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Research Article

Antigenotoxic effects of the phytoestrogen pelargonidin chloride and the polyphenol chlorogenic acid

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Pelargonidin (PEL), a common anthocyanidin with estrogenic activity, was tested in HL-60 cells for its genotoxicity and possible antigenotoxic effects against 4-nitroquinoline 1-oxide (NQO), a potent mutagen and carcinogen which induces oxidative stress. To take into account potential interactions between phytochemicals within normal human nutrition, we evaluated a combination of PEL with the nonestrogenic phytochemical chlorogenic acid (CLA), one of the most abundant polyphenols in the human diet. PEL ($\leq$2 μM) and CLA ($\leq$800 μM) were nongenotoxic in the micronucleus test. We observed significant antigenotoxic effects against NQO with both compounds, but no additive interaction of PEL and CLA. Comet assay results showed a nonsignificant reduction in NQO-induced DNA damage with both compounds and their combination. Flow cytometric analysis of oxidative stress revealed significant protection against NQO-induced oxidative stress by PEL, CLA, and their combination. Furthermore, PEL and CLA prevented the NQO-induced reduction in GSH level. This could be a mechanism for the observed reduction in genotoxicity. In conclusion, the phytoestrogen PEL revealed antioxidative and antigenotoxic properties in HL-60 cells, but no significant additive interaction with the abundant nutritional polyphenol CLA under the tested conditions.

Keywords: Antigenotoxicity / Comet assay / HL-60 cells / Micronuclei / Oxidative stress

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1 Introduction

Phytochemicals with substantial estrogenic activity are found in many commonly consumed fruits, vegetables, legumes, and whole grains [1–5]. The findings from epidemiological studies suggest that Asian populations consuming a diet rich in phytoestrogens have a lower incidence of osteoporosis, cardiovascular disease, postmenopausal symptoms, breast cancer, and prostate cancer when compared to western populations [1, 4]. As a result, there is extensive use of phytoestrogens as "natural" remedies against age-related diseases and hormone-dependent cancers [2]. The trend of using phytoestrogens is gaining prominence because of the concern over the potential adverse effects of traditional hormone replacement therapy with synthetic estrogens [1]. This situation calls for extensive investigations to assess the potential health effects associated with the large scale intake of natural remedies containing high levels of phytoestrogens because some of these compounds have shown carcinogenic effects in experimental animals [6, 7]. From the standpoint of genotoxicity, it should be emphasized that some of the popular phytoestrogens are known to exert genotoxic effects in mammalian cells [8–13]. On the other hand, there are reports on the antigenotoxic effects of phytoestrogens [14, 15]. Therefore, it would be of interest to know about the genotoxicity of phytoestrogens and their ability to modulate the DNA damaging effects of environmental agents. An additional area of importance is the interaction of phytoestrogens with other bioactive nonestrogenic phytochemicals present in the diet.

Anthocyanidins are aglycones of anthocyanins which give brilliant colors to fruits and vegetables. Both *in vitro* and *in vivo* studies have demonstrated the strong free radical scavenging and antioxidant properties of anthocyanins [16, 17]. At present there is substantial evidence for the chemo-

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Abbreviations: BN, binucleate; **CLA,** chlorogenic acid; **GSH,** glutathione; **MN,** micronucleated; **NQO,** 4-nitroquinoline 1-oxide; **PEL,** pelargonidin; **ROS,** reactive oxygen species

preventive effects of anthocyanins against carcinogenesis in experimental animals [18], and the induction of apoptosis in human cancer cells [19–21]. The most common anthocyanidins found in higher plants are cyanidin, delphinidin, malvidin, pelargonidin (PEL), peonidin, and petunidin [22]. Interestingly, PEL, cyanidin, and delphinidin are non-genotoxic anthocyanidins which have shown estrogenic activity [23]. The above observations and the abundance of anthocyanins/anthocyanidins in the human diet prompted us to initiate work to probe whether or not PEL, a nongenotoxic anthocyanidin with estrogenic activity can exert modulatory effects on oxidative stress and DNA damage induced by 4-nitroquinoline 1-oxide (NQO).

