Quercetin protects HCT116 cells from Dichlorvos-induced oxidative stress and apoptosis

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Abstract The present study was designed to assess the possible protective effects of Quercetin (QUER), a flavonoid with well-known pharmacological effects, against Dichlorvos (DDVP)-induced toxicity in vitro using HCT116 cells. The cytotoxicity was monitored by cell viability, reactive oxygen species (ROS) generation, anti-oxidant enzyme activities, malondialdehyde (MDA) production, and DNA fragmentation. The apoptosis was assessed through the measurement of the mitochondrial transmembrane potential ($\Delta\Psi_m$) and caspase activation. The results indicated that pretreatment of HCT116 cells with QUER, 2 h prior to DDVP exposure, significantly decreased the DDVP-induced cell death, inhibited the ROS generation, modulated the activities of catalase (CAT) and superoxide dismutase (SOD), and reduced the MDA level. The reductions in mitochondrial membrane potential, DNA fragmentation, and caspase activation were also attenuated by QUER. These findings suggest that dietary QUER can protect HCT116 cells against DDVP-induced oxidative stress and apoptosis.

Keywords Dichlorvos · Quercetin · Oxidative stress · Apoptosis · DNA fragmentation

Abbreviations

OP	Organophosphorous
DDVP	Dichlorvos
QUER	Quercetin
ROS	Reactive oxygen species
$\Delta\Psi_m$	Mitochondrial transmembrane potential

Introduction

Organophosphorus pesticides (OPs) have been widely used for applications in agricultural sites, public health, and individual households in order to raise efficiency of agricultural production and to maintain hygienic conditions (Bardin et al. 1994; Hirose et al. 2011).

Dichlorvos (O,O-dimethyl-2,2-dichlorovinyl phosphate (DDVP)) is one of the commonly used OPs all over the world. It enters into the environment mainly due to its use in agriculture and also as a major degradation product of other OPs, such as trichlorfon, naled, and metrifonate (Hai et al. 1997).

DDVP is a direct-acting inhibitor of acetylcholinesterase (AChE) (WHO 1989), the enzyme that degrades the neurotransmitter ACh in cholinergic synapses, and disrupts nerve function that can lead to the exposed organism death (Varo et al. 2003).

Several in vivo studies have shown the neurotoxic potential of DDVP in exposed organisms (Kaur et al. 2007; Binukumar et al. 2010; Yonguc et al. 2012; Wani et al. 2014). At the cellular level, DDVP-induced neurotoxic effects include reactive oxygen species (ROS) generation and subsequently increased oxidative stress that leads to neuronal cell death (Binukumar et al. 2010a, b; Wani et al. 2014). At organismal

Highlights

Quercetin prevents DDVP-induced toxicity in HCT116 cells.
Cell pretreatment with Quercetin reduces DDVP-induced oxidative stress.
Dietary Quercetin alleviates DDVP-induced apoptosis in HCT116 cells.

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level, DDVP-induced neurotoxicity includes altered motor function (locomotor activity), poor memory, and reduced olfaction (Sarin and Gill 1998; Watson et al. 2014; Ren et al. 2015). Besides, DDVP exposure has been also linked to substantial adverse health effects on other organ systems, including the reproductive system (Okamura et al. 2005; Oral et al. 2006) and respiratory system (Atis et al. 2002).

The prevention of DDVP toxicity involves reduction of pesticide levels in foodstuffs and increasing the intake of diet components such as vitamins and antioxidants. We have previously shown that oxidative stress was involved in DDVP-induced toxicity in HCT116 cells (Ben Salem et al. 2015). Thus, studies on the effect of antioxidants, especially those consumed in food, appear of great interest to prevent DDVP-induced cell damages.

