobis–2-nitrobenzoic acid to form 5-thiobis–2-nitrobenzoic acid which was detected spectrophotometrically at 412 nm. The extent of lipid peroxidation was determined by the method of Esterbauer and Cheeseman (1990). One of the markers for LP is the production of malondialdehyde (MDA). The LP level in the samples was calculated by using the extinction coefficient of MDA which is 1.56×10^5 M^{-1} cm^{-1}. The enzymatic method was used to determine the NOx level (Doganay et al., 2002). This method employs the NADH-dependent enzyme nitrate reductase for enzymatic reduction of nitrate (NO_3) to nitrite (NO_2) prior to the quantification of NO_2 using Griess reagent. The total amount of nitrite was calculated from a $NaNO_2$ (10-100 μM) standard curve.

Protein content was assayed by employing the method of Lowry et al. (1951) using bovine serum albumin as the standard.

Mitotic index and chromosomal aberrations analysis

Eight animals in each group were used for MI and CA analysis. Cytogenetic analysis of bone marrow cells was carried out according to Preston et al. (1981). Animals were administered an aqueous solution of 2 mg/kg colchicine 2 hr before scheduled euthanasia by cervical dislocation. Both femurs were dissected out and cleaned of any adhering muscle. Bone marrow cells were collected from both the femurs by flushing in isotonic, 0.9% NaCI. The cells were centrifuged at 1200 rpm for 10 min and the pellet was re-suspended in 0.56% KCl and incubated at 37°C for 25 min. Cells were re-centrifuged at 1200 rpm for 10 min and then fixed in chilled Carnoy's fixative (acetic acid: methanol. 1:3. v/v) three times. Fixed cells were re-suspended and dropped onto chilled slides, flame-dried and stained on the following day in 5% buffered Giemsa at pH 6.8.

Slides were examined using a Leica DM2500 light microscope at 1000X magnification. MI was calculated by scoring metaphase cells from 2000 interphase nuclei per animal. CA were calculated from one hundred, well spread, intact metaphase cells per animal and classified according to Savage (1976). The number of each type of aberration, the mean of CA and cells with aberrations were recorded and summarized.

Statistical analyses

Results are expressed as mean ± SD. All statistical comparisons were performed by using one-way analysis of variance (ANOVA) followed by Tukey's HSD multiple comparison test. The computer program (SPSS 12) was used for all procedures and $p < 0.05$ was regarded as statistically significant.

Results

Except for LDH activity, where there was a significant difference ($p<0.05$) for the VA treated group, neither VA nor QE had a significant ($p>0.05$) effect on the evaluated parameters when compared to the control group (Table 1). The intraperitoneal administration of a total of 80 mg/kg of CP 48 h prior to the termination of the experiment caused a significant ($p<0.05$) decrease in antioxidant enzyme activities (CAT, SOD, GPx and GST) and increase in XO activity and NOx level in the heart and LDH in the serum.

Groups that received CP together with QE entirely but CP together with VA partly restored examined parameters of heart as compared to control animals. Although not significant ($p>0.05$), the NO level was increased by the effects of VA, QE and CP alone treatment when compared to the control group. On the other hand, when CP was administered together with the VA or QE, the NO level significantly increased ($p<0.05$) compared to the control group (Table 1).

To examine oxidative stress and inflammation in CP-induced heart and bladder damage we determined the levels of LP, GSH and MPO activity in the heart and

Table 1. The Effect of Viscum Album extract (VA) and Quercetin (QE) on Cyclophosphamide (CP)-Induced Alterations in Catalase (CAT), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), Glutathione-S-Transferases (GST), Xanthine Oxidase (XO) and Nitric Oxide Level (NOx) in Heart and Lactate Dehyrogenase (LDH) Sctivities in Derum in the Control (C), VA, QE, CP, CP+VA and CP+QE Groups in Mice