Chlorogenic acid (CLA), the ester of caffeic acid with quinic acid is considered as one of the most abundant polyphenols in the human diet [24]. The antioxidant and anticarcinogenic properties of CLA have been demonstrated in animal models for cancer research [15, 25]. *In vitro* studies have shown its strong free radical scavenging and protective activities towards oxidative damage [26]. Recently, Feng *et al.* [24] demonstrated that CLA exerts its anticarcinogenic effects through up-regulation of cellular antioxidant enzymes and suppression of reactive oxygen species (ROS) mediated nuclear factor kappa-B, activator protein 1 and mitogen activated protein kinases [24]. Because of the presence of CLA in many of the agricultural products for human consumption, it is of interest to know about the possible outcome of an interaction between CLA and dietary phytoestrogens.

Currently, there is a growing awareness of the fact that the antioxidant and anticarcinogenic effects of a diet rich in fruits and vegetables can be attributed to the synergistic and additive effects of the complex mixtures of phytochemicals present in whole foods [27, 28]. *In vitro* studies have furnished evidence for the synergistic interaction among certain phytochemicals for antioxidant activity [29–31]. Furthermore, negative synergism among phytochemicals has also been reported for antioxidant capacity [32]. These findings have highlighted the need to probe whether some of the possible common combinations of phytochemicals can exert antioxidant and antigenotoxic activities which are different from that observed as individual effects of each compound. Therefore, in the present study experiments were carried out to evaluate the modulatory effects of PEL and CLA either individually or as a combination, on genotoxicity and oxidative stress induced by NQO in HL-60 cells.

In this study, we tested the modulatory effects of PEL and CLA against NQO, because it is a well-known directly acting genotoxic chemical carcinogen for inducing intracellular oxidative stress [33]. The recent work of Arima *et al.* [33] has shown that NQO induces ROS formation in mammalian cells. Therefore, NQO is an ideal genotoxin for evaluating the modulatory effects of dietary constituents on induced oxidative stress and DNA damage.

2 Materials and methods

2.1 Materials

If not mentioned otherwise, chemicals were purchased from Sigma–Aldrich, Germany. HL-60 cells were kindly donated by Professor Schinzel, Vasopharm, Würzburg, Germany.

2.2 Cell culture

HL-60 cells, human promyelocytic cells with a population doubling time of about 24 h, were grown at 37°C in a humidified atmosphere of 5% CO_2 in RPMI medium supplemented with 10% fetal calf serum, 1% glutamine, and antibiotics. Cells were routinely split twice a week to keep them in exponential-growth conditions. In all treatments, the final concentration of the solvent, DMSO, was limited to 1%, which was also added to the controls (solvent control).

2.3 Cell viability

HL-60 cells at a density of 2×10^5 cells/mL in 24 well plates were first treated with PEL and/or CLA, and 20 min later NQO was added. One hour later cells were assessed for viability. As a staining solution, 15 µL of fluorescein diacetate (5 mg/mL in acetone) and 30 µL of ethidium bromide (1 mg/mL in H_2O) were added to 2.5 mL of PBS. A 15-µL aliquot of this solution was mixed with 35 µL of cell suspension and after 3 min, 200 cells were counted at a 500-fold magnification using a fluorescence microscope. In viable cells esterases metabolize the colorless fluorescein diacetate to the green fluorescent fluorescein. Ethidium bromide can only enter membrane damaged cells providing a red fluorescent staining of nucleic acids. Thus, viable cells appear green fluorescent, while red fluorescence indicates a damaged cell.

2.4 *In vitro* cytokinesis-block micronucleus test

Exponentially growing HL-60 cells (2×10^5 cells/mL in 5 mL culture medium) were treated for 4 h with test substances. Cytochalasin B in a final concentration of 2 µg/mL was added to obtain binucleated cells. For harvesting, the cells were brought onto glass slides by cytospin centrifugation 20 h later and fixed with methanol (–20°C, 1 h). For the staining of binucleated cells, the slides were incubated with acridine orange (62.5 µg/mL in Sørensen buffer, pH 6.8) for 5 min, washed twice with Sørensen buffer for 5 min, and mounted for microscopy. The frequency of micronuclei was obtained after scoring two times 1000 binucleated cells *per* treatment. In addition, the percentage of binucleated cells was evaluated as a cell-proliferation marker.