Quercetin (3,5,7,3',4'-pentahydroxyflavon), proven to be the most potent scavenger of free radicals within the flavonoid family, is one of the most widely recognized dietary polyphenolic compounds (Natsume et al. 2009). It is ubiquitously present in foods and is claimed to exert antioxidant and anti-inflammatory activities (Perez-Vizcaino and Duarte 2010). There is evidence that Quercetin reduces low-density lipoprotein oxidation (Loke et al. 2008) and prevents the development of atherosclerotic lesions (Loke et al. 2010). It has also been reported that Quercetin inhibits the production of superoxide anion ($O_2^{\cdot-}$) in rat aorta and decreases protein expression of the NADPH oxidase subunit, p47phox (Sanchez et al. 2006; Romero et al. 2009).

The present study was designed to determine the effect of the dietary flavonoid, Quercetin, against DDVP-induced toxicity in HCT116 cells.

Materials and methods

Chemicals

DDVP, Quercetin, and pyrogallol were purchased from Sigma-Aldrich (St. Louis, MO, USA). 3-4,5-Dimethylthiazol-2-yl,2,5-diphenyltetrazolium bromide (MTT), cell culture medium (RPMI1640), fetal calf serum (FCS), phosphate-buffered saline (PBS), trypsin-EDTA, penicillin and streptomycin mixture, and L-glutamine (200 mM) were from GIBCO-BCL (UK). 2,7-Dichlorofluorescein diacetate (DCFH-DA) was supplied by Molecular Probes (Cergy Pontoise, France). Low-melting-point agarose (LMA) and normal-melting-point agarose (NMA) were purchased from Sigma (St. Louis, MO). All other chemicals used were of analytical grade.

Cell culture and treatment

Human colon carcinoma cells HCT116 were cultured in DMEM-F12, supplemented with 10 % FBS, 1 % L-glutamine

(200 mM), 1 % of mixture penicillin (100 IU/ml), and streptomycin (100 µg/ml), at 37 °C with 5 % CO_2 and 95 % O_2 .

Cell toxicity assay (MTT assay)

The MTT assay (a tetrazolium salt reduction assay) provides sensitive measurements of the normal metabolic status of cells, particularly that of the mitochondrion, where measurements reflect early cellular redox changes (Mosman 1983). HCT-116 cells (2.5×10^5 cells/well in 96-well plates) were incubated at 37 °C for 24 h with DDVP alone or combined to Quercetin (QUER) (5, 10, and 25 µM). A negative control containing only cells was also evaluated. After treatment, the plates were incubated in the MTT solution (final concentration of 0.5 mg/mL) for 3 h. The dark-blue formazan crystals that formed in intact cells were dissolved with DMSO, and the absorbance at 570 nm was measured with a spectrophotometer microplate reader (Biotek Elx 800). The results were expressed as the percentage of MTT reduction relative to the absorbances measured from negative control cells. All assays were performed in triplicate.

ROS determination and oxidative stress status

Reactive oxygen species (ROS) are essential intermediates in oxidative metabolism. Nevertheless, when oxidative stress occurs, ROS are generated in excess and consequently may damage cells by oxidizing lipids and disrupting DNA and proteins. The intracellular amounts of ROS were measured by a fluorometric assay with 2,7-dichlorofluorescein diacetate (DCFH-DA) used extensively to monitor oxidation in biological systems as a well-established compound to detect and quantify intracellular produced such as superoxide radical, hydroxyl radical, and hydrogen peroxide (Cathcart et al. 1983; Gomes et al. 2005; Chen and Wong 2009). The conversion of the non-fluorescent (DCFH-DA) to the highly fluorescent 2,7-dichlorofluorescein product (DCF) ($\lambda_{max}=522$ nm) happens in many steps. The fluorescent probe, after diffusing in the cell membrane, is hydrolyzed by intracellular esterases to non-fluorescent dichlorofluorescein (DCFH), which is trapped inside the cells then oxidized to fluorescent DCF through the action of peroxides in the presence of ROS (Le Bel et al. 1992). HCT-116 cells were seeded on 24-well culture plates (Polylabo, France) at 10^5 cells/well for 24 h of incubation. After, the cells were incubated with DDVP alone or combined to QUER (5, 10, and 25 µM), for 24 h at 37 °C. After incubation, the cells were treated with 20 µM DCFH-DA. Intracellular production of ROS was measured after 30-min incubation at 37 °C by fluorometric detection of DCF oxidation on a fluorimeter (Biotek FL 800×) with an excitation wavelength of 485 nm and emission wavelength of 522 nm. The DCF fluorescence intensity is proportional to the amount of ROS formed intracellularly.

