**Modulation of rat hepatic and pulmonary cytochromes P450 and Phase II
enzyme systems by erucin, an isothiocyanate structurally related to
sulforaphane.**

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Running Head: Modulation of xenobiotic-metabolising enzymes by isothiocyanates

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Abstract

Administration of dietary doses of the isothiocyanate erucin had no effect on rat hepatic cytochrome P450 activity or protein levels, but at higher doses a rise in CYP1A/B1 protein levels was evident. In lung, treatment with erucin, as well as sulforaphane, failed to modulate cytochrome P450 activities but elevated CYP1A/B1 protein levels. In liver, erucin stimulated quinone reductase activity accompanied by a rise in protein. Glutathione *S*-transferase activity was unaffected but GST α and GST μ protein levels increased. In lung, both isothiocyanates increased quinone reductase paralleled by a rise in protein levels; at the higher dose both isothiocyanates elevated moderately GST α levels. Hepatic microsomes converted both isothiocyanates to metabolites that impaired cytochrome P450 activity, which was antagonised by reduced glutathione. It may be concluded that erucin may protect against carcinogens by stimulating the detoxication of quinones but is unlikely to significantly influence reactive intermediate generation through modulation of cytochrome P450 activity.

Key words: Erucin; Sulforaphane; Isothiocyanates; Chemoprevention; Quinone reductase.

1 **Introduction**

2 Isothiocyanates comprise a class of phytochemicals present in cruciferous vegetables
3 and are considered as promising human chemopreventive agents. Epidemiological
4 studies have revealed the potential of these vegetables to protect against a number of
5 cancers (1-3), and indeed individual isothiocyanates have been linked, through
6 epidemiological studies, to lower cancer incidence at a number of sites (4,5).
7 Experimental evidence in animal models supports a chemopreventive role for these
8 chemicals; isothiocyanates can antagonise the carcinogenicity of chemicals, including
9 dietary carcinogens such as polycyclic aromatic hydrocarbons and heterocyclic
10 amines (6,7). Of importance are recent reports that major metabolites of
11 isothiocyanates, such as mercapturates, retain the chemopreventive activity (8).

12 In vegetables isothiocyanates exist in the form of glucosinolates but are
13 released following exposure to the enzyme myrosinase (β -thioglucoside
14 glucohydrolase) which comes into contact with these compounds during the
15 harvesting, chopping and mastication of these vegetables. Moreover, glucosinolates
16 that progress to the human intestine intact may be metabolised by microbial
17 myrosinases, further contributing to the release of isothiocyanates (9). Isothiocyanates
18 are well absorbed and achieve good bioavailability (10). Moreover, experimental
19 evidence has been recently presented that the glucosinolates of isothiocyanates can
20 also be absorbed, at least partly, intact (11).

21 The chemopreventive mechanism of action of isothiocyanates appears to be
22 multifactorial, influencing all stages of chemical carcinogenesis, including
23 impairment of the initiation stage (3,12). Indeed, the consumption of cruciferous
24 vegetables influenced the metabolism of carcinogenic heterocyclic amines in humans
25 (13). One of the most extensively studied isothiocyanate is sulforaphane (1-

1 isothiocyanato-4-(methylsulphinyl) butane) (**Figure 1**), encountered at substantial
 2 concentrations in broccoli, where it exists as the glucosinolate glucoraphanin. Even
 3 when administered at dietary doses, sulforaphane stimulates detoxication enzymes
 4 such as quinone reductase (14), and at higher doses it also enhances the activity of
 5 other deactivating enzyme systems such as glutathione *S*-transferase (GST) (15).
 6 However, sulforaphane also acts at the post-initiation stages, inducing apoptosis, cell
 7 cycle arrest, inhibition of histone deacetylase and suppressing the conversion of lung
 8 benign tumours to carcinomas in mice (8,16).

