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• *BASIC RESEARCH* •

Protective effects of Asian green vegetables against oxidant induced cytotoxicity

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Abstract

AIM: To evaluate the antioxidant and phase II detoxification enzyme inducing ability of green leaf vegetables consumed in Asia.

METHODS: The antioxidant properties of six commonly consumed Asian vegetables were determined using the ABTS, DPPH, deoxyribose, PR bleaching and iron-ascorbate induced lipid peroxidation assay. Induce of phase II detoxification enzymes was also determined for each respective vegetable extract. Protection against authentic ONOO- and HOCl mediated cytotoxicity in human colon HCT116 cells was determined using the MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasodium bromide) viability assay.

RESULTS: All of the extracts derived from green leaf vegetables exhibited antioxidant properties, while also having cytoprotective effects against ONOO- and HOCl mediated cytotoxicity. In addition, evaluation of the phase II enzyme inducing ability of each extract, as assessed by quinone reductase and glutathione-S-transferase activities, showed significant variation between the vegetables analyzed.

CONCLUSION: Green leaf vegetables are potential sources of antioxidants and phase II detoxification enzyme inducers in the Asian diet. It is likely that consumption of such vegetables is a major source of beneficial phytochemical constituents that may protect against colonic damage.

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Key Words: Cruciferous vegetables; Lipid peroxidation; Free radicals; Isothiocyanates

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INTRODUCTION

Reactive oxygen, nitrogen and chlorine species are generated *in vivo* by a diverse array of mechanisms including inflammatory responses, aerobic metabolism, and exposure to ionizing radiation. For example, the interaction of nitrogen monoxide ($^{\cdot}NO$) and superoxide ($O_2^{\cdot -}$) forms the cytotoxic product peroxynitrite, ($ONOO^-$) (Eq. 1)[1]. $NO + O_2^{\cdot -} \rightarrow ONOO^-$ (1)

Under physiological conditions ONOO- is converted to its protonated form peroxynitrous acid, ONOOH, which in turn decays to generate multiple toxic products with reactivities resembling those of the nitryl cation (NO_2^+), nitrogen dioxide radical ($^{\cdot}NO_2$), and hydroxyl radical ($^{\cdot}OH$). Similarly, at sites of chronic inflammation, neutrophils secrete hydrogen peroxide (H_2O_2) and the enzyme myeloperoxidase (MPO) which catalyzes the formation of hypochlorous acid (HOCl) (equation 2).

$H_2O_2 + Cl^- \rightarrow HOCl + OH^-$ (2)

Up to 80% of the H_2O_2 generated by activated neutrophils is used to form 20-400 μmol/L HOCl an hour[2-4]. Throughout this paper we use the term "hypochlorous acid" (pKa = 7.46) to refer to the approximately 50% ionized mixture of HOCl and OCl- species that exists at physiological pH 6[5]. Both peroxynitrite and HOCl and species derived from it can oxidize lipids, proteins, DNA, and carbohydrates[6-11]. Indeed, the addition of ONOO- or HOCl to biological fluids leads to the depletion of antioxidants including ascorbate, urate, and thiols. Depletion of *in vivo* antioxidant level by RS can initiate and promote cellular damage leading to genotoxicity and disease progression[12]. For example, RS may participate in the carcinogenesis by inducing genetic mutations. Therefore, due to the cytotoxicity of RS considerable interest in identifying chemical agents or dietary constituents that can interfere with RS mediated damage has been sought[13,14]. Plant derived antioxidants have been proposed to fulfill this role[15,16] and much research has focused on the potential antioxidant and cytoprotective or anti-carcinogenic properties of numerous phytochemical compounds[17,18]. These studies have been further supported by epidemiological investigations indicating that dietary habits

Table 1 Total phenolic content and inhibition of PR bleaching mediated by ONOO- and HOCl by green leaf vegetables used in this study.