2.5 Comet assay

For this assay fully frosted microscope slides, coated with a layer of high melting agarose (1.5%, diluted in calcium and magnesium free, PBS), were used. HL-60 cells (2×10^5 cells/mL in 5 mL culture medium) were treated with PEL and/or CLA for 15 min and then NQO was added. After 1 h (which had been found to be optimal in preliminary experiments) cells were harvested, 20 μL of the cells were suspended in 180 μL of low melting point agarose (0.5% diluted in calcium and magnesium free, PBS), and 45 μL of the suspension was embedded on the frosted microscope slides. Slides were then immersed in a jar, containing fresh cold lysing solution (1% Triton X-100, 10% DMSO, 89%: 10 mmol/L Tris, 1% Na-sarcosine, 2.5 mol/L NaCl, 100 mmol/L Na_2EDTA) for lysis, at 4°C in a dark chamber for 1 h. Next, slides were placed into a horizontal electrophoresis tank with fresh alkaline electrophoresis buffer (300 mmol/L NaOH, 1 mmol/L Na_2EDTA; pH 13) and left for 20 min at 4°C in the dark, to allow DNA unwinding and alkali labile damage expression. Electrophoresis was then carried out, at 4°C in the dark, for 20 min in a 25 V and 300 mA electrical field. Afterwards the slides were neutralized for 5 min in 0.4 mol/L Tris (pH 7.5) and then the DNA was stained by adding 20 μL of 20 μg/L propidium iodide (Molecular Probes, Eugene/USA) to each slide. A fluorescence microscope at 200-fold magnification and a computer-aided image analysis system (Komet 5, Kinetic Imaging) was used for analysis. Fifty cells in total (25 *per* slide) were analyzed and the mean tail DNA (percentage of staining in the tail-region) is given.

2.6 Flow cytometric analysis of oxidative stress

2′,7′-Dichlorodihydrofluorescein diacetate (H_2DCF-DA) was used to detect ROS production in cells. The fluorescence of this cell-permeable agent significantly increases after oxidation [34, 35]. HL-60 cells at a density of 1×10^6 cells/mL in 24 well plates were preincubated with 10 μM H_2DCF-DA for 5 min at 37°C and then PEL and/or CLA, and 20 min later NQO was added. One hour after that – a time that had been determined in preliminary experiments – cells were harvested, washed three times with PBS/1% BSA, and analyzed (3×10^5 cells/sample) by flow cytometry using a FACS LSR I (Becton-Dickinson, Mountain View, CA) after incubation for 10 min on ice with 1 μg/mL propidium iodide (PI). The percentage of PI-positive cells served as a control for toxicity (not shown), the location of the fluorescein-signal on the *x*-axis represents the extent of cellular ROS.

2.7 Glutathione (GSH) determination

Prior to incubation cell density was adjusted to 1.5 – 2.0×10^5 cells/mL in 1 mL treatment volume. DL-buthionin-sulfoximine (500 μM) was added for 24 h to achieve partial glutathione-depletion and thus enhance cellular sensitivity. Test substances (NQO, PEL, and CLA) were then added for 4 h. After incubation cells were washed twice with PBS and resuspended in 400 μL of 1% sulfosalicylic acid. After 15 min incubation on ice, cells were centrifuged ($5000 \times g$). Twenty microliters of the supernatant was mixed with 260 μL of 100 mM phosphate buffer, 20 μL of 2.5 mM dithiobis-2-nitrobenzoic acid, and 300 μL of reductase solution (1.3 U reductase/mL, 50 mM phosphate buffer, 0.5 mM EDTA, 0.3 mM NADPH). The kinetics was determined at 410 nm.