9 Erucin (1-isothiocyanato-4-(methylthio)-butane) (**Figure 1**) is another
 10 isothiocyanate, structurally related to sulforaphane, being its sulphide analogue. It is
 11 found as the glucosinolate, glucoerucin, and is a major glucosinolate in rocket salad
 12 (roquette; *Eruca sativa*); moreover there is experimental evidence to suggest that
 13 glucoraphanin can be reduced to glucoerucin by mammalian enzymes (11). Erucin
 14 itself is also an important metabolite of sulforaphane formed from the reduction of the
 15 sulphinyl group; similarly, sulforaphane is a principal metabolite of erucin, generated
 16 following sulphur oxidation (11,17). Erucin appears to share many of the biological
 17 activities of sulforaphane related to its chemopreventive activity, in many cases being
 18 far more potent (18,19).

19 Vegetables are usually cooked by boiling, and this procedure results in
 20 marked loss of glucosinolates which leach into the cooking water, although other
 21 cooking processes such as steaming and microwaving have minimal effects (20).
 22 More importantly, cooking of vegetables denatures myrosinase, the enzyme that
 23 converts the glucosinolates to the active isothiocyanates (21). As a result of these two
 24 effects, the bioavailability of isothiocyanates is drastically decreased. Indeed, the
 25 bioavailability of isothiocyanates from broccoli markedly decreased after steaming

(22). An advantage of rocket salad, the major source of the erucin glucosinolate, is that it is largely consumed raw and, consequently, glucosinolate levels and myrosinase activity are not adversely affected. As modulation of the xenobiotic-metabolising enzyme systems, resulting in lower availability of the genotoxic metabolites, is a major mechanism of the anticarcinogenic effects of isothiocyanates, a study has been undertaken to evaluate the potential of erucin to modulate these enzyme systems in the liver of rats. Previous studies have been reported in murine hepatoma cells and in mice treated with erucin by gavage, focussing only on Phase II enzyme systems (23) but, to our knowledge, this is the first study investigating the modulation of cytochrome P450 enzymes. Moreover, the ability of erucin to modulate carcinogen-metabolising enzymes in the lung, a target tissue of isothiocyanates, has been investigated and compared to sulforaphane.

Materials and Methods

Sulforaphane and erucin (LKT Laboratories, Minnesota, USA), NADPH, ethoxyresorufin, pentoxyresorufin, resorufin, 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene, glutathione reductase, peroxidase-linked anti-rabbit and anti-goat antibody raised in rabbits (Sigma Co. Ltd., Poole, Dorset, UK), 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (Fluka, Buchs SG, Switzerland), 7-benzyloxyquinoline and 7-hydroxyquinoline (BD Biosciences, California USA), 2-amino-3-methylimidazo-(4,5-f)quinoline (IQ) (Toronto Research Chemicals, Toronto, Canada) were all purchased. Rat anti-CYP1A1, recognising both CYP1A1 and CYP1A2, anti-CYP1B1, anti-CYP2B and anti-CYP3A2 antibody were obtained from BD Biochemicals (Oxford, UK), antibodies to human GST P1-1, A1-1 and M1-1

1 (Calbiochem, Merck, UK) and antibody to human quinone reductase (abcam
2 Cambridge, UK) were similarly purchased.