Protein extraction

Cells (10^6 cells/well) were cultured for 24 h in six-well multidishes (Polylabo, France) at 37 °C; then, cultures were incubated for 24 h at 37 °C in the presence of DDVP with/without QUER (5, 10, and 25 μ M). Cells were rinsed with ice-cold PBS, scrapped, collected in a lysis buffer (Hepes 0.5 M containing 0.5 % Nonidet-P40, 1 mM PMSF, 1 μ g/ml aprotinin, 2 μ g/ml leupeptin, pH 7.4), and incubated for 20 min in ice before centrifugation. Protein concentrations were determined in cell lysates using Protein Bio-Rad assay (Bradford 1976).

Measurement of SOD activity

Superoxide dismutase (SOD) activity was determined according to the method described by Marklund and Marklund (1974) by assaying the autooxidation and illumination of pyrogallol at 440 nm for 3 min. One unit of SOD activity was calculated as the amount of protein that caused 50 % pyrogallol autooxidation inhibition. The SOD activity is expressed as units per milligram protein.

Measurement of CAT

Catalase (CAT) activity was measured according to the method described by Aebi (1984) by assaying the hydrolysis of H_2O_2 and the resulting decrease in absorbance at 240 nm over a 3-min period at 25 °C. The activity of CAT was calculated using the molar extinction coefficient (0.04/mM/cm). The results were expressed as micromole per minute per milligram protein.

Lipid peroxidation

Lipid peroxidation was assayed by the measurement of malondialdehyde (MDA) according to the method of Ohkawa et al. (1979). Cells were seeded on six-well plates at 7.5×10^5 cells/well. After 24 h of incubation, they were exposed to DDVP with/without QUER (5, 10, and 25 μ M), for 24 h at 37 °C. Cells were then collected and lysed by homogenization in ice-cold 1.15 % KCl. Samples containing 0.1 ml of cell lysates were combined with 0.2 ml of 8.1 % SDS, 1.5 ml of 20 % acetic acid adjusted to pH 3.5, and 1.5 ml of 0.8 % thiobarbituric acid. The mixture was brought to a final volume of 4 ml with distilled water and heated to 95 °C for 120 min. After cooling to room temperature, 5 ml of mixture of *n*-butanol and pyridine (15:1, v/v) was added to each sample and the mixture was shaken vigorously. After centrifugation at 4000 rpm for 10 min, the supernatant fraction was isolated and the absorbance measured at 546 nm. The concentration of MDA was determined according to a standard curve.

Mitochondrial membrane potential assay

The uptake of the cationic fluorescent dye rhodamine-123 has been used for the estimation of mitochondrial membrane potential (Debbasch et al. 2001). In a typical experiment, the seeded cells in 96-well culture plates were treated with DDVP alone or combined to QUER for 24 h; then, the cells were carefully rinsed with phosphate-buffered saline (PBS), and 100 μ L of rhodamine-123 (1 μ M) in PBS was replaced on the plates. Cells were returned to the incubator (37 °C, 5 % CO_2) for 15 min. Next, the supernatant PBS (containing unuptaken rhodamine-123) was removed and replaced by fresh PBS. The uptake of rhodamine-123 was measured by fluorimetric detection. The results were expressed as the percentage of uptaken rhodamine fluorescence relative to the fluorescence measured from negative control cells.