Table 1. Cell viability of HL 60 cells after treatment with NQO, CLA, and PEL chloride. As positive control, cells were treated with 0.1 mM hydrogen peroxide (H_2O_2) for 30 min

Treatment (μM)	Nonviable cells (%)
Control	8.4 ± 3.8
Positive control (H_2O_2)	47.1 ± 29.7*
NQO (0.53)	8.3 ± 4.3
NQO (2.63)	7.5 ± 3.3
PEL (0.5)	8.4 ± 5.0
PEL (1.0)	9.1 ± 3.8
PEL (2.0)	8.6 ± 4.3
CLA (200)	8.8 ± 3.7
CLA (400)	6.9 ± 3.7
CLA (800)	8.3 ± 2.3
NQO (0.53) + PEL (0.5) + CLA (200)	9.1 ± 3.1
NQO (2.63) + PEL (0.5) + CLA (200)	9.8 ± 3.2

Means ± SDs from four independent experiments are shown. From each sample, 200 cells were analyzed for the presence of fluorescein (revealing esterase activity) and ethidium bromide (revealing membrane damage) signals. H_2O_2 = hydrogen peroxide; PEL = pelargonidin, CLA = chlorogenic acid; NQO = 4-nitroquinoline 1-oxide.

* Significantly different from the control at $p < 0.05$ (*t*-test).

3 Results

3.1 Cell viability

For assessing cell viability, the percentage of ethidium bromide negative and fluorescein-positive cells was analyzed following a 1 h treatment of HL-60 cells with PEL, CLA, NQO, and a combination of NQO with PEL and CLA (Table 1). Treatment of HL-60 cells with the applied concentrations of PEL, CLA, and the combination of NQO with PEL and CLA did not reveal any substance toxicity when compared to the control. As in the control, more than 90% of the treated cells showed esterase activity (revealed by green fluorescein fluorescence) and intact cell membranes (revealed by the absence of red ethidium bromide fluorescence). The positive control, hydrogen peroxide, induced a significant increase in the percentage of nonviable cells.

Table 2. Induction of micronuclei by NQO, CLA, and PEL chloride in HL-60 cells

Treatment (μM)	BN cells (%)	MN cells in 1000 BN cells
Control 1	70/73	3.5 ± 2.1
Control 2	67/67	3.5 ± 2.1
NQO (0.53)	53/55	11.0 ± 1.4
CLA (25)	69/71	4.0 ± 1.4
CLA (50)	72/72	4.0 ± 0.0
CLA (100)	72/71	5.0 ± 4.2
CLA (200)	77/74	4.5 ± 0.7
CLA (400)	74/74	3.5 ± 0.7
CLA (800)	73/71	4.0 ± 1.4
PEL (0.0625)	68/70	3.0 ± 1.4
PEL (0.125)	71/71	3.5 ± 2.1
PEL (0.25)	68/70	4.0 ± 1.4
PEL (0.5)	71/71	3.0 ± 0.0
PEL (1)	66/75	3.5 ± 2.1
PEL (2)	67/73	2.5 ± 2.1

Each micronucleus value is the mean ± SD from two slides. One thousand BN cells were evaluated from each slide. For percentage of BN cells (BN cells (%)), individual values are given.

3.2 Assessment of the genotoxic effects of PEL and CLA

The cytokinesis block micronucleus test was carried out to evaluate the genotoxicity of PEL and CLA in HL-60 cells. The data presented in Table 2 show that PEL (0.0625 – 2 μM) and CLA (25 – 800 μM) are nongenotoxic since there is no significant increase in the incidence of micronucleated binucleate (MN BN) cells, when compared to the control. Table 2 also shows that treatment with NQO (0.53 μM) induced an increase in the frequency of micronuclei. The percentage of BN cells – reflecting cell proliferation capacity – was reduced by NQO, but remained unaffected after treatment with PEL or CLA (Table 2).

3.3 Antigenotoxic effects of PEL and CLA against NQO

Modulatory effects of PEL and CLA on the genotoxicity of NQO were assessed by performing the cytokinesis block micronucleus test. The data presented in Table 3 show the results obtained from two independent experiments. Both PEL and CLA exerted significant antigenotoxic effects ($p < 0.05$) against NQO (2.63 μM). The combinations of PEL and CLA (0.5 μM PEL + 200 μM CLA and 0.25 μM PEL + 100 μM CLA) showed significant antigenotoxic effects ($p < 0.05$) against NQO (2.63 μM). However, the level of inhibition of genotoxicity remained similar to that with either PEL (0.5 μM) or CLA (200 μM) alone. There was no indication of an additive effect. No effects on cell proliferation as indicated by the percentage of BN cells was observed after any of the treatments (Table 3).

