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Camelina sativa Defatted Seed Meal Contains Both Alkyl Sulfinyl Glucosinolates and Quercetin That Synergize Bioactivity

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ABSTRACT: *Camelina sativa* L. Crantz is under development as a novel oilseed crop, yet bioefficacy of camelina phytochemicals is unknown. Defatted camelina seed meal contains two major aliphatic glucosinolates (GSLs), glucoarabin (9-(methylsulfinyl)nonylglucosinolate; GSL 9) and glucocamelinin (10-(methylsulfinyl)decylglucosinolate; GSL 10), with traces of a third, 11(methylsulfinyl)undecylglucosinolate and several flavonoids, mostly quercetin glycosides. In Hepa1c1c7 cells, hydrolyzed GSLs (hGSLs) 9 and 10 upregulated the phase II detoxification enzyme quinone reductase (NQO1), with no effect on cytochrome P450 (CYP) 1A1 activity. Isobologram graphs revealed synergy of NQO1 induction for a combination of hGSL 9 and quercetin. These findings suggest that defatted camelina seed meal should be evaluated for anticancer activity, similar to broccoli and other Brassicaceae family members. Interestingly, synergy of NQO1 induction was also seen for physiologically relevant doses of sulforaphane (SF) and quercetin, two key bioactives present in broccoli. This suggests that SF within broccoli may be more potent than purified SF.

KEYWORDS: *camelina, glucosinolate, quercetin, NQO1, synergy, sulforaphane*

INTRODUCTION

There is significant interest in developing novel oilseed crops in both the United States and Canada. *Camelina sativa* L. Crantz, a flowering plant belonging to the Brassicaceae family and commonly known as gold-of-pleasure or false flax, is native to Northern Europe and Central Asia, has been cultivated as an oilseed crop. Although, camelina was introduced more recently into North America, radiocarbon dating of archaeobotanical samples identifies its presence from 720 to 520 cal B.C.[1] Because of its high polyunsaturated C18-fatty acid content, less weather dependency, consistent yields, low requirements for tillage and weed control, and relatively cheap production cost, interest in camelina is growing rapidly for use as an oil crop, source of biodiesel production, and renewable feedstock.[2] Camelina oil consists of about 45% polyunsaturated fatty acids, 35% monounsaturated fatty acids, 10% saturated fatty acids, and up to 10% free fatty acids, tocopherols, sterols, other terpenes, and volatiles. High levels of omega-3 fatty acids and antioxidants such as tocopherols have been the focus of potential health benefits of camelina to date.[3,4] Defatted camelina seed consists of approximately 10% residual oils, 45% protein, up to 15% insoluble fiber, up to 10% soluble carbohydrates, 5% minerals, approximately 0.2% nucleic acids, and 14% or more of a mixture of phytochemical components consisting mostly of glucosinolates (GSLs) and flavonoids.[5] Camelina accumulates significant levels of two aliphatic GSL in its seeds: glucoarabin (9-(methylsulfinyl)nonylglucosinolate; GSL 9) and glucocamelinin (10-(methylsulfinyl)decyl-glucosinolate; GSL 10), with a trace amount of a third, 11(methylsulfinyl)undecylglucosinolate (GSL 11).[6] Moreover, mature camelina seed contains several flavonoids, the most plentiful of which is the flavonol quercetin.[7]

GSLs occur as secondary metabolites, mainly in the Brassicaceae. They act as natural pesticides, preventing herbivory.[8,9] Myrosinase, a thiohydrolase present in GSL-containing plants, can cleave glucose from GSL in the presence of water to form an unstable intermediate that rapidly rearranges to a thiocyanate, isothiocyanate, or nitrile; these serve to defend the plant.[10] Importantly, when ingested by mammals, isothiocyanates exhibit chemoprotective activity against the initiation of tumors, inducing detoxification enzymes, inhibiting enzyme activation, modifying steroid hormone metabolism, and generally improving the host-defense system.[11]

The bioactivity of the isothiocyanate sulforaphane (SF) from broccoli has been studied in detail.[12] Although SF and other hydrolysis products may have multiple sites of action, upregulation of the phase II detoxification enzyme NAD(P)-H:quinone oxidoreductase 1 (NQO1), is often used as a biomarker of beneficial bioactivity.[13] In addition to NQO1, aliphatic GSL hydrolysis products are typically associated with upregulation of antioxidant, anti-inflammatory, and anticancer effects. In contrast, aromatic and indolyl GSLs exert mixed effects, often including upregulation of cytochrome P450 (CYP) 1A1 as well as phase II enzymes, such as NQO1. Possibly associated with CYP1A1 induction, the aromatic and indolyl GSLs have been reported to promote some cancers, even though they protect against others.[14] In contrast, aliphatic GSL hydrolysis products have been found to act as inhibitors of CYP activation of carcinogens.[15]