Common name	Latin name	Total phenoliccontent (GAE mg/g Dwt)	% Inhibition of PR bleaching
Watercress "Rorripa"	*Rorripa nasturtium aquaticum*	133.6±15.1	75.6±3.2[a] 68.6±3.4
Broccoli	*Brassica oleracea var. italica*	61.03±13.1	85.7±10.36[a] 0.6±0.5
Choi Sum	*B.chinensis var. parachinensis*	163.7±2.11	104±6.6[a] 53.3±1.2
Pa Po	*B. chinensis var. parachinensis*	65.7±3.6	91.1±7.2[a] 50.1±1.6
Pheuy leng	*Amaranthus tricolor*	56.2±3.0	102±3.5[a] 50.7±0.2
SioPek	*B. chinensis*	111.0±11.9	76.9±7.8[a] 58.3±0.6

[a]$P<0.05$ *vs* others.

play a significant role in the risk of developing cancer[19]. High consumption of fruits and vegetables, reduced red meat intake and low alcohol consumption appears to be inversely correlated with colon cancer development. In Asia the lower prevalence of degenerative disease like cancer and heart disease are thought to be due to the high consumption of fruits and vegetables[20]. However, little information is available on the antioxidant properties of green vegetables that are widely consumed in Asia. These food stuffs are perhaps a major source of antioxidants and antioxidant like compounds in the Asian diet. Therefore, in this report, we determined the antioxidant properties and phase II enzyme inducing the ability of several green vegetables commonly consumed in Asia. In addition, each vegetable was evaluated for its protective effects against both authentic ONOO- and HOCl mediated cellular toxicity in human colon cells.

MATERIALS AND METHODS

Chemicals

Glutathione (GSH, reduced form), 4-nitrobenzaldehyde (4-NBA), h-nicotinamide adenine dinucleotide (NAD), NADP, NADPH, NADH, 1-chloro-2,4-dinitrobenzene (CDNB), flavin adenine dinucleotide (FAD), 2,6-dichloroindophenol (2,6-DCIP), were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Protein assay kit was purchased from Bio-Rad labs (Hercules, CA, USA).

Plant material and extract preparation

Individual vegetables, broccoli (B), *Rorripa* (R), Sio Pek (SP), Pa Po (PP), Pheuy leng (PL) and Choi Sum (CS) were collected over a 3 mo period from the local supermarkets (Table 1). *Rorripa* and watercress are used interchangeably through the current text. All individual varieties were placed on dry ice and freeze dried immediately to preserve freshness. All individual representative vegetable samples were then pooled, this being conducted to eliminate variation. Extracts were then prepared using the procedure detailed[23,24]. In brief, 100 mg of freeze-dried tissue was weighed into a 50 mL polypropylene tube, hydrated with 2.0 mL of deionised water and homogenized for 15 s (Ultraturrax homogeniser) and left at room temperature for 1 h with occasional vortexing. Boiling 700 mL/L methanol (3.0 mL) was added to the mix and incubated for a further 15 min at +70 °C. The mixture was cooled to room temperature, and centrifuged at 3 000 r/min for 5 min. After centrifugation 1 mL aliquots were removed and vacuum condensed to 200 μL volumes. The resultant concentrates were filtered through sterile non-pyrogenic filters (0.2 μm; Millipore) and stored at -70 °C prior to testing. Extracts gave an equivalent concentration of 100 mg/mL for each sample. All extracts were analyzed for their respective ITC composition using a Finnigan- LCQ LC-MS system using the method previously described.

Cell culture and treatments

Human HCT116 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were grown in complete DMEM (containing 100 mL/L FBS, 100 000 U/L penicillin, 100 mg/L streptomycin, pH 7.4) in 75/cm^2 culture flasks at 37 °C in 50 mL/L CO_2. HOCl concentration was quantified immediately before use spectrophotometrically at 290 nm (pH 12, ε = 350 mol/L/cm). HOCl was diluted in ice cold EBSS to a concentration of 10 mmoL/L and stored on ice for no longer than 1 min. To expose the cells to HOCl, cells were washed twice with PBS and once with EBSS warmed to 37 °C. Fresh EBSS was then added followed by oxidant addition as described. The addition of HOCl did not significantly alter the pH of the reaction mixture. Hydrogen peroxide-free peroxynitrite (ONOO-) was synthesized as described[24], respectively. ONOO- solution was quantified immediately before use using a molar absorption coefficient of 1670/cm/mol/L[5].