3 Male Wistar albino rats (about 180g) were obtained from B&K Universal Ltd
4 (Hull, East Yorkshire, UK). The animals were housed at 22 ± 2 °C, 30-40% relative
5 humidity, in an alternating 12-h light:dark cycle with light onset at 07.00 hr. Rats
6 were randomly divided into four groups, each comprising five animals. One group
7 served as control, two groups were maintained on drinking water containing 30 or 150
8 mg/L erucin, corresponding to approximate daily doses of 3 and 15 mg/kg (18.6 and
9 93.2 μ mole/kg) respectively, and the final group was exposed to water containing
10 sulforaphane (150 mg/L, approximate daily dose of 15 mg/kg, i.e. 88.2 μ mole/kg);
11 animals were maintained on these regimes for 10 days. At the end of this period, rats
12 were killed by cervical dislocation, livers and lungs were immediately excised,
13 homogenised and post-mitochondrial fractions prepared by centrifugation (9000 g for
14 20 minutes) and stored at -80°C until use. When required, samples were thawed and
15 resolved to microsomal and cytosolic fractions by centrifugation (105 000g x 1 hour).
16 The dealkylations of methoxy- (24), ethoxy- (25), pentoxy-resorufin (26) and 7-
17 benzyloxyquinoline (27) were determined using the microsomal fraction. The
18 following assays were performed in the cytosol; quinone reductase using MTT [3-(4,-
19 5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide] as substrate (28),
20 glutathione *S*-transferase activity using as accepting substrates 1-chloro-2,4-
21 dinitrobenzene, 1,2-dichloro-4-nitrobenzene (29) and 7-chloro-4-nitrobenzo-2-oxa-
22 1,3-diazole (30), and total glutathione (31). Protein concentration was determined in
23 both fractions using bovine serum albumin as standard (32). Finally, in order to
24 determine changes in enzyme protein expression, hepatic and pulmonary microsomal
25 proteins from pooled animals were resolved by SDS-PAGE before being transferred

1 electrophoretically to Hybond-P polyvinylidene difluoride membrane. Immunoblot
2 analysis was carried out using antibodies to cytochrome P450 or Phase II enzymes,
3 followed by the appropriate peroxidase-labelled secondary antibody. Molecular
4 markers were always run concurrently. Immunoblots were quantitated by
5 densitometry using the GeneTool software (Syngene Corporation, Cambridge, UK).

6 To induce the hepatic CYP1A and CYP2B subfamilies of cytochrome P450,
7 rats were treated with a single intraperitoneal dose of Aroclor-1254 (500 mg/kg), the
8 animals being killed on the 5th day following administration. In studies aimed at
9 establishing the effect of erucin (25 μ M) on the *in vitro* O-dealkylation of
10 ethoxyresorufin, microsomes from Aroclor-1254-induced animals were incubated
11 with erucin or sulforaphane in the presence of NADPH for various periods of time,
12 and reaction was initiated with the addition of the substrate. In studies aimed at
13 studying the concentration-dependent effects, microsomes from Aroclor-1254-
14 induced rats were incubated with a series of concentrations of erucin or sulforaphane
15 in the presence of NADPH for 30min, and reaction was initiated with the addition of
16 the substrate.

17 Mutagenic activity induced by IQ was monitored using the Ames mutagenicity
18 assay (33) in the presence of an activation system containing 10% (v/v) hepatic S9
19 preparations from control, erucin- and Aroclor 1254-treated rats, and employing
20 *Salmonella typhimurium* YG1024 as the indicator strain.

21 Statistical evaluation was carried out employing Student's t-test.

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Results

Intake of neither erucin nor sulforaphane influenced animal body weight gain, and water consumption did not differ between control and isothiocyanate-treated animals (results not shown).

Treatment of rats with erucin failed to modulate the hepatic dealkylations of methoxy-, ethoxy- or pentoxyresorufin, catalysed selectively by CYP1A2, CYP1A1 and CYP2B respectively, and of 7-benzoyloxyquinoline catalysed by CYP3A (**Table1**). Immunoblot analysis employing antibodies to rat CYP1A1 recognised a single band, presumably CYP1A2, whose levels were modestly (30%) elevated by the higher dose of erucin (**Figure 2A**). A single band was also recognised by the antibodies to CYP1B1, and its expression increased (90%) following treatment of the animals with the higher dose of erucin (**Figure 2A**). Treatment with erucin failed to modulate the levels of the single band detected by anti-rat CYP2B1 and CYP3A2 antibodies (**Figure 2A**).