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Much research is ongoing to establish camelina as a biofuel crop, yet little is known about the potential bioefficacy of camelina GSLs and flavonoids. Preparative and countercurrent chromatography has been used to purify GSLs and flavonoids from camelina seed extract.[16] The GSLs in camelina are methyl sulfinyl aliphatics, similar to glucoraphanin the parent GSL to SF, suggesting that they may provide health benefits similar to those of SF. However, they are far larger molecules than the bioactive compounds found in common cruciferous vegetables: 9−11 carbons in length, rather than 3−5. For this reason, we decided to evaluate induction of phase I and phase II detoxification enzymes CYP1A1 and NQO1, as biomarkers of potential bioactivity. Because camelina seed extract also contains the bioactive flavonoid quercetin in several glycosidic forms including rutin, we chose to evaluate the impact of GSL 9, GSL 10, quercetin, and rutin individually, in combination, and as the defatted seed meal extract. We did not evaluate GSL 11, as it is present in negligible amounts (<2 mg/g of seed meal). We also evaluated interactive induction of NQO1 activity between major camelina GSLs and the flavonoid quercetin, comparing this to SF and quercetin, to determine if these were additive, synergistic, or antagonistic in their effect on NQO1 induction. A synergy of action has been reported previously for SF and quercetin in the inhibition of self-renewal of pancreatic cancer stem cells.[17] Our aim was to determine if defatted camelina seed meal might be a health promoting byproduct of biofuel production.

MATERIALS AND METHODS

Chemicals. SF was purchased from LKT Laboratories (St. Paul, MN, USA). Glucose-6-phosphate dehydrogenase was obtained from Roche diagnostics (Indianapolis, IN, USA). Fetal bovine serum (FBS) was purchased from Atlanta Biologicals (Flowery Branch, GA, USA). All other chemicals including myrosinase from *Sinapis alba*, quercetin, and rutin were of the highest grade available and purchased from Sigma (St. Louis, MO, USA).

Analytical Sample Preparation and Purification of Bioactive Compounds from Camelina Sseeds. Camelina seeds were harvested according to the method described previously.[16] Camelina GSLs were purified from methanol extracts of defatted meal, using flash chromatography, followed by semipreparative reverse-phase HPLC,[16] or according to the modified counter-current chromatography method described by Toribio et al.[18] Purification of GSLs (GSL 9 > 95%; GSL 10 > 97%), confirmed by NMR addition analysis using acetone as a comparison standard, has been reported in detail elsewhere.[16]

Cell Culture and Treatments. Hepa1c1c7 mouse hepatoma cells (10^4 cells/mL) were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were cultured in α-MEM, enriched with 10% heated and charcoal-inactivated FBS, supplemented with 2.2 g/L $NaHCO_3$, penicillin (100 U/mL), and streptomycin (100 μg/mL), and maintained at 37 °C in 95% air and 5% CO_2. After 24 h incubation, SF (dissolved in DMSO at 0.1% final concentration), GSL 9 or GSL 10 (dissolved in cell culture medium), quercetin (dissolved in DMSO at 0.1% final concentration), and rutin (dissolved in water) were added to the culture medium alone or in combination according to the experimental design and incubated for 24 h. Myrosinase (20 mU/mL) was added to media containing camelina GSLs immediately before adding the media to cultured cells, since early studies showed that, without hydrolysis, GSLs were without effect. Medium treated with GSL 9 and GSL10 plus myrosinase formed the expected isothiocyanate hydrolysis products, confirmed by GC/MS.[16]

MTT Cytotoxicity Assay. Any adverse effects of SF or hydrolyzed GSLs (hGSLs) 9 or 10 on cell growth were determined using a standard MTT assay. Hepa1c1c7 cells were placed in 96-well culture plates (10^4 cells/well) in complete medium and incubated at 37 °C. After 24 h, the medium was replaced with treatment media containing camelina GSLs plus myrosinase (20 mU/mL) and incubated for another 24 h. Cytotoxicity of the hGSLs and SF was then evaluated using the MTT assay,[19] and results were quantified using a μQuant microplate reader (Bio-Tek Instruments, Winooski, VT, USA) at 595 nm. Data are reported as percent cell survival relative to respective controls.