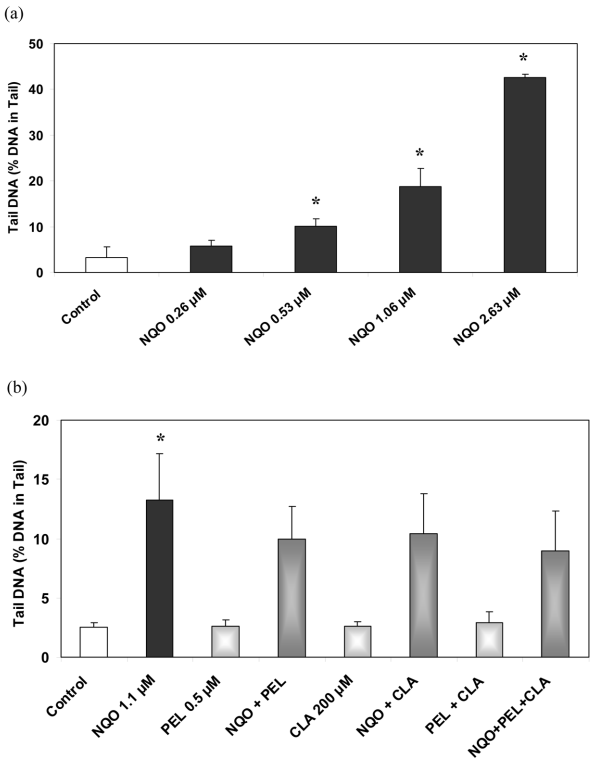

Figure 1. Induction of damage in the comet-assay, (a) by NQO (1 h treatment), and (b) by NQO, PEL chloride, CLA, and combinations (PEL and CLA were added 15 min before NQO). Shown are means of 2 – 3 independent experiments for (a) and of 3 – 4 experiments for (b) combination. The induction of damage was significant for NQO ≥ 0.53 μM ($p \leq 0.05$; *t*-test;*); reduction of NQO-induced damage by PEL and CLA acid did not reach significance.

3.4 Assessement of DNA damage in the comet assay

The comet assay was performed using HL-60 cells to assess DNA damage induced by NQO alone and the modulation of its effects by cotreatment with PEL, CLA, and PEL + CLA. Figure 1a shows the DNA damage induced by four concentrations of NQO alone. A dose-related increase has been observed in DNA damage. Figure 1b illustrates the modulatory effects of PEL, CLA, and PEL + CLA on NQO-induced DNA damage. Both PEL CLA and the combination of PEL and CLA reduced the effect of NQO. However, these reductions did not reach significance.

3.5 Modulation of oxidative stress

Flow cytometric analysis of oxidative stress was carried out following treatment of HL-60 cells with solvent, NQO, PEL, CLA, and PEL + CLA. As shown in Fig. 2, significant reductions in NQO-induced oxidative stress were observed with CLA alone and CLA in combination with PEL. The