Groups	CAT	SOD	GPx	GST	XO	NOx	LDH
C	18.2±1.9	9.3±1.5	23,87±1,2	0.8±0.1	2.1±0.5	0.6±0.1	128.6±9.2
VA	19.1±2.4	8.9±1.0	24,36±2,0	0.8±0.1	1.9±0.2	0.7±0.1	109.3±7.8[a]
QE	20.8±2.4	9.3±0.9	25,52±1,7	0.8±0.1	1.8±0.2	0.7±0.1	115.8±8.3
CP	10.6±0.8[a]	3.9±0.6[a]	12,61±1,5[a]	0.4±0.0[a]	5.5±1.0[a]	0.7±0.1	254.7±18.2[a]
CP+VA	16.0±1.8[b]	6.7±1.0[a,b]	19,66±2,4[a,b]	0.7±0.0[b]	3.6±0.6[a,b]	0.9±0.1[a,b]	170.0±9.2[a,b]
CP+QE	17.2±1.5[b]	7.9±1.3[b]	21,98±3,1[b]	0.8±0.0[b]	2.9±0.5[b]	0.8±0.1[a,b]	175.7±9.6[a,b]

VA and QE were given orally to mice 250 and 50 mg/kg/day, respectively for 10 consecutive days and CP (40 mg/kg; i.p.) was administered on the eighth and ninth days. Results are expressed as U/mg protein for CAT, SOD, GPx, GST and mU/ g w.t for XO. Units- CAT:μmoles H_2O_2 decomposed min^{-1} mg^{-1} protein; SOD:50% inhibition of nitroblue tetrazolium min^{-1} mg^{-1} protein; GPx: nmoles of NADPH consumed min^{-1} mg^{-1} protein; GST: μmole of GSH-CDNB conjugate formed min^{-1} mg^{-1} protein; XO: μmol/ min of urate formed at 25 ˚C and pH 7.8. NOx and LDH level expressed as μmol/g w.t (U/L), respectively. Data are the mean ± SD of 8 mice. [a]$p < 0.05$: Compared with control, difference is statistically significant, [b]$p < 0.05$: Compared with CP group, difference is statistically significant.

Table 2. The Effect of Viscum Album Extract (VA) and Quercetin (QE) on Cyclophosphamide (CP)-Induced Alterations in Lipid Peroxidation (LP) and Reduced Glutathione (GSH) Levels and Myeloperoxidase (MPO) Activity in Heart and Bladder in the Control (C), VA, QE, CP, CP+VA and CP+QE Groups in Mice

Groups	Heart			Bladder		
	LP	GSH	MPO	LP	GSH	MPO
C	48.1±5.2	1.2±0.2	23.5±3.4	136.1±26.7	0.2±0.0	4.7±0.6
VA	46.7±5.0	1.3±0.2	19.0±2.8	126.2±30.4	0.2±0.0	4.4±0.5
QE	45.7±4.9	1.3±0.2	19.7±2.9	119.5±25.5	0.2±0.0	4.5±0.5
CP	82.8±8.9[a]	0.8±0.2[a]	38.0±5.0[a]	367.6±72.1[a]	0.1±0.0[a]	10.5±1.4[a]
CP+VA	58.5±8.8[a,b]	1.0±0.1	27.8±2.8[b]	235.9±29.1[a,b]	0.1±0.0[b]	7.1±0.9[a,b]
CP+QE	56.2±7.2[b]	1.2±0.1[b]	25.7±2.8[b]	192.4±42.2[b]	0.2±0.0[b]	5.7±0.8[b]

VA and QE were given orally to mice 250 and 50 mg/kg/day, respectively for 10 consecutive days and CP (40 mg/kg; i.p.) was administered on the eighth and ninth days. Results are expressed as mU/g w.t for MPO activity. Unit-µmol hydrogen peroxide split (1.13 x 10-2/min). LPa and GSH level expressed as nmol/g w.t and GSH µmole/g w.t., respectively. Data are the mean ± SD of 8 mice. [a]p < 0.05: Compared with control, difference is statistically significant, [b]p < 0.05: Compared with CP group, difference is statistically significant.