NQO1 Inductive Activity. The NQO1 inducing activity of different samples was estimated as previously described.[20] Hepa1c1c7 cells, grown in α-MEM and enriched with 10% FBS, were seeded in a 96-well plate at a density of 10^4 cells/well and maintained at 37 °C in 95% ambient air and 5% CO_2. After 24 h, cells were exposed to fresh media containing SF, hGSL 9, hGSL 10, quercetin, or rutin for a further 24 h, alone or in combination. Concentrations used for the treatments with hGSL 9, hGSL 10, quercetin, and rutin were 1.25, 2.5, and 5 μM. In a second study, cells were treated with 2.5 mg seed extract/mL and with the individual components, at the concentration present in 2.5 mg seed extract/mL: 0.86 μM hGSL 9, 2.2 μM hGSL 10, together with 1.46 μM quercetin and 0.48 μM rutin. Treated cells were rinsed with phosphate buffer at pH 7.4, lysed with 50 μL 0.8% digitonin in 2 mM EDTA, incubated, and agitated for 10 min. A 200 μL aliquot of reaction mix was added to the lysed cells. Readings were made at five time points, 50 s apart, using a μQuant microplate reader at 610 nm. Immediately after completion of the readings, 50 μL of 0.3 mM dicumarol in 25 mM Tris−Cl buffer was added to each well, and the plate was read again (five time points, 50 s apart) to determine nonspecific MTT reduction. Total protein content was measured by the BioRad assay (Bio-Rad, Hercules, CA, USA) following manufacturer's instruction. Activity was expressed as NQO1 specific activity (nmol MTT reduced/min/mg protein). For each study, incubations were carried out in triplicate; studies were repeated three times.

Measurement of CYP1A1 Catalytic Activity. Hepa1c1c7 cells were seeded on 96-well plates at a density of 10^4 cells/well in α-MEM supplemented with 10% FBS and stabilized for 24 h. Cells were then treated for 24 h with 1.25, 2.5, and 5 μM GSLs 9 or 10 plus myrosinase (20 mU/mL) alone, in combination, and in the presence or absence of the CYP1A1 inducing agent β-naphthoflavone (5 μM). The catalytic activity of 7-ethoxyresorufin-*O*-deethylase (EROD) was measured as described elsewhere.[21] Briefly, monolayers were washed with PBS and then treated with 100 μL of serum free medium/well, containing 8 mM 7-ethoxyresorufin and 10 mM dicumarol (to inhibit cytosolic NQO1) followed by 150 μL Tris−MeOH (15% methanol in 50 mM Tris−Cl buffer). Fluorescence was measured at 530 nm excitation and 590 nm emission, using a FLx800 universal microplate reader. Specific activity was expressed as picomoles of resorufin formed per minute per milligram of protein, comparing the resorufin formed to the standard curve of known concentrations of resorufin.

Synergy Study Using Major Camelina GSLs and Flavonoids. To evaluate synergy between camelina bioactives, we developed an isobologram[22] using concentrations of GSL 9 and quercetin, individually or together, that doubled NQO1 activity in Hepa1c1c7 cells after 24 h incubation, compared to activity in untreated cells. We compared this to synergy between SF and quercetin, major bioactives present in broccoli, under similar conditions.

Statistical Analysis. One way analysis of variance (ANOVA) was used to identify differences among treatment groups. Between individual groups, statistical significance was assessed using Student's *t*-test with a *p* value of less than 0.05 indicating a significant difference between data sets.

RESULTS

Impact of Defatted Camelina Seed Extract and Its Individual Bioactive Components on Cellular Survival. We tested the cytotoxicity of hGSL 9, hGSL 10, quercetin, and rutin for three doses 1.25, 2.5, and 5 μM as well as the existing concentration ratios in the seed extract (2.5 mg/mL) (Figure 1A). We also tested the cytotoxicity of the seed extract itself at three doses, 0.5, 1.25, and 2.5 mg/mL (Figure 1B). Neither the