ABTS assay

This was carried out as described in Ref.[25] 2,2'-Azinobis[3-ethylbenzothiazoline-6-sulfonate] (ABTS) in water (7 mmol/L final concentration) was oxidized using potassium persulfate (2.45 mmol/L final concentration) for at least 12 h in the dark. The ABTS$^{\cdot}$+ solution was diluted to an absorbance of 0.8±0.05 at 734 nm (Beckman UV-VIS spectrophotometer, Model DU640B) with phosphate-buffered saline (PBS). Absorbance was measured every 1 min for 5 min after initial mixing of extracts of different concentrations or Trolox standard with 1 mL of ABTS$^{\cdot}$+ solution. Trolox was used as a reference standard. Antioxidant properties of extracts were expressed as Trolox equivalent antioxidant capacity (TEAC).

Ascorbate-iron induced lipid peroxidation

Peroxidation of bovine brain extract was performed as described[26]. Briefly, bovine brain extract (BBE, 100 mg) was dissolved with PBS, and sonicated in an ice bath until dissolved. The BBE (0.2 mL) was preincubated with vegetable extracts in the presence of PBS and $FeCl_3$ (1 mmol/L). Lipid peroxidation was initiated by adding ascorbate (1 mmol/L), and the mixture was then incubated

for 1 h at 37 °C. The reaction was stopped by adding butylated hydroxytoluene (2 g/L in 950 mL/L ethanol), followed by the addition of trichloroacetic acid (28 g/L) and 2-thiobarbituric acid (TBA, 1 g/L). The mixture was heated at 80 °C for 20 min in a water bath. The (TBA) 2-MDA (malondialdehyde) chromogen formed was measured at 532 nm after extraction into 1-butanol using a SpectraMax190 microplate reader (Molecular Devices). Results were expressed as percentages of control.

Scavenging of 2,2-diphenyl-1-picrylhydrazyl

Scavenging activity was determined as described in Ref.[27]. DPPH solution (200 µmol/L in 800 mL/L ethanol) was mixed with an equal volume of extracts, and the absorbance at 520 nm was measured after 20 min at room temperature using the microplate reader. Results were expressed as percentages of control (100%).

Assessment of pyrogallol red bleaching by peroxynitrite

Bleaching of PR was performed as described in Ref.[28], respectively. PR (100 µmol/L final concentration) was dissolved in K_2HPO_4-KH_2PO_4 buffer. Compounds to be tested were added into the PR solution and incubated at room temperature for 10 min; addition of ONOO- (200 µmol/L) or HOCl (125 µmol/L) followed and the mixture was vortexed immediately for 10 s. The decrease in absorbance at 542 nm was determined using a microplate reader.

Total phenolic content

Total phenolic content of extracts was assessed approximately by using the Folin-Ciocalteu phenol reagent as described in Ref.[29]. The extracts (100 µL) were mixed with the Folin-Ciocalteu phenol reagent (0.2 mL), water (2 mL), and Na_2CO_3 (15 g/L, 1 mL), and absorbance at 765 nm was measured 2 h after incubation at room temperature using the microplate reader specified above. Gallic acid was used as a reference standard, and the total phenolics were expressed as milligrams per milliliter of gallic acid equivalents (GAEs).

Determination of phase II enzymatic induction and cellular glutathione

Glutathione-S-transferase and quinone reductase activities were determined as previously described[30]. Reduced glutathione levels in HCT116 cells were determined using the procedure described[31]. The formation of the GSH-phthalaldehyde conjugate was measured fluorometrically (excited at a wavelength of 350 nm, and the fluorescence measured at 420 nm).

Statistical analysis

All statistical analysis of data was conducted using MINITAB version 10.1 software package. ANOVA analysis was performed to determine the variation within and between selected populations.

RESULTS

Total phenolic content

The amounts of total phenolics varied widely in the vegetable extracts evaluated, these ranging between 56.2 to 163.7 mg GAE/g dry weight material (Table 1). Among the vegetable extracts, Choi Sum (*B. chinensis var. parachinensis*) contained the highest total amount of phenolics (163.2 mg GAE/g), whereas lower levels were found in Pa Po (*B. chinensis*), broccoli (*Brassica oleracea. var. italica*), and Pheuy leng (*Amaranthus tricolor*), (65.7, 61.0, and 56.2, respectively).

An additional assay, pyrogallol red (PR) bleaching, was used as an initial screening for the protective effects of each vegetable extract (10 mg/mL concentration) against ONOO- and HOCl induced PR bleaching. As shown in Table 1, all extracts showed some degree in preventing both ONOO- and HOCl mediated bleaching.