Caspase activation

The measure of caspase-3 activity was performed using a commercially available kit, according to the manufacturer's instructions and according to Rjiba-Touati et al. (2013). Fifty milligrams of total protein was incubated along with acetylated tetrapeptide (Ac-DEVD) substrate labeled with the chromophore p-nitroaniline (pNA) and seeded in a 96-well microplate. In the presence of active caspase-3, cleavage and release of pNA from the substrate occur. Free pNA produces a yellow color detected by a spectrophotometer at 405 nm. A standard curve was realized in order to determine the correspondence between optical density and pNA concentration; then, the results were expressed as caspase-3-specific activity (pmol pNA/h/mg protein) calculated as indicated by the manufacturers (Promega, France).

DNA damage assessed by the comet assay

Single-cell gel electrophoresis (SCGE) is a visual and sensitive technique for measuring DNA breakage in individual mammalian cells. HCT116 cells were seeded on six-well culture plates (Polylabo, France) at 7.5×10^5 cells/well for 24 h of incubation and were re-incubated as described above in the presence of DDVP for 24 h at 37 °C. Approximately 2×10^4 cells were mixed with 1 % low-melting-point (LMP) agarose in PBS and spread on a microscope slide previously covered with a 1 % normal-melting-point (NMP) agarose in PBS layer. After agarose solidification, cells were treated with an alkaline lysis buffer (2.5 M NaCl, 0.1 M EDTA, 10 mM Tris, pH 10, 1 % (v/v) Triton X-100 and 10 % (v/v) DMSO) for 1 h at 4 °C; then, the DNA was allowed to unwind for 40 min in the electrophoresis buffer (0.3 M NaOH, 1 mM EDTA, pH >13). The slides were then subjected to electrophoresis in the same buffer for 30 min at 25 V and 300 mA. Slides were then neutralized using a Tris buffer solution (0.4 M Tris, pH 7.5) for 15 min.

After staining the slides with ethidium bromide (20 $\mu\text{g/ml}$), the comets were detected and scored using a fluorescence microscope. The experiment was done in triplicate. The damage is represented by an increase of DNA fragments that have migrated out of the cell nucleus during electrophoresis and formed an image of a “comet” tail. A total of 100 comets on each slide were visually scored according to the intensity of fluorescence in the tail and classified by one of five classes as described by Collins et al. (1996). The total score was evaluated according to the following equation: (% of cells in class 0 $\times$ 0)+(% of cells in class 1 $\times$ 1)+(% of cells in class 2 $\times$ 2)+(% of cells in class 3 $\times$ 3)+(% of cells in class 4 $\times$ 4).

Statistical analysis

Data are expressed as the mean $\pm$ standard deviation (SD) of the means. The analysis parameters were tested for homogeneity of variance and normality, and they were found to be normally distributed. The data were therefore analyzed using a one-way analysis of variance (ANOVA) with a post hoc Tukey–Kramer test to identify significance between groups and their respective controls. In all cases, $p < 0.05$ was considered statistically significant.

Results

Measurement of cell viability

HCT116 cells were exposed to increasing concentrations of DDVP (100 to 500 μM) or QUER (100 to 500 μM) for 24 h, and cell viability was determined by MTT assay. Our results indicate that DDVP induced a dose-dependent decrease in cell viability ($P < 0.05$) (Fig. 1a). The IC_{50} value determined after 24 h of cell treatment was about 300 μM . In addition, while QUER alone exhibited no toxicity toward HCT116 cells (Fig. 1a), pretreatment with this flavonoid at 5, 10, and 25 μM significantly decreased DDVP-mediated cytotoxicity. Indeed, the percentage of cell death was about $49.2 \pm 2.4\%$ in the presence of DDVP alone and about 36.3 ± 3.1 , 28.6 ± 2.3 , and $19.6 \pm 1.8\%$ when the cells were pretreated with QUER at 5, 10, and 25 μM , respectively, 2 h before DDVP treatment (Fig. 1b).