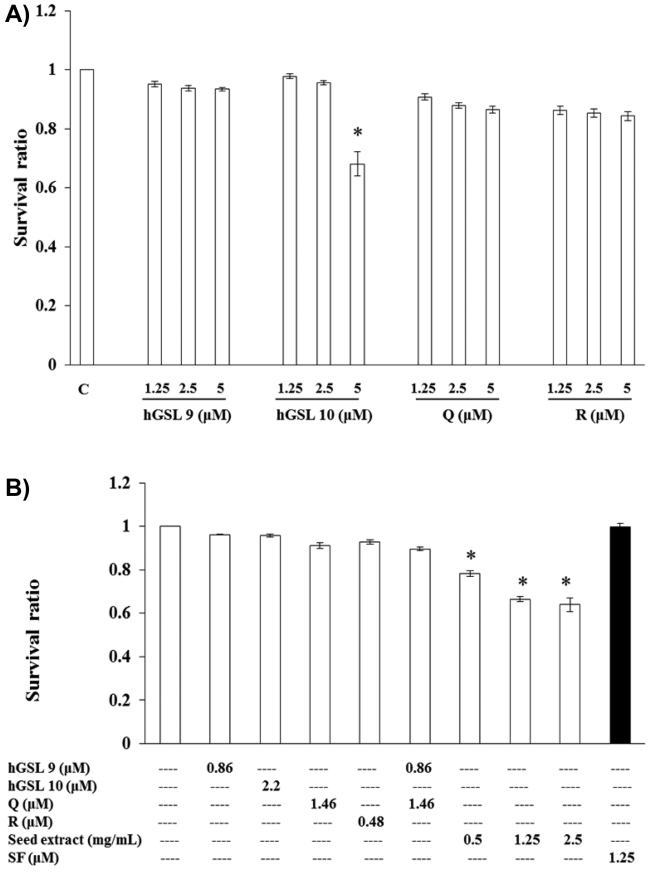

Figure 1. Cytotoxicity of camelina seed extract and of individual bioactives from camelina. (A) Individual cytotoxicity dose–response of hydrolyzed GSLs 9 (hGSL 9) and 10 (hGSL 10), quercetin (Q), and rutin (R). (B) Cytotoxicity of the camelina extract and of the components present in 2.5 mg extract/mL. Survival of treated cells was compared to the survival of the untreated control, which was set to 1. Values are mean ± SD; $n = 3$. *Different from control ($p < 0.05$, using Student's t test).

hGSLs nor the flavonoids from the camelina seed extract exhibited cytotoxicity at the concentrations used to determine bioactivity. However, the highest concentration of hGSL 10 (5 μM) and all the doses of the seed extract showed some cytotoxicity.

Induction of NQO1. We found dose-dependent NQO1 induction with hGSL 9, hGSL 10, quercetin, and rutin, four major bioactives of defatted camelina seed meal (Figure 2). Treatment with 1.25, 2.5, and 5 μM hGSL 9 showed 2.6-, 3.2-, and 3.6-fold induction of NQO1 activity, respectively. Similar inducing activity was seen for hGSL 10, which showed 1.98-, 2.7-, and 3.3-fold induction, respectively. Quercetin also showed a similar range of activity, 1.7-, 2.7-, and 3-fold induction, respectively, as did rutin, with 1.4-, 2.25-, and 2.8-fold induction, respectively (Figure 2). Used as a positive control, SF (1.25 μM) showed 3.5-fold induction over control (Figure 2). In the study of NQO1 induction by the two GSL at the concentrations present in 2.5 mg/mL camelina seed meal extract (0.86 μM GSL 9 and 2.2 μM GSL 10), we found that induction was 1.42-fold and 2.1-fold by hGSLs 9 and 10, respectively. When used in combination at these low concentrations, these two GSLs caused 3.6-fold NQO1 induction, not different from the expected, theoretical value of 3.52 from addition of the individual values (Figure 3). All four major bioactives of camelina seed (GSL 9, GSL 10, quercetin, and rutin) caused NQO1 induction alone and in combination (Figure 4). The combination treatment of bioactives, reflecting that found in 2.5 mg defatted seed extract/mL medium (0.86 μM GSL 9 and 2.2 μM GSL 10, together with 1.46 μM quercetin and 0.48 μM rutin) caused 4.8-fold induction of NQO1, not significantly different from the 5.1-fold induction caused by the 2.5 mg extract/mL (Figure 4).

Interestingly, combination of only hGSL 9 and quercetin at these concentrations caused a far greater than additive, 4-fold induction of NQO1 (Figure 5). No other combination of camelina seed bioactives showed greater than the expected value from the theoretical addition of data from the individual treatments. Rather, combination of hGSL 10 and quercetin

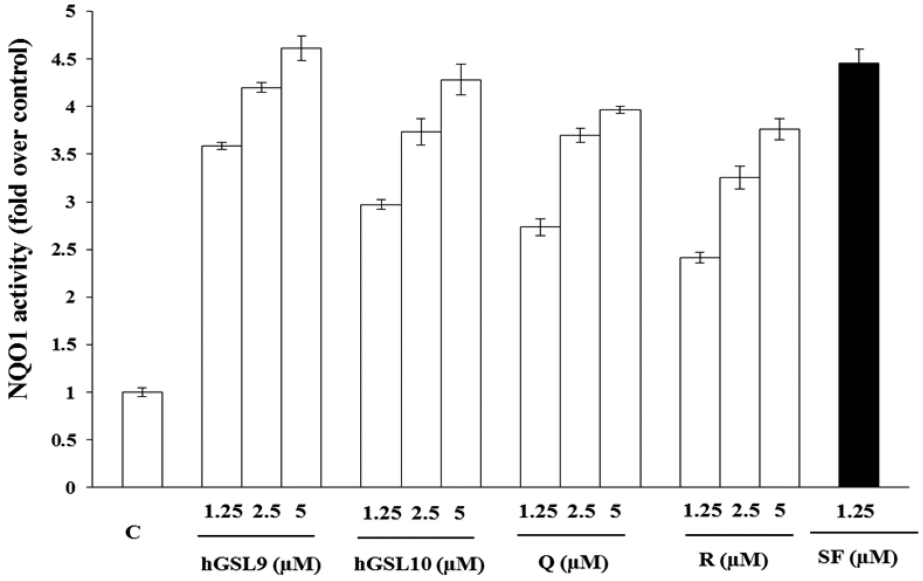

Figure 2. Induction of NQO1 activity by hydrolyzed GSLs 9 (hGSL 9) and 10 (hGSL 10), quercetin (Q), and rutin (R). Sulforaphane (SF) was used as a positive control. Myrosinase (20 mU/mL) was added to the medium for GSL hydrolysis. Relative NQO1 activity was calculated in relation to the untreated control, which was set to 1. Values are mean ± SD; $n = 3$ ($p < 0.05$).

