Modulatory influence of *Brassica compestris* Linn var sarson on phase-II carcinogen metabolizing enzymes and glutathione levels in mice

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The present study reports the modulatory influence of 95% ethanolic extract from the seeds of *B. compestris* on the activity of phase-II enzymes such as glutathione S-transferase (GST), DT-diaphorase (DTD) and reduced glutathione (GSH) level in the skin, lung, kidney and forestomach of the mouse. Oral treatment with the seed extract at 800 mg/kg body wt. for 15 days significantly elevated GST in lung and forestomach and DT-diaphorase in forestomach and skin and GSH level in lung, kidney forestomach and skin. The lower dose 400 mg/kg body wt was effective only in inducing GST and DT-diaphorase activity in forestomach and reduced glutathione level in lung. The findings suggest that *B. compestris* seed extract may block or suppress the events associated with chemical carcinogenesis at least in part, by inducing metabolic detoxification of the carcinogen.

**Keywords**: *Brassica compestris*, Carcinogen, Glutathione, Modulatory influence.

Cancer chemoprevention is defined as the use of chemicals or dietary components to block, inhibit or reverse the development of cancer in normal or preneoplastic tissue. A large number of potential chemopreventive agents have been found to function by a variety of mechanisms directed at all major stages of carcinogenesis. Induction of phase II drug-metabolising enzymes such as DT-diaphorase (DTD) and glutathione S-transferase (GST) is considered as a major mechanism of protection against chemical stress and initiation of carcinogenesis.

The chemopreventive action of *Brassica compestris* seed extract on 7, 12-dimethylbenz (a) anthracene (DMBA) induced skin papillomagenesis in Swiss albino mice was studied. A significant reduction in tumor formation (average number of tumor per mouse and the cumulative number of papillomas) was observed in mice treated through gastric intubation with the seed extract.

In order to understand the mechanism of chemoprevention, an attempt has been made to study alcoholic extract of mustard seed of *Brassica compestris* var sarson on phase II detoxification enzymes and glutathione level in skin, lung, kidney and forestomach in Swiss albino mice.

**Animals**—Male Swiss albino mice (7-8 weeks old) were obtained from the animals facility (JNU, New Delhi). The animals were provided with standard mice feed (Hindustan Lever Ltd., India) and tap water ad libitum.

**Chemicals**—1-chloro-2,4-dinitrobenzene (CDNB), 5,5-dithio-bis-2-nitrobenzoic acid (DTNB), bovine serum albumin (BSA), reduced nicotiamide adenine dinucleotide phosphate (NADPH), reduced nicotiamide adenine dinucleotide (NADH), 2,6-dichlorophenol-indophenol (DCPIP) and 3(2)tero-butyl-4-hydroxy anisole(BHA) were obtained from Sigma chemicals (St. Louis, MO, USA). The other chemicals obtained from local firms were at the highest purity.

**Brassica compestris seed extract**—Seeds of *B. compestris*, collected locally were powdered and extracted with 95% ethanol solvent by refluxing for 36 hr (12 hr×3). Finally the extract was lyophilized, weighed and preserved at 4°C and used for treating the animals. The extract was given in suspension at two dose levels of 400 and 800 mg/kg body wt of *Brassica* extract dissolved in double distilled water (DDW), on the basis of our previous report ⁰.05 ml of dissolved extract was given to each mouse by oral gavage daily.

**Experimental design**—Animals were randomly sorted into the following groups: *Group 1* (*n* = 8) —
Animals were put on a normal diet and sham-treated with 0.05 ml double distilled water by oral gavage daily for 15 days. This group of animals served as a negative control. Group II (n = 8)—Animals were put on a normal diet and treated with 400 mg/kg body wt of Brassica seed extract in 0.05 ml double distilled water by oral gavage daily for 15 days. Group III (n = 8)—Animals were put on a normal diet and treated with 800 mg/kg body wt of Brassica seed extract in 0.05 ml double distilled water by oral gavage daily for 15 days. Group IV (n = 8)—Animals were put on special diet containing 0.75% butylated hydroxanisole (BHA) for 15 days. This group of animals served as a positive control. The body weights of the mice were recorded initially and at the end of the experiment.