Radical scavenging by Asian green leaf vegetable extracts

The antioxidant activities of each vegetable extract were determined using four different chemical assays, ABTS, DPPH, deoxyribose and iron ascorbate lipid peroxidation assay. All the methods have been extensively used for the screening of antiradical activities of fruit and vegetable juices and extracts.

The antioxidant properties of each individual vegetable extract are represented in Figure 1A and F. Extracts of each vegetable were examined and compared for their free radical scavenging activities against radical cation ABTS+. The ABTS assay has been widely used to determine the radical scavenging ability of both synthetic chemicals and plant extracts. All extracts showed ABTS+ scavenging capacity as determined by the reduction in absorbance at 734 nm, as previously reported (Figures 1A and B). In addition, comparison with the water soluble analog Trolox allowed us to determine the total antioxidant capacity TEAC value for each extract, none of the extracts were more effective than Trolox alone (Figure 1C). Likewise, as demonstrated in Figure 1D, all vegetable extracts showed antioxidant scavenging potential in the DPPH assay. In additional assays, we also examined the effects of extracts on hydroxyl radicals and lipid peroxidation (Figures 1E and F). Again all extracts showed some positive inhibition in each assay however, we were unable to show any correlation between phenolic acid content and scavenging ability. Kahkonen *et al*[32]. and Shahidi *et al*[33]. reported that differences in antioxidant activities of plant extracts are a likely result of differences in the types of phenolic acids and flavonoid compounds and their derivatives present with in the plant extracts. For example, the antioxidant activities of phenolic acids and their esters are dependant on the number of hydroxy groups in the molecules. Perhaps such limitations are also apparent in our study.

Protection Against ONOO- and HOCl mediated cytotoxicity in HCT116 cells by asian green leaf vegetables

Incubating HCT116 colon cells in the presence of each vegetable extract (0.2-10 mg/mL extract) for 1 h did not result in any significant cytotoxicity measured using the MTT assay (data not shown) whereas the addition of 200 µmol/L ONOO- or 125 µmol/L HOCl led to