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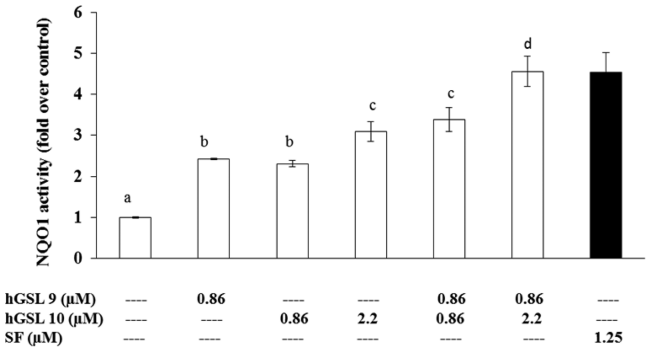

Figure 3. NQO1 activity of hydrolyzed GSLs 9 (hGSL 9) and 10 (hGSL 10) alone and in combination. Sulforaphane (SF) was used as a positive control. Relative NQO1 activity was calculated in relation to the untreated control, which was set to 1. Values are mean ± SD; n = 3. Values with different superscripts are different ($p < 0.05$).

caused a small but nonsignificant loss of activity compared to theoretical addition of their individual activities.

Synergistic Action of hGSL9 and Quercetin and Its Similarity to That of SF and Quercetin from Broccoli. To evaluate in more detail the interactive activity between GSL 9 and quercetin, we developed an isobologram. First, a five-point concentration range was used to determine the dose needed to double NQO1 activity with GSL 9 or quercetin individually (data not shown). We found that individually, 0.48 μM hGSL 9 and 2.07 μM quercetin were necessary to double NQO1 activity in 24 h. By using different concentrations of hGSL9 ($^1/_{10}$, $^2/_{10}$, and $^3/_{10}$ of 0.48 μM hGSL 9, the concentration needed to double the NQO1 activity), a range of quercetin concentrations was evaluated at each of the GSL 9 doses, to determine the concentration of quercetin needed to enhance the GSL 9 effect sufficiently to double NQO1 activity. Synergy was apparent only at the $^3/_{10}$ and $^2/_{10}$ concentrations of GSL 9, whereas antagonism appeared at the $^1/_{10}$ concentration of hGSL 9 (Figure 6A). Because we had used SF as a positive control and because quercetin is present in broccoli, we evaluated synergy between SF and quercetin (Figure 6B), developing a similar isobologram. We found that 0.38 μM SF was required to double NQO1 activity. Then, we set the concentration combinations as we did for hGSL 9 and quercetin and found synergy with all three combinations evaluated, but again, we found the greatest synergy at lower quercetin concentrations.

CYP1A1 Activity. Although we evaluated CYP1A1 inducing activity of all four camelina bioactives under study, none of these compounds caused induction of CYP1A1 alone or in combination (Figure 7A). Further, we determined if any of these compounds were able to inhibit β-naphthoflavone-mediated induction of CYP1A1 activity, since β-naphthoflavone is a potent CYP1A1 inducer. However, we did not find any inhibitory action by camelina bioactives against β-naphthoflavone-mediated induction of CYP1A1 activity (Figure 7B).

DISCUSSION

To our knowledge, this is the first article to evaluate the impact of camelina seed bioactives on detoxification enzymes, as a measure of bioactivity. We chose to evaluate defatted camelina seed meal as a potential bioactive food, not only because it is a byproduct of camelina fuel oil production but because it contains both GSLs and flavonoids, well-known for their health benefits. The content of GSLs can vary over a range of approximately 10−25 μmol/g defatted seed meal, due to variation in the growing environment, in the genotype, and in processing losses.[23] This is similar to the 20−75 μmol/g freeze-dried brassica vegetables and thus considerably greater than the GSL content of fresh brassica vegetables.[24] The dried methanol extract that we used for these studies contained ∼250 μmol GSL/g extract, about a 10-fold increase in concentration over the defatted seed meal. A dried ethanol extract, appropriate for human consumption, could be expected to be similar. The flavonoids in the extract are mainly quercetin glycosides, almost entirely in the form of rutin (quercetin rutinoside) or more complex glycosides of rutin.[23] Rutin is only partly absorbed whole, and no free quercetin was evident in the extract. However, we used the aglycone quercetin in these studies, since this is the form that is considered the active form within cells.