Cytosolic glutathione *S*-transferase activity in the liver, determined using 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole, 1-chloro-2,4-dinitrobenzene or 1,2-dichloro-4-nitrobenzene as substrates, and total glutathione levels were unaffected by the erucin treatment, whereas quinone reductase activity was stimulated at both dose levels (**Table 1**). Immunoblot analysis indicated that erucin, at both dose levels, up-regulated the levels of GST α and GST μ , as well as quinone reductase, but GST π was unaffected (**Figure 2B**).

In lung, neither isothiocyanate modulated the *O*-dealkylation of the two alkoxyresorufins (**Table 2**); 7-benzyloxyquinoline demethylase was not detectable in this tissue. Immunoblot studies revealed that erucin, at both doses, as well as sulforaphane elevated pulmonary CYP1A1 protein levels (**Figure 3A**). Similarly, the

1 higher erucin dose and sulforaphane increased the band recognised by antibody to
2 CYP1B1 antibody by 80 and 70% respectively (**Figure 3A**). No changes were evident
3 in the case of CYP2B1 or CYP3A2. Glutathione *S*-transferase activity, whether
4 monitored with 1-chloro-2,4-dinitrobenzene or 7-chloro-4-nitrobenzo-2-oxa-1,3-
5 diazole, and total glutathione levels were not influenced by the isothiocyanate
6 treatment. Quinone reductase activity, which was higher in the lung compared with
7 the liver, was clearly elevated by the treatments with erucin or sulforaphane (**Table**
8 **2**). The increase in quinone reductase activity was paralleled by a similar rise in
9 protein levels determined by immunoblot, being 165 and 150% higher in the animals
10 treated with the high erucin dose and sulforaphane respectively, in comparison with
11 controls (**Figure 3B**). Erucin, at the higher dose, similar to sulforaphane, raised the
12 levels of GST α by 25-30% whereas GST π and GST μ were unaffected (**Figure 2B**).

13 Both isothiocyanates, following pre-incubation in the presence of NADPH,
14 suppressed the hepatic *O*-deethylation of ethoxyresorufin in Aroclor 1254-induced
15 hepatic microsomes to a similar degree (**Figures 4A and 4B**); extent of inhibition was
16 time-dependent with respect to pre-incubation time (**Figure 4C**). Reduced glutathione
17 prevented the suppression of *O*-deethylation of ethoxyresorufin following pre-
18 incubation with the isothiocyanate (**Figure 5**); oxidised glutathione had no effect
19 (results not shown). Finally, in the absence of pre-incubation, neither compound, at
20 least up to a concentration of 25 μ M, influenced the *O*-deethylation of ethoxyresorufin
21 (results not shown).

22 The bioactivation of the heterocyclic amine IQ to genotoxic intermediates in
23 the Ames test was determined in the presence of hepatic post-mitochondrial
24 preparations from control, erucin- and sulforaphane-pretreated rats. A concentration-
25 dependent mutagenic response was evident in the presence of all activation systems;

1 treatment with either isothiocyanate caused a modest decrease in mutagenic response
2 **(Figure 6).**

4 **Discussion**

5 One of the major mechanisms through which isothiocyanates are believed to elicit
6 their anticarcinogenic effects is by limiting the amount of genotoxic metabolites of
7 chemical carcinogens by either stimulating their detoxication and/or impairing their
8 generation (3). Consequently, the aim of this study was to evaluate the potential of the
9 isothiocyanate erucin to modulate glutathione *S*-transferase and quinone reductase,
10 two pivotal enzymes in the detoxication of reactive intermediates and prevention of
11 oxidative stress, and cytochromes P450, the most important enzyme in the generation
12 of these intermediates; studies were conducted in the liver, the principal site of
13 metabolism, and lung, a target tissue for the anticarcinogenic effect of isothiocyanates
14 (9). The effects of erucin on pulmonary enzymes were compared with those of
15 sulforaphane, as the influence of the latter has not been studied in this tissue following
16 exposure to dietary doses. It has been established that sulforaphane is metabolised to
17 erucin in the rat, and erucin can be readily formed from the reduction of sulforaphane
18 (11,17).