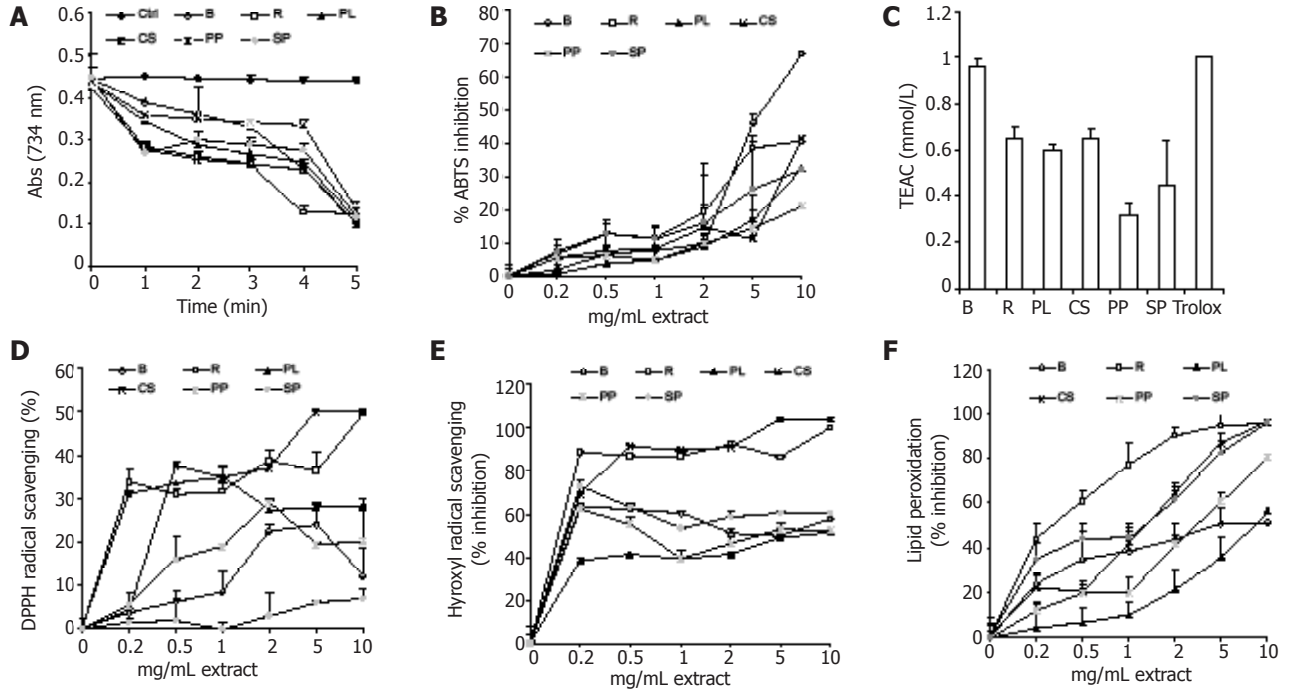

Figure 1 Kinetics of reactions of ABTS radicals in the presence of 10 mg/mL of each vegetable extract (**A**), (**B**) the effects of increased concentration of the vegetable extracts on the inhibition of the ABTS radical represented as % ABTS inhibition, (**C**) total antioxidant activity of extracts from green leaf vegetables as compared to trolox, (**D**) DPPH radical scavenging, (**E**) hydroxyl radical scavenging, and (**F**) iron-ascorbate induced lipid peroxidation. Values are presented as means±SD (n = 6).

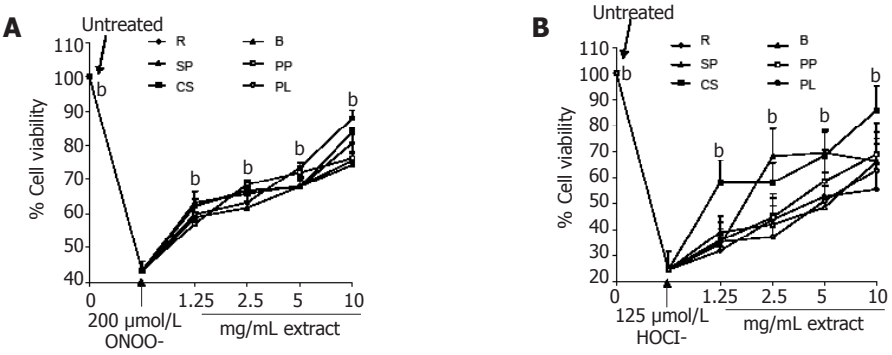

Figure 2 Concentration dependant inhibition of ONOO–- and HOCl mediated cytotoxicity by green leaf vegetable extracts. HCT116 cells were treated with each extract for 5 min and ONOO– or HOCl added. Cell viability was assayed using MTT and measurement of the solubilized formazan dye at Abs 595 nm. Experiments were conducted as described in Materials and methods and data are expressed as mean±SD (n = 6). ^{b}P<0.01 comparing extracts to ONOO– or HOCl treatment alone.

substantial decrease in viability (Figures 3A and B). All extracts significantly inhibited ONOO–- and HOCl mediated cytotoxicity in a concentration-dependent manner.

Induction of phase II detoxification enzymes by Asian green leaf vegetable extracts inhibits ONOO- and HOCl mediated cytotoxicity

The induction of phase II detoxification enzymes QR and GST varied widely among the vegetable extracts evaluated. As shown in Figures 3A and B, both the broccoli and watercress extracts, two species previously been shown as potent inducers of phase II enzymes, showed the most significant induction of QR and GST at 0.02-0.1 mg/mL extract in human HCT116 cells. In contrast, only extracts of Choi Sum (*B. chinensis var. parachinensis*) and Pheuy leng (*Amaranthus tricolor*), showed any potential in inducing QR and GSTs in the current study, all be it at a 10 fold higher concentration (0.1-10 mg/mL extract) than that of broccoli or watercress. Neither Pa Po (*B. chinensis var. parachinensis*) nor Sio Pek (*B. chinensis var. parachinensis*) showed any ability to induce QR or GST (Figure 3C and D). In addition, analysis of each vegetable extract using LC-MS, we were unable to find any putative ITCs present except in any extracts except for broccoli (4-methylsulfinylbutyl ITC) and *Rorripa* (7-methylsulfinylheptyl ITC), respectively. All LC-MS data corresponded to that previously published[23-24]. This may suggest that the other vegetable extracts did not contain any ITCs or that the levels were below the level of detection in our method (Figures 3E and F). To