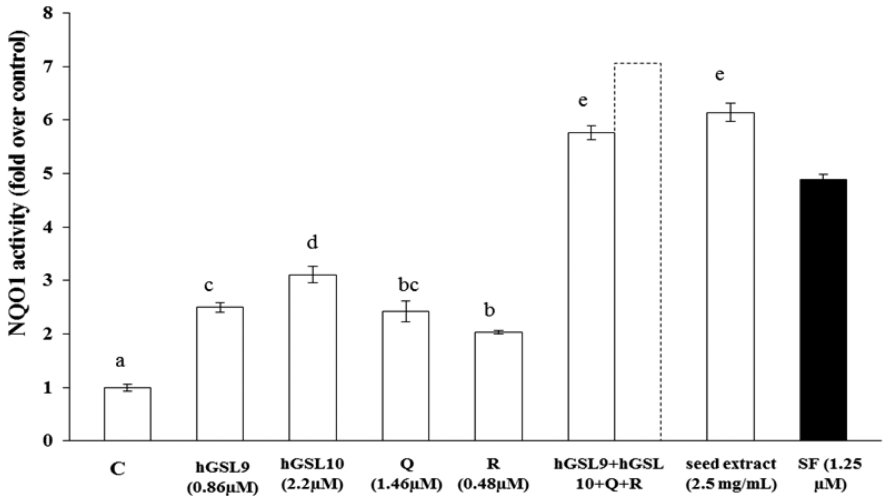

Figure 4. NQO1 activity of camelina seed extract and major components. Cells were treated with the four bioactives individually or combined, at the concentrations found in 2.5 mg extract/mL, and compared to the activity of whole extract, 2.5 mg/mL. Hydrolysed GSLs 9 (hGSL 9) and 10 (hGSL 10), quercetin (Q), rutin (R), and sulforaphane (SF). Relative NQO1 activity was calculated in relation to the control, which was set to 1. Values are mean ± SD; n = 3. Values with different superscripts are different ($p < 0.05$). Dashed bars show the theoretical value, calculated by addition of values derived from individual agents.

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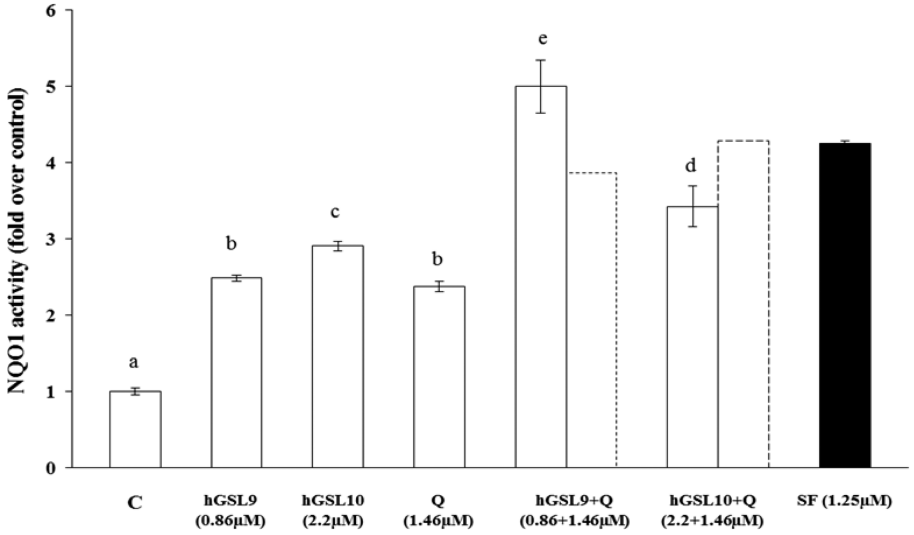

Figure 5. Interactive NQO1 activity between glucosinolates and quercetin. Relative NQO1 activity was calculated in relation to the untreated control, which was set to 1. Hydrolysed GSLs 9 (hGSL 9) and 10 (hGSL 10), quercetin (Q), and sulforaphane (SF).Values are mean ± SD of three experiments performed in triplicate. Values with different superscripts are different ($p < 0.05$). Dashed bars show the theoretical value, calculated by addition of values derived from individual agents.