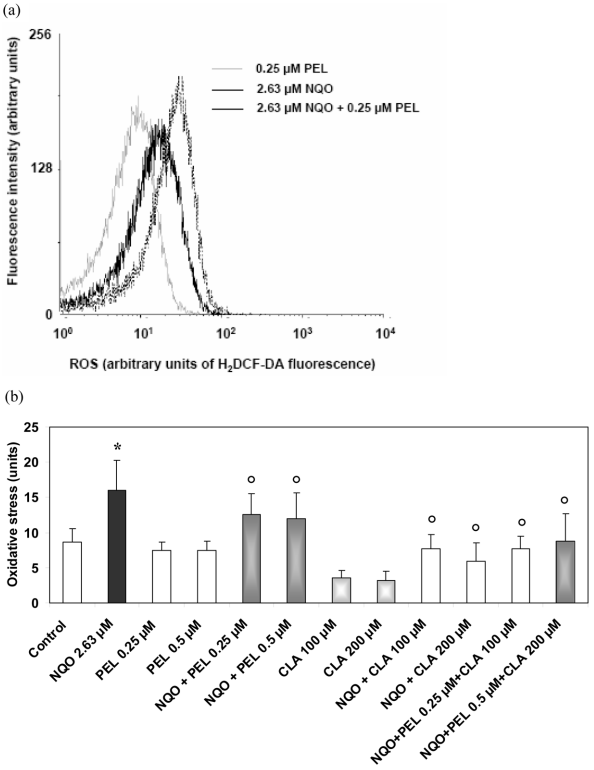

Figure 2. Oxidative stress induction by NQO, PEL chloride, CLA, and combinations after 1 h treatment with NQO (PEL and CLA were added 20 min before NQO). (a) Sample picture of a flow cytometry plot. The location of the cell population distribution curve on the *x*-axis indicates the levels of cellular ROS. (b) Median cellular ROS-levels after the indicated treatments with NQO or NQO and PEL/CLA. Shown are means of at least five samples from at least four independent experiments. Induction of damage by NQO (*) and reduction of NQO-induced damage by PEL, CLA, or combinations (°) were significant ($p \geq 0.05$; *t*-test).

reduction in oxidative stress with PEL alone was less when compared to CLA and CLA + PEL.

Another endpoint for oxidative stress, glutathione-depletion (Fig. 3), yielded a significant reduction in cellular glutathione level after 4 h treatment with NQO. This effect was significantly reduced by PEL and CLA, while the observed reduction by PEL + CLA did not reach statistical significance.

4 Discussion

Our present study has demonstrated that PEL is a nongenotoxic phytoestrogen which reduces NQO-induced oxidative stress and genotoxicity. The intake of such phytoestrogens would possibly be beneficial from the point of human health.

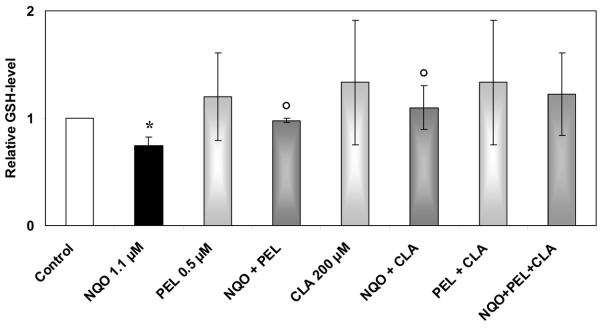

Figure 3. GSH depletion induced by NQO and reduced by PEL chloride, CLA, and combinations after 4 h treatment. Shown are means of three measurements. The effect of NQO (compared to the control; *) and the reduction of the NQO-induced effect by PEL and CLA (°) were significant ($p \geq 0.05$; *t*-test).

An ideal chemopreventive agent against mutagenesis and carcinogenesis is one with no toxicity, high efficacy, capability of oral administration, low cost and known mechanism of action [36]. PEL is endowed with some of the above qualities like absence of toxicity, suitability for oral intake as a constituent of common fruits and known mechanisms of protection like antioxidant and free radical scavenging activity. From the present toxicity tests, it is evident that treatment of HL-60 cells with PEL has not induced a significant increase in *in vitro* cytotoxicity. In addition the *in vitro* genotoxicity tests have demonstrated the nongenotoxic effects of PEL.

Oxidative stress induced by free radicals can cause DNA damage leading to gene mutation and chromosomal alterations. This potentially harmful genomic instability mediated by oxidative damage can be prevented or minimized by the intake of dietary antioxidants. Results obtained from our present study have demonstrated that PEL and CLA can significantly reduce the oxidative stress induced by NQO. Together with these antioxidant effects, we observed antigenotoxic effects of PEL and CLA against NQO in the micronucleus test. These observations indicate that the inhibition of oxidative stress is a possible mechanism which led to the antigenotoxic effects of these phytochemicals. At present there are many reports on the antioxidant activity of PEL [37–40]. Similarly, the role of CLA as an important dietary constituent which reduces oxidative stress is well established [26, 41–43].