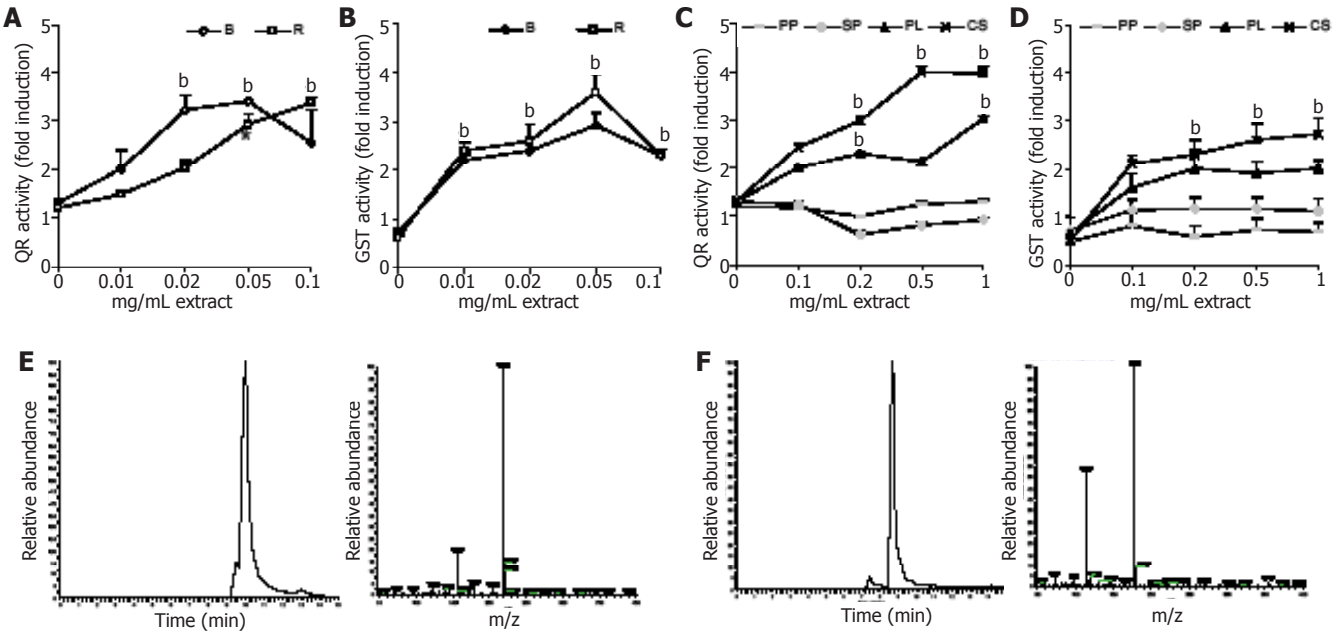

Figure 3 Effect of green vegetable extracts on QR and GST induction in the human colon HCT116 cell line. Cells were treated with each respective extract at 0.01-1 mg/mL (broccoli and *Rorripa*) and 1-10 mg/mL Choi Sum, Pa Po, Pheuy leng and Sio Pek for 24 h. (**A**) induction of QR and (**B**) GST induction by broccoli and *Rorripa*, (**C**) and (**D**) induction of QR and GST by Choi Sum, Pa Po, Pheuy leng and Sio Pek, (**E**) *Rorripa* ITC, 7-methylsulfinylheptyl ITC and (**F**) broccoli ITC, 4-methylsulfinylbutyl ITC as determined using LC-MS. Each data point represents the mean±SD for four separate experiments. ^{b}P<0.001 *vs* the control cells.

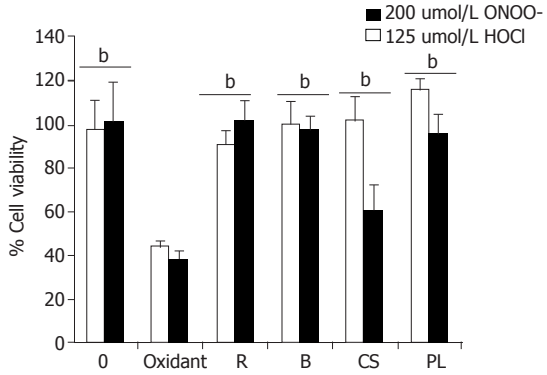

Figure 4 Protective effects of vegetable extracts on ONOO-- and HOCl mediated cytotoxicity in HCT116 cells. Cells were pre-treated with each extract for 24 h to induce phase II detoxification enzymes prior to the addition of ONOO- or HOCl for 30 min. Cell viability was assayed using MTT and measurement of the solubilized formazan dye at Abs 595 nm. Experiments were conducted as described in Materials and methods and data are expressed as mean±SD (n = 6). ^{b}P<0.001 *vs* extracts to ONOO- or HOCl treatment alone.