Table 3. Mitotic Index, Distribution of the Different Types of Chromosomal Aberrations and Aberrant Cells Observed in Mouse Bone Marrow Pre-Treated with Viscum Album (VA) Extract and QE, Alone or in Combination with Cyclosphamide (CP).

Compounds	MI (Mean ± SD)	Chromosomal Aberrations								CA (Mean ± SD)	Aberrant Cells (Mean ± SD)
		P	SCU	B'	B''	F	CF	CE	Total CA		
C	72.6±12.1[b]	1	2	2	-	3	-	-	8	1.6±0.8[b]	1.6±0.8[b]
VA	68.3±7.5[b]	4	1	1	-	3	-	-	9	1.8±0.4[b]	1.6±0.5[b]
QE	63.8±5.4[b]	5	1	1	-	6	1	-	14	2.8±1.0[b]	2.8±1.0[b]
CP	37.1±11.3[a]	14	20	32	4	54	7	10	141	28.2±8.1[a]	23.8±6. 1[a]
VA + CP	62.8±7.5[b]	10	14	12	4	38	13	2	93	18.6±2.7[a,b]	17.4±2.4[a,b]
QE + CP	60.6±5.0[b]	15	13	14	1	37	6	2	88	17.6±3.9[a,b]	16.2±3.1[a,b]

MI: Mitotic index; CA: Chromosomal aberrations; P: Polyploidy; SCU: Sister chromatid unions; B': Chromatid breaks; B'': Chromosome breaks; F: Fragments; CF: Centric fusions; CE: Chromatid exchanges. Data are the mean ± SD of 8 mice. [a]p < 0.05: Compared with control, difference is statistically significant, [b]p < 0.05: Compared with CP group, difference is statistically significant.

bladder tissues of mice. The LP levels in heart and bladder increased significantly by 1.72 and 2.70 fold, respectively, after CP administration (Table 1). Also, the activity of MPO increased ($p<0.05$) by 1.61 and 2.23 fold in heart and bladder tissue, respectively. Furthermore, the levels of GSH in the heart and bladder decreased by 66.66 and 50.00 %, respectively (Table 2).

As shown in Table 2 by the efects of VA and QE (in the groups CP+VA and CP+QE) LP and MPO levels decreased and GSH levels increased significantly ($p<0.05$) in the heart and bladder, as compared to the CP- alone treated group. However the ameliorating effects seen in the QE were more pronounced than in the VA. Lipid peroxidation, MPO activity and GSH level were restored to near normalcy ($p<0.05$) in the CP+QE group but in the CP+VA group only GSH level in the heart caused a significant increase ($p<0.05$) as compared to the control group (Table 2).

The bone marrow chromosomal aberration assay is the widely used test to assess the clastogenic/aneugenic potential of chemicals. Table 3 summarizes the effects of VA extract and QE, singularly or in combination with CP on the MI and CA in mouse bone marrow cells. As expected, CP treatment significantly decreased the MI and induced CA and aberrant cells ($P<0.05$). CP-induced CA consisted mainly of fragments, chromatid breaks and sister chromatid unions. No significant difference in MI values was observed between the animals that received the VA or QE alone or in combination with CP, compared to the control. Pre-treatment with VA and QE improved the mitotic activity against CP. In addition, when mice were pre-treated with VA and QE together with CP, CA and aberrant cell counts were found to significantly decrease compared to CP alone ($p<0.05$). However, the decreased CA and aberrant cell values in the CP+VA and CP+QE groups were still higher ($p<0.05$) than the control group. In other words, pre-treatment with VA and QE in combination with CP did not completely reverse the increased frequency of CA and aberrant cells, as compared to the control group.