19 Previous studies in mice have indicated the potential of erucin and
20 sulforaphane, at least at high doses administered by gavage, to stimulate quinone
21 reductase and glutathione *S*-transferase activities in the liver and other tissues (23),
22 but to our knowledge this is the first study evaluating the potential of this
23 isothiocyanate to modulate cytochrome P450 enzymes. Both isothiocyanates were
24 given to rats in the drinking water, and a dose was employed that corresponds to the
25 human intake. Since, to our knowledge, the human consumption levels of erucin or

1 glucoerucin have not been defined, the doses employed in these studies was based on
2 the consumption of sulforaphane, a structurally similar isothiocyanate. A 250g serving
3 of fresh broccoli will release 93-187 mg of sulforaphane (34), so that the intake for a
4 70 kg individual would be 1.3-2.7 mg/kg. The lower dose of erucin employed in the
5 current study of approximately 3 mg/kg represents such dietary level of intake.

6 None of the cytochrome P450 activities studied were modulated by treatment
7 with erucin in the liver or lung, or with sulforaphane in the lung. In the lung, however,
8 both compounds elevated the levels of the band recognised by the antibody to
9 CYP1A1. CYP1B1 could not be monitored at the activity level as a selective substrate
10 is not available, but immunoblot analysis indicated that treatment with erucin, at the
11 higher dose, elevated the levels of this enzyme in the liver. In the lung, both
12 isothiocyanates led to an increase in the single band detected by the CYP1B1
13 antibody. Neither compound had any effect on CYP2B or CYP3A2 protein levels.
14 Up-regulation of the CYP1 family, albeit at the mRNA level, has been reported
15 following exposure of HepG2 cells to 4-methylthio-3-butenyl isothiocyanate (35).

16 Erucin, at both dose levels, gave rise to a significant increase in hepatic
17 quinone reductase activity as previously described for sulforaphane (14). For both
18 tissues, Western blot analysis indicated that this was due to increased cytosolic
19 concentration of quinone reductase. Contrary to the present observations, exposure of
20 rats to phenethyl isothiocyanate failed to enhance this activity in the lung (36),
21 highlighting the importance of the side chain; it is of interest that sulforaphane and
22 erucin have an aliphatic side chain whereas in the case of phenethyl isothiocyanate it
23 is aromatic. Glutathione *S*-transferase activity was monitored using three substrates;
24 1-chloro-2,4-dinitrobenzene is a substrate for a number of the cytosolic transferases
25 (37). The much higher activity in the liver compared with the lung reflects the higher

1 capacity of the former tissue to detoxicate electrophiles. Both compounds failed to
2 influence activity in lung and, similarly, in the liver erucin had no effect. 1,2-
3 Dichloro-4-nitrobenzene is a substrate associated with the μ -family, but no activity
4 was detectable in lung as a result of poor expression in this tissue (37). Hepatic
5 activity was not influenced by erucin treatment although a rise in protein levels was
6 clearly visible. Finally, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole has been reported to
7 be selective for the α -class (30), and the very low activity in the lung demonstrates the
8 poor expression of this class in this tissue (37). A rise in GST activity was not seen
9 with any of the three accepting substrates studied, whereas GST α and GST μ were up-
10 regulated at the protein level. Similarly, in lung no change in activity was manifested
11 by treatment with either isothiocyanate, but GST α levels increased, GST π was not
12 modulated by either isothiocyanate in either tissue. Thus, the effects of
13 isothiocyanates on the GST system are both compound and tissue specific.