Phytochemicals like quercetin, apigenin, and phloretin are known to inhibit cancer cell growth through induction of apoptosis [44]. From our present study, there is no indication of apoptosis induction when HL-60 cells are treated with either PEL or CLA (annexin V-staining, data not shown). This observation is similar to the findings from recent investigations. Hou *et al.* [22] reported that the induction of apoptosis in HL-60 cells by anthocyanidins

Table 3. Inhibitory effects of PEL chloride and CLA either alone or in combination on the induction of micronuclei by NQO in HL 60 cells

Treatment (μM)	Experiment no.	BN cells (%)	MN cells in 1000 BN cells[a)]	% Of NQO alone
Control	1	87–90	5.3 ± 1.2	
	2	81/84	6.5 ± 0.7	
PEL (0.5)	1	87–89	6.5 ± 2.1	
CLA (200)	1	87–89	8.0 ± 2.6	
NQO (0.53)	1	88–91	16.6 ± 2.1*	
NQO (0.53) + PEL (0.5)	1	83–89	11.3 ± 1.2	68
NQO (0.53) + CLA (200)	1	86–87	7.5 ± 0.7°	45
NQO (2.63)	1	86–92	24.6 ± 1.5*	
	2	82/85	27.0 ± 1.4	
NQO (2.63) + PEL (0.5)	1	87–89	10.0 ± 2.6°	41
	2	78/83	14.5 ± 0.7	53
NQO (2.63) + CLA (200)	1	84–89	7.3 ± 2.5°	30
	2	79/83	13.5 ± 2.1	50
NQO (2.63) + PEL (0.5) + CLA (200)	2	78/80	15.0 ± 1.4	55
NQO (2.63) + PEL (0.25) + CLA (100)	2	82/83	14.5 ± 4.9	54

a) Each value is the mean ± SD from either three slides (Experiment 1) or two slides (Experiment 2). One thousand BN cells were evaluated from each slide. For percentage of BN cells (BN cells (%)), individual values (Experiment 2, from two slides) or ranges (Experiment 1, from three slides) are given.

°/* = Significantly different at $p < 0.05$ (*t*-test) from the control (*) or from NQO (°) in the corresponding experiment. Significance calculated only for Experiment 1 (three slides).

showed a structure activity relationship. Anthocyanidins containing single hydroxy group at B-ring such as PEL, peonidin, and malvidin provide no activity; whereas delphinidin, a compound with three hydroxy groups exhibited the highest activity in apoptosis induction. Zheng *et al.* [45] observed that CLA had little modifying effect on apoptosis. Their findings suggest that the tumor preventive effect of CLA might be apoptosis-independent.

Chemical constituents of common fruits and vegetables are known to have complementary and overlapping mechanisms of action which includes antioxidant and scavenging free radicals; regulation of gene expression in cell proliferation, cell differentiation, oncogenes and tumor suppressor genes; induction of cell-cycle arrest and apoptosis; and modulation of enzyme activities in detoxification, oxidation, and reduction [27]. These overlapping mechanisms could lead to additive, synergistic or even antagonistic interaction [15]. Because of the possibility of mechanistic interaction, there is need for studies on the effect of combinations of polyphenolics. A review of literature shows that there are reports on synergistic interaction. Rossetto *et al.* [29] demonstrated how malvidin 3-glucoside recycling by catechin strongly increases the antioxidant efficiency of these two antioxidants. Studies on anticancer effect have shown synergistic interaction between grape extracts and green tea extracts [31]. Furthermore, phytochemicals within cruciferous vegetables have shown synergistic interaction [30]. Antagonistic interaction has been observed for antioxidant effect with combinations of catechin, resveratrol, and quercetin [32]. Both PEL and CLA are well known for their antioxidant activity [26, 40]. However, the results of our present study show that the interaction of these phytochemicals has not led to any significant enhancement of protection against the genotoxicity of NQO, when compared to that observed with PEL/CLA alone.

The absorption, metabolism, and tissue distribution of chemopreventive phytochemicals can play an important role in determining their beneficial effects. There are reports showing that anthocyanins are absorbed unmodified from the diet and distributed to the blood [46–49]. A recent study based on HPLC and LC-MS analyses showed that PEL is absorbed and present in plasma following oral gavage to rats [50]. The main structurally related PEL metabolite identified in plasma and urine was PEL glucoronide.

5 Conclusion

Our present study has demonstrated that PEL, a common anthocyanidin with estrogenic activity is a nongenotoxic phytochemical which can reduce the oxidative stress and genotoxicity induced by NQO in HL-60 cells. Furthermore, there is no clear indication of a significant additive interaction with CLA for inhibition of the genotoxicity of NQO. These findings have highlighted the importance of further studies to evaluate the potential beneficial role this nongenotoxic phytoestrogen can play in reducing the genotoxicity of environmental chemicals.

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