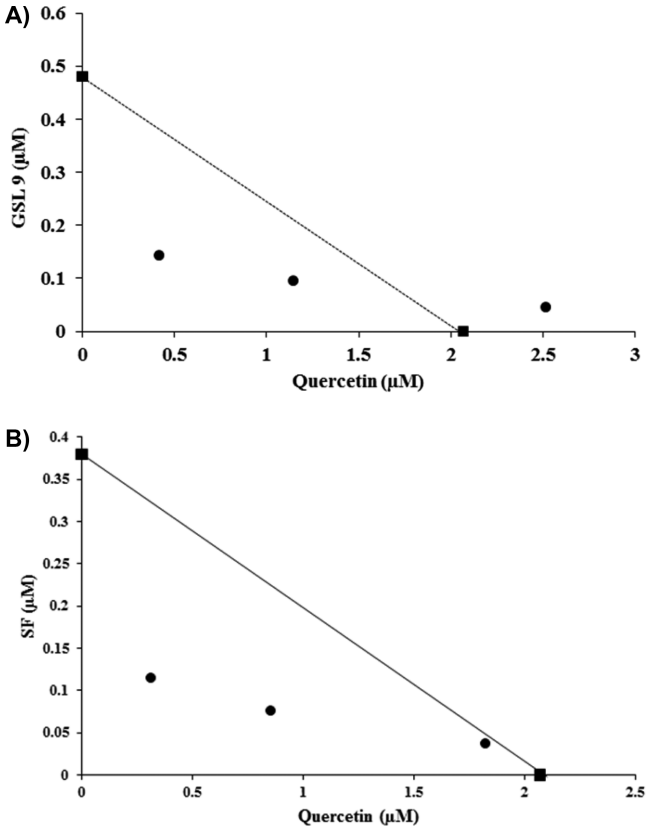

Figure 6. Isobolographic representation of interactive NQO1 inducing activity between different concentration combinations of (A) hGSL 9 and quercetin, (B) sulforaphane (SF) and quercetin. The squares are the concentrations of each individual bioactive necessary to double NQO1 activity in 24 h; the line joining them described the theoretical concentrations necessary to double NQO1 activity if activities are additive. The circles show concentrations found to double NQO1 activity when both compounds were present at the concentrations shown. For details on isobologram construction, see the Materials and Methods section.

Most quercetin glycosides are hydrolyzed in the gut, either by lactase phlorizin hydrolase, an enzyme of the brush border, or β-glucosidases present intracellularly.[25] The free quercetin is then conjugated within the cell, mainly to glucouronide or sulfate conjugates, and thought to be released at the surface of cells by a β-glucuronidase before it enters the cell in the free form to exert its activity.[26] We therefore chose to evaluate bioactivity of rutin and free quercetin, over a range representing that present in camelina seed meal.

Initially, we evaluated possible cytotoxicity of the four major bioactive components, GSL 9, GSL 10, quercetin, and rutin. At the concentrations tested, only hGSL 10 exhibited any toxicity, causing an approximately 30% loss of cell count at 24 h at the largest dose tested, 5 μM (Figure 1). The complete extract, at the dose containing this level of hGSL 10 (2.5 mg extract/mL culture medium) caused a similar loss in viability. However, lower doses also caused some losses, suggesting that, whereas hGSL 10 was a major cause of toxicity, it was not solely responsible for this loss of viability.

Following hydrolysis of the two GSLs, all four of the camelina bioactives under study caused a dose-dependent induction of NQO1, in the concentration range 1.25−5 μM, Figure 2. The two GSLs each induced NQO1 activity in a similar dose range to SF, and this was additive when they were both present, Figure 3.

When cells were treated with a mixture of all four bioactives, the effect on NQO1 induction was similar to that of the whole extract, Figure 4. In an effort to identify the major bioactives responsible for this combinatorial effect, we examined the interaction between hGSL 9 and free quercetin and found that together these gave a significantly greater than additive effect, although this was possibly countered by hGSL 10 and quercetin together causing less induction than expected, Figure 3. Focusing on the effect of hGSL 9 and quercetin, we decided to develop an isobologram, to determine if there was any synergy in the NQO1 inducing activity shown by their combination. The isobologram showed synergy, particularly strongly at the 20% and 30% of maximal GSL 9 doses. Although there are several ways to assess synergy, an advantage