examine whether the induction of phase II enzymes could protect against oxidative stress, human HCT116 cell were pre-incubated for 24 h with each respective extract at a concentration that induced a 2 fold induction of QR and GST [(0.01mg/mL) broccoli or *Rorripa* and 1 mg/mL for Pheuy leng, and Choi Sum)], respectively. Consequently, intracellular glutathione was determined prior to the addition of the oxidants ONOO- and HOCl. In all the treatment groups particularly broccoli, Rorripa and Choi Sum, intracellular GSH was elevated (Table 2). Moreover, pre-treated cells were more resistant against ONOO- and HOCl mediated toxicity (Figure 4A).

Table 2 Pre-treatment with green leaf vegetables increases intracellular glutathione levels in HCT116 cells. ^{b}P<0.01 comparing treated to control cells (mean±SD).

Vegetable	Total GSH ($nmol^{-1}/mg^{-1}$protein)
Ctrl	15.2±0.4
Watercress "*Rorripa*"	33.2±2.7^{b}
Broccoli	28.7±0.5^{b}
Choi Sum	25.1±1.9^{b}
Pheuy leng	25.2±2.2^{b}

DISCUSSION

In the present investigation we evaluated green leaf vegetables that are commonly consumed in Singapore, this being a representative group of vegetables commonly consumed in the region. Recent epidemiological studies have highlighted a protective effect of vegetable consumption, particularly cruciferous vegetables, on colon cancer development[34-37]. Thus, knowledge of the antioxidant properties of local vegetables may partly explain their beneficial health effects.

The generation of $ONOO^{-}$ and HOCl *in vivo* is implicated in a wide range of human diseases ranging from cancer and cardiovascular diseases to chronic inflammation[12]. The *in vivo* formation of ONOO- in patients with colorectal cancer and a corresponding reduction in plasma antioxidant status has been reported[38]. While increased expression levels of inducible nitric oxide sythase and the formation of ONOO- is observed in patients with ulcerative colitis[39]. Similarly, HOCl produced by inflammatory phagocytic leukocytes reportedly contribute to gastrointestinal mucosal damage[40]. These data being suggestive that

oxidative stress contributes to the pathogenesis of colonic inflammation and cancers. Indeed, supplementation with antioxidants in numerous gastrointestinal model systems has been shown to reduce oxidant induced damage[41-45]. Therefore, agents that are able to protect against ONOO- and HOCl dependent damage may be particularly useful in the diet.

Till date, much is known about the dietary sources and antioxidant properties of vegetables commonly consumed in the Western diet however, much less is known about the antioxidant properties of green leaf vegetables consumed in Asia. In our study, we examined six local vegetables, five being members of the family Cruciferae, and an additional specimen being a spinach substitute used in the region, a member of the genus *Amaranthus* (Table 1). Analysis of total phenolic content using the Folin–Ciocalteu phenol reagent method showed considerable variation among the vegetables studied. Chye Sim had the highest total phenolic content (163.7±2.1 mg/g sample) whereas Pheuy leng had the lowest (56.2±3.0 mg/g sample). Dietary derived phenolic compounds like flavonoids, phenolic acids and condensed tannins are all reported to function as antioxidants. Indeed, in all the antioxidant assays used in the current study, PR bleaching, lipid peroxidation, ABTS, DPPH and hydroxyl radical scavenging; all vegetable extracts, to a varying degree, possessed antioxidant properties. Moreover, co-treatment of human colon cancer HCT116 cell line with individual vegetable extract inhibited both ONOO- and HOCl mediated toxicities in a concentration dependant manner. Several investigators have correlated the antioxidant potential of plant extracts with the content of individual compounds. As well as phenolic constituents many vegetables also contain fat-soluble vitamins and precursors, such as tocopherols and carotenoids, along with the water-soluble vitamin ascorbic acid that also poses antioxidant properties. Indeed, the antioxidant potential of Broccoli has been partially attributed to both flavonoid as well as hydroxycinnamic acid constituents[46]. Flavonoids along with other phenolic constituents are widely distributed in higher plants and exhibit diverse biological activities. For example, the antioxidant action of flavonoids in the GI tract could be mediated by the suppression of ROS formation either by inhibition of enzymes or chelating trace elements involved in free radical production, direct scavenging ROS or the upregulation of antioxidant defense system. Structure function analysis has revealed that the antioxidant properties are dependant upon the extent, type and position of functional group substitutions on the ring structures. In our study we were unable to find any correlation between the antioxidant potential of individual vegetable extracts and their total phenolic content. Our findings are in-agreement with that of Kurilich *et al*[47]. 2002, who demonstrated that the antioxidant properties of different broccoli cultivars did not correlate with their respective ascorbic acid or flavonoid composition. We propose that such discrepancies are a likely result of differences in the chemical composition of each vegetable and that such effects may influence the antioxidant properties as observed in the current study. Also, given that recent evidence suggests that many phenolic compounds such as flavonoids are not absorbed to any appreciable levels *in vivo*, perhaps the major site of action is in the lumen of the GI tract as has previously been suggested[48]. We assume that the observed variation in antioxidant properties and cytoprotective effects are perhaps due to the composition of phenolic compounds present in each vegetable, although this requires further study.