QUER inhibits DDVP-induced ROS generation

The toxicity of DDVP has been linked to the generation of oxidative stress in HCT116 cells (Ben Salem et al. 2015). Therefore, we evaluate whether Quercetin (QUER) exerts its proposed anti-oxidant properties and modulates the level of ROS induced by DDVP in HCT116 cells. The level of intracellular ROS was measured after DDVP treatment in the absence or presence of QUER (5, 10, or 25 μM) by recording the

Fig. 1 **a** Cytotoxic effect of DDVP and QUER on HCT116 cells. Cells were treated with DDVP or QUER at the indicated concentrations for 24 h. Cell viability was determined using the MTT assay and expressed as percentages of viability. Values are significantly different ($P < 0.05$) from control. **b** Quercetin reduces DDVP-induced cytotoxicity in HCT116. Cells were pretreated for 2 h with QUER (5, 10, and 25 μM) before DDVP treatment for 24 h (300 μM). Data are expressed as the mean $\pm$ SD of three independent experiments. ** $P < 0.01$ vs. DDVP alone

fluorescence of DCF, which is the result of DCFH oxidation mainly by H_2O_2 . As shown in Fig. 2, DDVP treatment induced a higher level of ROS production to about 2.27-fold to control. Pretreatment with QUER at different doses, 2 h before DDVP treatment, totally abolished the intracellular ROS generated by DDVP in HCT116 cells.

QUER modulates anti-oxidant enzyme activities following DDVP-treatment

SOD catalyzes the dismutation of the highly reactive superoxide anion from oxidative stress to H_2O_2 , which can further be decomposed to water and oxygen by CAT or GPx. Changes in the levels of anti-oxidant enzyme activity can be considered as a fairly sensitive biomarker of the cellular response to oxidative stress. To further characterize the protective effects exerted by QUER, we measured changes in the activities of intracellular anti-oxidant enzymes: SOD and CAT (Fig. 3). Treatment of HCT116 cells with 300 μM DDVP induced significant increases in the enzyme activities; SOD activity

Fig. 2 Effects of Quercetin on DDVP-induced ROS generation. HCT116 cells were pretreated with QUER (5, 10, and 25 μ M) for 2 h before DDVP treatment for 24 h (300 μ M). The relative intracellular ROS production was evaluated by recording the fluorescence of DCF, the product of DCFH oxidation mainly by H_2O_2 . Data are expressed as the mean $\pm$ SD of three separate experiments. ### P <0.01 vs. control, *** P <0.001 vs. DDVP alone

increased from 3.72 ± 1.75 $U_{SOD}/\text{min}/\text{mg}$ of prot in control to 24.13 ± 2.82 $U_{SOD}/\text{min}/\text{mg}$ of prot (Fig. 3a), and CAT activity raised from 5.23 ± 2.34 $\mu\text{mol}/\text{min}/\text{mg}$ of prot in control to

Fig. 3 Effects of Quercetin on superoxide dismutase (a) and catalase (b) activities. HCT116 cells were pretreated with QUER (5, 10, and 25 μ M) for 2 h before DDVP treatment for 24 h (300 μ M). Data are expressed as the mean $\pm$ SD of three separate experiments. ### P <0.001 vs. control, ** P <0.01 and * P <0.05 vs. DDVP alone

38.65 ± 2.76 $\mu\text{mol}/\text{min}/\text{mg}$ of prot (Fig. 3b). However, pretreatment with QUER (5–25 μ M) modulated these activities in a dose-dependent manner. In fact, when the cells were treated with QUER at 25 μ M, 2 h before DDVP treatment, SOD activity was 7.25 ± 1.98 $U_{SOD}/\text{min}/\text{mg}$ of prot (Fig. 3a) and CAT activity was 12.76 ± 2.34 $\mu\text{mol}/\text{min}/\text{mg}$ of prot (Fig. 3b).

Effect of QUER on DDVP-induced lipid peroxidation

The intracellular concentration of MDA was measured in HCT116 cells, as an index of lipid peroxidation. Our results indicate that DDVP treatment significantly increased the intracellular concentration of MDA (0.78 ± 0.03 $\mu\text{mol}/\text{mg}$ prot) which indicates an oxidative insult to cell lipids. However, pretreatment of cells with QUER, 2 h before DDVP treatment, reduced the level of lipid peroxidation. In fact, the MDA level was 0.64 ± 0.02 , 0.42 ± 0.04 , and 0.21 ± 0.04 $\mu\text{mol}/\text{mg}$ prot in the presence of QUER at 5, 10, and 25 μ M, respectively (Fig. 4).