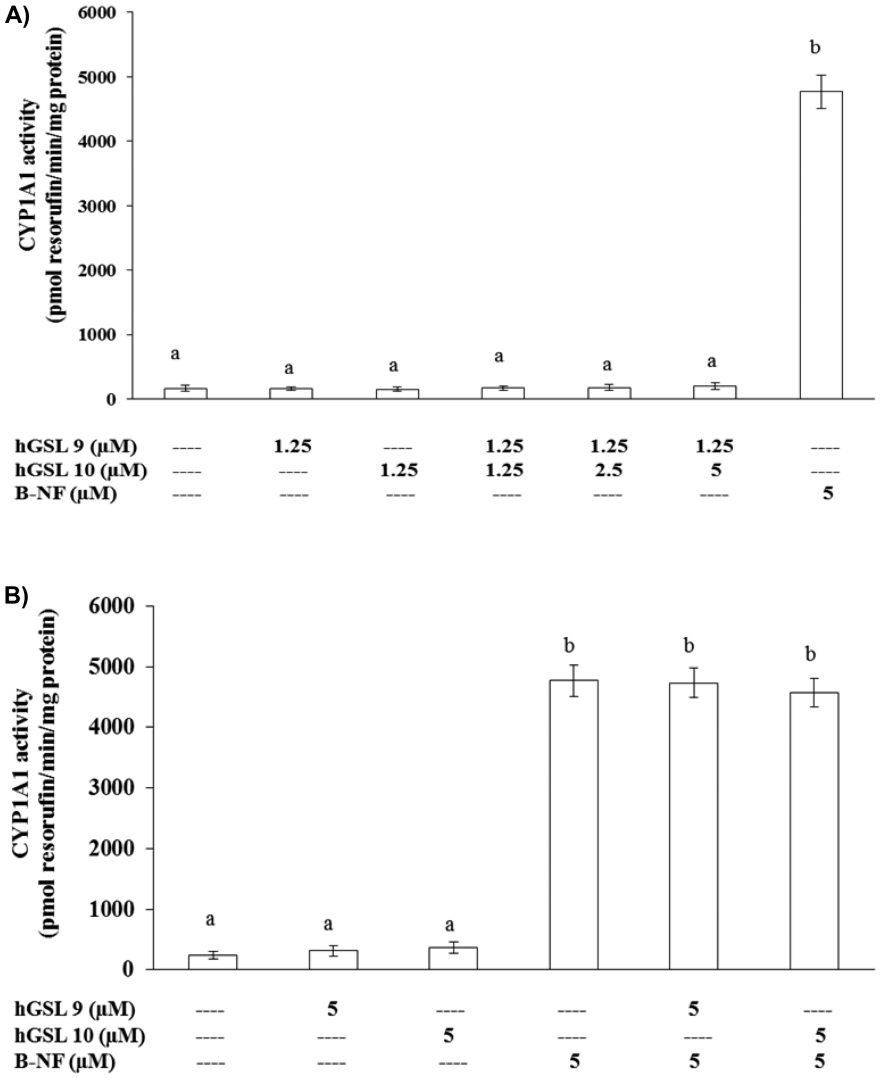

Figure 7. Influence of hGSL 9 and hGSL 10 on ethoxyresorufin-*O*-deethylase (CYP1A1) activity. (A) Cells were treated with GSLs or *β*-naphthoflavone (*β*-NF, 5 *μ*M) as positive control. (B) Cells were treated with hGSLs and *β*-NF, to evaluate the potential of GSLs to inhibit *β*-NF-mediated CYP1A1 induction. Values are mean ± SD of three experiments performed in triplicate. Values with different superscripts are different ($p < 0.05$).

in using isobologram construction is that it is independent of the mode of action and thus applies under many conditions. An isobole is an "iso-effect" curve, in which a combination of constituents is represented on a graph, the axes of which are the dose axes of the individual agents.[22] A line is then drawn joining the individual concentrations that cause a chosen, specific effect; here, we chose doubling of NQO1 at 24h. If the two compounds do not cause an interactive effect (synergy or antagonism) when used in combination, then resulting dose combinations required to cause doubling will fall on the line joining the points. If there is a synergy, that is, the effect of the combination is greater than expected from their individual dose−response curves, the doses of the combination needed to produce the same effect will be less than for the sum of the individual components, and the curve will appear concave. The opposite applies for antagonism, in which the dose of the combination is greater than expected, and will produce a convex isobole. We found that, when hGSL 9 was provided at a very low dose (only 10% of that needed to provide doubling), the quercetin required was greater than that required individually to double induction, showing an antagonism or interference. Whereas we did not evaluate the mechanism further, one explanation would be that, when quercetin is at very high doses, it is competitive with hGSL 9.

In all our experiments, we used SF as a positive control for comparison, as it is known to have potent NQO1 inducing ability. We therefore did the same synergy evaluation for SF with quercetin, developing a second isobologram, Figure 6B. Just as we found that quercetin synergizes the upregulation of NQO1 by hGSL 9, we also saw synergy between quercetin and SF. This is particularly interesting, given that broccoli is a good source of quercetin as well as SF. We interpret this to mean that whole broccoli may exert a stronger potency than can be explained based on purified SF of the same concentration. The impact of quercetin on bioefficacy of broccoli is worthy of further study.

Because indolyl, but not aliphatic, GSLs have been reported to upregulate CYP1A1, we had hypothesized that camelina GSLs would not increase CYP1A1 activity. None of the camelina bioactives tested induced CYP1A1 or inhibited *β*-naphthoflavone-mediated induction of CYP1A1 activity. Compounds upregulating phase II detoxification enzymes

such as NQO1 but not the phase I CYP1A1 enzyme have been termed monfunctional. Monofunctional inducers are considered to have greater health promoting activity than bifunctional inducers that upregulate both phase I and phase II enzymes. This is based on the finding that some precarcinogens are activated by CYP1A1.[15] In this regard, camelina has even greater potential to be viewed as a food with positive health benefits. In summary, we found the *C. sativa* seed meal contains bioactivity stemming from two alkyl sulfinyl GSLs and quercetin. We also found that the major bioactive from broccoli, SF, exhibits a synergistic upregulation of NQO1 with quercetin, a flavonoid that is naturally present in broccoli.

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Notes

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