Discussion

Results presented showed that 80 mg/kg total cumulative dose of CP increased serum LDH level, which is indicated CP cardiotoxicity, 48 h later. The ameliorating effects of QE on LDH level were also reported (Ikizler et al., 2007; Nagi et al., 2010) as consistent with the present study. The heart is particularly vulnerable to damage induced by ROS because protective enzymes such as SOD and CAT are present at a lower level there than in other tissues of the body (Mojzisová et al., 2006). XO, a flavoprotein which catalyses the oxidation of hypoxanthine to xanthine and generates superoxide and uric acid is one of the main enzymatic sources of ROS *in vivo* (Hayashi et al., 1988). We observed a significant

increase in heart XO activity in mice administered CP, which could be due to increased free radical production and decreased antioxidant enzymes in response to CP. CP- induced cardiotoxicity and decreased antioxidant enzyme levels also were demonstrated in recent reports (Mythili et al., 2005; Senthilkumar et al., 2006; Todorova et al., 2009; Motawi et al., 2010).

GSH is a key regulator of the cellular redox state and the redox environment within the tumor cells determines the response of tumors (and protection of the normal cells) to chemotherapy and radiation (Todorova et al., 2009). CP-induced decrease in GSH level and increased lipid peroxidation were demonstrated in cardiac tissue in previous studies (Mythili et al., 2005; Senthilkumar et al., 2006; Todorova et al., 2009; Nagi et al., 2010). The decreased availability of GSH due to CP partly may be responsible for the decreased activity of GST and GPx in heart tissue because of oxidative modification of their protein structure. Since the bladder is the site for storage of urine and acrolein is excreted via the urine, the overall exposure of the bladder is increased, resulting in a decrease in GSH in the cells as acrolein can pass through the uroepithelium (Bhatia et al., 2008). Our findings show a decrease in the levels of GSH and an increase in lipid peroxidation in the bladder of mice administered CP, which is consistent with other reports recently (Bhatia et al., 2006; Bhatia et al., 2008; Arafa, 2009; Motawi et al., 2010).

The increased antioxidant enzyme activities may reflect an improved antioxidant status of animals pretreated with QE or VA, as indicated by elevation of GSH level and reduction in LP. This changes may be may militate against CP-induced cell damage and oxidative stress. Earlier studies revealed that QE prevented changes in heart mitochondrial enzyme activities and damage to the outer mitochondrial membrane in animals after daunorubicin application (Guzy et al., 2003). A similar cardioprotective effect of QE was also documented by Mojzisová et al. (2006). Recently, it was reported that quercetin attenuates oxidative stress in experimental settings of myocardial I/R (Ikizler et al., 2007; Ahmed et al., 2009; Annapurna et al., 2009). The reported protective action of QE may be related to its antioxidant and metal-chelating activities (Hayashi et al., 1988; Robak and Gryglewski, 1988; Ikizler et al., 2007). Inhibition of XO by QE in the present study may have been caused by its direct scavenging of the superoxide anion (O^{2-}) (Robak and Gryglewski, 1988) or inhibition of O^2-generating enzymes, XO (Hayashi et al., 1988). QE not only has a proven antioxidant effect *in vitro* and *in vivo*, but it may also stimulate glutathione synthesis by modulating gene expression of glutamylcysteine synthase (Moskaug et al., 2005). A possible explanation for the observed physiological and biochemical effects of VA have been attributed to its phytoconstituents, especially those having antioxidant activity, such as the phenolics. The antioxidant activity of phenolics is due to their redox properties which allow them to act as reducing agent, metal chelators and free radical scavengers (Rice-Evans et al., 1996). The antioxidant properties of methanolic extracts of VA have also been demonstrated in previous studies (Onay-Ucar et al., 2006; Oluwaseun and Ganiyu, 2008).