QUER attenuates DDVP-induced loss of mitochondrial transmembrane potential ($\Delta\Psi_m$)

We have previously reported that DDVP induces cell death through the activation of the mitochondrial pathway of apoptosis (Ben Salem et al. 2015). Thus, we assessed the effect QUER on the mitochondrial alterations induced by DDVP. As shown in Fig. 5, DDVP treatment induced a lower level of Rh123 uptake to 44.6 ± 2.1 % indicating a loss of mitochondrial potential. However, when the cells were pretreated with QUER at 5, 10, and 25 μ M, 2 h before DDVP, the percentages of Rh123 uptake were 69.1 ± 2.3 , 77.3 ± 2.4 , and 83.3 ± 2.9 %, respectively. These results indicate that QUER reduces the mitochondrial alterations induced by DDVP in a dose-dependent manner.

Fig. 4 Effects of Quercetin on DDVP-induced lipid peroxidation. HCT116 cells were pretreated with QUER (5, 10, and 25 μ M) for 2 h before DDVP treatment for 24 h (300 μ M). The peroxidation of lipids was recorded by measuring the accumulation of MDA. Data are expressed as the mean $\pm$ SD of three separate experiments. ### P <0.001 vs. control, ** P <0.01 and *** P <0.001 vs. DDVP alone

Fig. 5 Effects of Quercetin on DDVP-induced loss of mitochondrial transmembrane potential. HCT116 cells were pretreated with QUER (5, 10, and 25 μ M) for 2 h before DDVP treatment for 24 h (300 μ M). The mitochondrial potential was assessed by measuring the uptake of rhodamine-123. Data are expressed as the mean $\pm$ SD of three separate experiments. ## P <0.01 vs. control, ** P <0.01 vs. DDVP alone

QUER reduces DDVP-induced caspase activation and DNA fragmentation

Next, we examined the ability of QUER to modulate DDVP-induced caspase activation and DNA fragmentation, two apoptotic hallmarks.

Cell treatment with DDVP alone induced a higher activity of caspase-3 to about 17.58 ± 0.77 pmol pNA/h/ μ g as compared to untreated cells in which the caspase-3 activity was about 2.14 ± 0.33 pmol pNA/h/ μ g of protein. This activity was about 12.76 ± 0.53 , 8.98 ± 0.37 , and 6.34 ± 0.33 pmol pNA/h/ μ g when the cells were pretreated with QUER at 5, 10, and 25 μ M, respectively (Fig. 6a).

DNA damages were analyzed using the alkaline Comet assay. The high sensitivity of the Comet assay allows measurement of DNA fragmentation in individual cells. As shown in Fig. 6b, DDVP induced 121 ± 10 DNA fragmentations, as compared to 29 ± 3.4 DNA fragmentations in controls. Cell pretreatment with QUER induced lower levels of DNA fragmentation which was about 49 ± 7.2 in the presence of QUER at 25 μ M. Taken together, these data demonstrate that dietary QUER protects HCT116 cells from DDVP-triggered caspase activation and DNA fragmentation.

Discussion

Organophosphate (OP) pesticides, also known as cholinesterase inhibitors, are widely used for household control and agricultural pests. Experimental and epidemiological data indicate that the OP compounds, including DDVP, being lipid soluble, can be quickly absorbed from the skin, gastrointestinal tract, and pulmonary route. As previously demonstrated, cell exposure to pesticides evokes an imbalance in the cellular oxidative status and leads to an apoptotic cell death.