14 As isothiocyanates, with either aliphatic or aromatic side chain, can be
15 metabolically converted to intermediates that cause cytochrome P450 inhibition, the
16 ability of erucin to act as a mechanism-based inhibitor was evaluated and compared to
17 sulforaphane. The two isothiocyanates did not differ in their ability to suppress, in a
18 time-dependent manner, the *O*-deethylation of ethoxyresorufin. The fact that
19 inhibition was prevented by reduced, but not oxidised, glutathione concords with the
20 generation of an electrophilic metabolite(s). A more reactive isocyanate, derived from
21 the cytochrome P450-mediated isothiocyanate oxidation (38), has been postulated as
22 the possible entity that interacts and inactivates cytochromes P450. Isocyanates are
23 known to be capable of modifying proteins. Indeed, α -naphthylisothiocyanate was
24 converted by rat liver microsomes, in the presence of NADPH, to metabolites that
25 bound irreversibly to microsomal proteins (39).

1 In order to assess whether the erucin treatment had any impact on the ability of
2 liver microsomes to bioactivate chemical carcinogens, the metabolic conversion of the
3 heterocyclic amine 2-amino-3-methylimidazo-(4,5-f)quinoline (IQ) to mutagens was
4 investigated using the Ames mutagenicity assay. This compound was used as model
5 carcinogen as previous studies showed that sulforaphane inhibits the genotoxicity and
6 DNA-adduct formation of heterocyclic amines in cell cultures *in vitro* (40,41). Erucin
7 treatment caused a modest decrease in the bioactivation of IQ but no clear
8 concentration-dependent effect was evident. Sulforaphane had a similar, modest effect
9 as we previously reported (14).

10 In summary, the present studies have demonstrated that following intake of
11 erucin, at a dose corresponding to human intake, the only enzyme modulated of those
12 studied was quinone reductase; an increase was seen in both liver and lung. No
13 cytochrome P450 enzyme was perturbed by the erucin treatment implying that
14 attenuated generation of genotoxic intermediates is unlikely to be a chemopreventive
15 mechanism at dietary dose levels. These observations allow us to conclude that erucin
16 may exert a chemopreventive effect by affording protection against the
17 carcinogenicity of quinones, such as those formed by the oxidation of polycyclic
18 aromatic hydrocarbons. At higher doses, which may be achieved by the consumption
19 of dietary supplements, the expression of other enzymes was modulated, at least at the
20 protein level. In general, no marked differences were observed between erucin and
21 sulforaphane. However, as the major source of erucin is rocket salad which is largely
22 consumed uncooked, it may prove a more promising chemopreventive agent than
23 sulforaphane as it is likely to achieve a higher bioavailability. The principal sources of
24 sulforaphane are vegetables which are consumed cooked, resulting in lower
25 conversion of the glucosinolate to the isothiocyanate. Finally, to our knowledge, this

1 is the first paper to report the ability of dietary isothiocyanates to up-regulate CYP1B1
2 expression in the liver and lung.

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Legends to figures

Figure 1: Chemical structures of erucin and sulforaphane

Figure 2: Modulation of rat hepatic cytochrome P450 and Phase II enzymes by treatment with erucin

Figure 3: Modulation of rat pulmonary cytochrome P450 and Phase II enzymes by treatment with erucin and sulforaphane

Figure 4: Effect of pre-incubation on the inhibition of the hepatic *O*-deethylation of ethoxyresorufin by erucin and sulforaphane.

Results are presented as mean $\pm$ SD of triplicate determinations.

*P<0.05; **P<0.01; ***P<0.001

Figure 5: Effect of glutathione on the erucin- and sulforaphane-mediated inhibition of the *O*-deethylation of ethoxyresorufin

Results are presented as mean $\pm$ SD of triplicate determinations.

*P<0.05; **P<0.01; ***P<0.001

Figure 6: Effect of erucin treatment on the activation of IQ to mutagenic intermediates in the Ames test.