In contrast to direct antioxidant scavenging properties, a secondary mechanism that may protect against oxidative stress is the stimulation of cellular protective pathways. The co-ordinate induction of phase 2 detoxification enzymes provides protection against electrophilic and oxidant induced cellular damage. Interestingly, cruciferous vegetables contain phytochemicals known as glucosinolates that under the action of plant or bacterial myrosinases (thioglucoside glucohydrolase; EC 3.2.3.1) following tissue disruption, are converted to bioactive isothiocyanates (ITC)[49]. ITCs are potent inducers of phase II detoxification enzymes in mammals[23-52]. Indeed, induction of phase II enzymes by the ITC sulforaphane has recently been shown to protect human adult retinal pigment cells, epithelial keratinocytes and murine leukemia cells against oxidant induced damage[50]. Mechanistic studies have also indicated that, depending upon the specific structure of the ITC, these compounds can act at three stages of carcinogenesis. Firstly, they can prevent carcinogen activation through inhibition of phase I enzymes such as cytochrome P450s[51]. Secondly they can induce phase II enzymes such as quinone reductase (QR) [NAD(P)H: (quinone-acceptor) oxidoreductase, EC 1.6.99.2], glutathione S-transferases (GSTs) [EC 2.5.1.18] and UDP-glucuronosyltransferases [EC 2.4.1.17] , resulting ultimately in the excretion of the potential carcinogens[52]. Thirdly, they can induce apoptosis[53-56]. The putative anticarcinogenic activity of ITCs is consistent with the results of epidemiological studies, which have suggested a reduction in risk of cancer, particularly of the gastrointestinal tract, through the consumption of cruciferous vegetables[34,36]. In our, study, we found a 10 fold difference in the ability of the vegetables examined to induce both QR and GSTs in human colon HCT116 cells. Both broccoli and *Rorripa* were the strongest enzyme inducers, this previously being attributed to there ITC composition[23-24]. Of the local vegetables only Choi Sum and Pheuy leng induced any significant increase in QR and GST activity. We assume that such variation in phase II enzyme induction is likely to be a result of the composition and content of ITCs present within each vegetable. Jiao *et al*[57]. 1998, previously demonstrated that considerable variation exists between local cruciferous asian vegetables (low ITC content) when compared to broccoli or *Rorripa* (High ITC content). Moreover, Hecht *et al*[58]. 2004, also reported that cruciferous vegetables collected in Singapore contained high levels of glucobrassicins (70-90%) and relatively low levels of alkyl GSLs, the progenitor compounds of chemicals like sulforaphane. Glucobrassicins, upon tissue disruption form indole-3-carbinols, these compounds have previously been shown to be poor inducers of phase II detoxification enzymes[59].

In summary, our data supports the finds that cruciferous vegetables can decrease *in vitro* and *in vivo* oxidant induced genotoxicity[60] by being potent source of antioxidants that may offer protection against oxidant induced damage in human beings. Moreover, we also found that the phase II enzyme inducing ability varied considerably among the vegetables analyzed, these data suggesting that biomarkers of exposure to cruciferous vegetables may be a more important inclusion into epidemiological studies than previously thought.

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