Fig. 6 **a** Effects of Quercetin on DDVP-induced caspase-3 activation. **b** Effects of Quercetin on DDVP-induced DNA damage. HCT116 cells were pretreated with QUER (5, 10, and 25 μ M) for 2 h before DDVP treatment for 24 h (300 μ M). Data are expressed as the mean $\pm$ SD of three separate experiments. ### P <0.001 vs. control, * P <0.05 and ** P <0.01 vs. DDVP alone

In this study, we aimed to evaluate the protective effect of Quercetin (QUER), a common food component, against DDVP-induced toxicity in HCT116 cells. Our results show that QUER significantly reduces DDVP-induced cell death; it ameliorates the oxidative status by decreasing ROS generation, modulating the anti-oxidant enzyme activities, and inhibiting lipid peroxidation, and it alleviates DDVP-induced apoptosis.

Our data show that treatment of HCT116 cells with DDVP results in induction of oxidative stress as demonstrated by robust increase in ROS generation. ROS are excessively produced in oxidative stress and cause cell injuries leading to cell death if this balance is disturbed (Kang et al. 2005). On this purpose, the use of anti-oxidants and radical scavengers has been suggested to facilitate the recovery of cell injuries.

In the present study, we demonstrated that cell pretreatment with QUER at low doses (5, 10, and 25 μ M) significantly protected cells from DDVP-induced oxidative stress. In fact, DDVP-induced ROS generation was totally inhibited by QUER.

Cells are equipped with innate anti-oxidant defense system, including non-enzymatic and enzymatic anti-oxidants. SOD and CAT are the most important defense mechanisms against toxic

effects of ROS. SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide, whereas CAT helps in the removal of the H_2O_2 formed during the reaction catalyzed by SOD (Kanbur et al. 2008; Mansour and Mossa, 2009; Liu et al. 2010; Karabacak et al. 2011). Several studies have demonstrated that OP pesticides can increase SOD and CAT activities in different tissues (Tuzmen et al. 2008; Uzun et al. 2010), whereas in other studies, the activities of these enzymes were found to be decreased (Karaoz et al. 2002; Ajiboye 2012). In our study, ROS production as an outcome of oxidative stress induced by DDVP increased the activities of SOD and CAT in HCT116 cells. The increased SOD and CAT may be considered as an adaptive cell response to free radical attack. However, these increases were not sufficient to protect the membrane lipids, since the level of MDA was significantly increased following DDVP treatment. Furthermore, SOD/CAT activities and MDA levels were significantly reduced when the cells were pretreated with QUER, 2 h before DDVP treatment. This may be because QUER can directly scavenge free radicals and/or modulate the biochemical markers of oxidative stress and anti-oxidant enzymes (Gargouri et al. 2011). These results confirm another study conducted in vivo showing that QUER partly protects rats from DDVP-induced renal injuries (Hou et al. 2014).

We have previously reported that apoptosis was executed in response to DDVP-induced oxidative stress. Therefore, to understand whether pretreatment with QUER can also alleviate apoptosis following DDVP exposure, some key factors involved in apoptotic pathway were evaluated in this study.

In fact, when combined to DDVP (300 μ M), QUER (5, 10, and 25 μ M) significantly reduced the apoptosis induced by this pesticide by reducing the loss of membrane mitochondrial potential, mitigating DNA fragmentation, and reducing caspase-3 activation. These findings support that QUER induces an anti-apoptotic activity as described by Zhang et al. (2014) who demonstrated that QUER inhibited microcystin (MCLR)-induced apoptosis in lymphocytes by decreasing the Bax/Bcl-2 ratio.

Inhibition of AChE by OPs is closely linked to oxidative stress (Milatovic et al. 2006; Kaur et al. 2007). Here, we demonstrated that QUER inhibited DDVP-induced cell death by suppressing oxidative stress and alleviating apoptosis. However, more experiments are needed to determine the effect of QUER on DDVP-induced AChE inhibition.

In conclusion, this study provides insight into ways to reduce the toxicity of the commonly encountered pesticide DDVP. We show that QUER, the common anti-oxidant food component, protects HCT116 cells from DDVP-induced cell death. In relation to the public health, we should consume food rich in QUER since there is always a possibility of pesticide contamination in our diet.

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Conflict of interest The authors assure that there are no conflicts of interest.

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