Aroclor 1254-induced activation system (positive control) elicited a mutagenic response of 7562 ± 1240 revertants/plate at an IQ concentration of 1000 ng/plate. The spontaneous reversion rate of 48 ± 8 has already been subtracted. Results are presented as mean $\pm$ SD for triplicate plates.

*P<0.05; **P<0.01

Table 1: Effect of treatment with erucin on rat hepatic cytochrome P450 and phase II enzyme activities^a

Enzyme activity	Control	Erucin (Low dose)	Erucin (High dose)
Methoxyresorufin <i>O</i> -demethylase (pmol/min/mg protein)	23 ± 6	20 ± 2	16 ± 6
Ethoxyresorufin <i>O</i> -deethylase (pmol/min/mg protein)	30 ± 7	24 ± 3	20 ± 9
Pentoxyresorufin <i>O</i> -depentylase (pmol/min/mg protein)	3.3 ± 0.7	2.5 ± 0.5	3.1 ± 0.7
Benzoyloxyquinoline <i>O</i> -debenzylase (pmol/min/mg protein)	327 ± 84	369 ± 60	291 ± 63
Quinone reductase (nmol/min/mg protein)	679 ± 298	1074 ± 125**	1270 ± 130***
Glutathione <i>S</i> -transferase (1-chloro-2,4-dinitrobenzene) (nmol/min/mg protein)	2137 ± 190	2181 ± 258	1947 ± 236
Glutathione <i>S</i> -transferase (7-chloro-4-nitrobenzo-2-oxa-1,3-diazole) (nmol/min/mg protein)	463 ± 112	414 ± 116	477 ± 116
Glutathione <i>S</i> -transferase (1,2-dichloro-4-nitrobenzene) (nmol/min/mg protein)	73 ± 5	67 ± 11	66 ± 11
Total glutathione (mM)	5.13 ± 0.67	4.56 ± 0.51	4.76 ± 0.56

^a Results are presented as mean ± SD for five rats. ** Significantly different compared with control (**P<0.01). *** Significantly different compared with control (P<0.001).

Table 2: Effect of treatment with erucin and sulforaphane on rat pulmonary cytochrome P450 and phase II enzyme activities^a

Rats were maintained on erucin- or sulforaphane-supplemented diets for 10 days. Results are presented as mean $\pm$ SD for five rats.

*P<0.05; **P<0.01

Enzyme activity	Control	Erucin (Low dose)	Erucin (High dose)	Sulforaphane
Ethoxyresorufin <i>O</i> - deethylase (pmol/min/mg protein)	0.91 $\pm$ 0.35	0.71 $\pm$ 0.21	0.98 $\pm$ 0.27	0.71 $\pm$ 0.29
Pentoxoresorufin <i>O</i> - deethylase (pmol/min/mg protein)	3.26 $\pm$ 0.70	2.53 $\pm$ 0.50	3.09 $\pm$ 0.69	2.59 $\pm$ 1.02
Quinone reductase (nmol/min/mg protein)	1288 $\pm$ 298	1709 $\pm$ 238*	2526 $\pm$ 40**	2775 $\pm$ 497**
Glutathione <i>S</i> -transferase (1-chloro-2,4- dinitrobenzene) (nmol/min/mg protein)	24 $\pm$ 5	23 $\pm$ 4	32 $\pm$ 6	31 $\pm$ 6
Glutathione <i>S</i> -transferase (7-chloro-4-nitrobenzo-2- oxa-1,3-diazole) (nmol/min/mg protein)	2.84 $\pm$ 0.58	2.16 $\pm$ 0.68	3.45 $\pm$ 0.61	3.79 $\pm$ 0.78
Total glutathione (mM)	0.82 $\pm$ 0.28	0.66 $\pm$ 0.08	0.82 $\pm$ 0.22	0.79 $\pm$ 0.09

^a Results are presented as mean $\pm$ SD for five rats. * Significantly different compared with control (**P<0.05). ** Significantly different compared with control (P<0.01).

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6