Nitrite levels in our study indicated higher levels of NO in the myocardial tissue treated with QE and VA together with CP. Quercetin causes positive effect on the cardiovascular system due to promotes relaxation of cardiovascular smooth muscle through antihypertensive and antiarrhythmic effects. Quercetin and other grape flavonoids increase endothelial NO levels and smooth muscle relaxation through the up-regulation of cyclic GMP and induce vasodilatation (Fitzpatrick et al., 1993).

VA also has been widely studied for the management of cardiac problems due to its antihypertensive, antiarrhythmic effects. Tenorio et al. (2005) and Tenorio-Lopez et al. (2006) reported that an aqueous extract of Viscum album has hypotensive and vasodilatory effects on the isolated and perfused heart model and that its vasodilatory activity is mediated by increases in NO production.

ROS also contribute to vascular dysfunction and remodeling through oxidative damage. In hypertension, increased production of O^{2-} and hydrogen peroxide (H_2O_2) but decreased NO synthesis and bioavailability of antioxidants. As a consequence of oxidative stress the reduced vascular bioavailability of NO, the most important endogenous vasodilator agent, leads to vasoconstriction, increasing the blood pressure (Fitzpatrick et al., 1993). The increased cardiac NO level caused by VA and QE in this study may be due to their ROS scavening activity. The mitigating effects of both VA and QE on the elevation of cardiac NO levels attributable to CP in the present study brings forward the pharmacological preconditioning inducing property that contributes to the cardioprotective effects of QE and VA.

In the present investigation, CP elevated MPO activity in both heart and bladder tissue, indicating neutrophil accumulation in these tissues. The enhancement of MPO activity following CP administration in rats was demonstrated earlier in bladder (Linares-Fernández and Alfieri, 2007) and heart tissue (Motawi et al., 2007). Inhibition of elevated MPO activity by QE and VA in this study suggests that neutrophil infiltration was restricted. The dominant mechanism for such protection appears to be related to increase NO levels through the effects of QE and VA. The released NO would inhibit platelet and neutrophil aggregations and hence attenuate the elevated MPO activity (Mollace et al., 1991). The other mechanisims is via the ability of QE to effectively inhibit MPO activity, as well as directly scavenge HOCl, may limit the vascular injury associated with inflammatory reactions (Pincemail et al., 1988).

Although CP affects virtually every organ system in the body, the cell populations that typically exhibit rapid cell turnover such as those of the bone marrow and gastrointestinal mucosa, are the most sensitive (Selvakumar et al., 2006). As expected, mice treated with CP in the present study showed a significant decrease in MI and increases in CA and aberrant cells in bone marrow cells when compared to the control group. The improvement in mitotic activity of bone marrow cells of animals pre-treated with VA and QE may further focus attention on the beneficial effect of these substances in overcoming two of the most serious problems in cancer

chemotherapy, which are bone marrow suppression and related immunosuppression. Similar observations related to the antigenotoxic/antimutagenic effects of QE (Farombi and Onyema, 2006; Attia, 2010, Gupta et al., 2010 and VA (Kuttan et al., 1990; Bussing et al., 1995; Kovacs, 2002) have been reported.

In conclusion by scavenging free radicals and bolstering the heart and bladder antioxidant defence system VA and QE were effective in alleviating the oxidative injury to the heart and bladder imposed by CP. Consequently, it is most likely that the cardio- uro- and genoprotective effects of VA extract are due mainly to the antioxidative effects of the respective compound quercetin. These beneficial effects of quercetin in the present study seemed almost comparable with those obtained with VA extract, although recovery in the CP+VA group was lower than for CP+QE. This is probably due to better absorption of QE in comparision to VA, or alternatively, the antioxidant capacity of VA extract at 250 mg/kg/day is less than that of pure phenolic QE at 50 mg/kg/day. The improvement in animals pretreated with VA and QE may focus attention on the beneficial effect of these substances to overcome one of the most serious problems in chemotherapy.

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