**Preparation of homogenates and supernatant fraction**

**Skin**—Animals were sacrificed by cervical dislocation after the termination of the experiment. Skin was carefully removed, trimmed free of extraneous collagen material attached underneath by scraping with a scalped blade and cut into small pieces. It was then weighed, blotted dry and homogenized in ice cold Tris KCl buffer (pH 7.4) to yield a 10% (w/v) homogenate by using apotron homogenizer. Aliquot (0.5 ml) of this homogenate was used for assaying reduced glutathione while the remainder was centrifuged at 10,000 g for 20 min (model RC-5B). The supernatant fraction after discarding any floating lipid layer was used for assaying glutathione S-transferase and DT-diaphorase activities.

**Liver**—Animals were sacrificed by cervical dislocation after the termination of the experiment. The liver was perfused in situ immediately with cold 0.9% NaCl and thereafter carefully removed and rinsed in chilled 0.15 M Tris KCl buffer (pH 7.4) to yield a 10% (w/v) homogenate. Aliquots (0.5 ml) of this homogenate were used for assaying reduced glutathione, while the remainder of the homogenate was centrifuged by refrigerated super speed centrifuge (model RC-5B) at 10,000 g for 20 min. The resultant supernatant was transferred into pre-cooled ultracentrifugation tubes and centrifuged at 105,000 g for 60 min in a Beckman ultracentrifuge (model 7-800 M). The supernatant (cytosol fraction), after discarding any floating lipid layer was used for assaying glutathione S-transferase, DT-diaphorase.

**Lung, forestomach and kidney**—The lung, forestomach and kidney were carefully removed, trimmed and rinsed in chilled 0.15 M Tris-KCl buffer (pH 7.4). The forestomach was slit opened longitudinally, cleaned and flushed with the buffer 5-6 times. The lung and kidney were cut into small pieces. The lung forestomach and kidney were then blotted dry, weighed and homogenized in ice-cold 0.15 M Tris KCl buffer (pH 7.4) to yield 10% (w/v) homogenate. Aliquot (0.5 ml) of this homogenate was used for assaying GSH. The remainder was centrifuged at 15,000 g for 30 min. The resultant supernatant obtained was used for assaying glutathione S-transferase and DT-diaphorase activities.

The cytosolic glutathione S-transferase activity was determined spectrophotometrically at 37°C according to the procedure of Habig et al. The reaction mixture (3 ml) contained 1 ml of (100 mM) phosphate buffer (pH 6.5), 1.7 ml of distilled water, 0.1 ml of (30 mM) CDNB and 0.1 ml of (30 mM) GSH. After pre-incubating the reaction mixture at 37°C for 2 min the reaction was started by the addition of 0.1 ml diluted cystosol and the absorbance was followed 3 min at 340 nm. Reaction mixture without the enzyme was used as the blank. The specific activity of GST is expressed as μmole of GSH-CDNB conjugate formed/min/mg protein using an extinction coefficient of 9.6 μM⁻¹ cm⁻¹.

The DT-diaphorase activity was measured as described by Ernster et al. with NADPH as the electron donor and 2, 6-dichlorophenol-indophenol (DCPIP) as the electron acceptor at 600 nm. The DTD activity was calculated by reading the absorbance after 3 min at 600 nm with an extinction coefficient value of 21 μM⁻¹ cm⁻¹. One unit of enzyme activity has been defined as amount of enzyme required to reduce 1 μmole of DCPIP per min.

Glutathione level was determined by method as described by Moron et al. Homogenates were immediately precipitated with 0.1 ml of 25% TCA and the precipitate was removed after centrifugation. Free-SH groups were assayed in a total 3 ml volume by the addition of 2 ml of 0.6 mM DTNB and 0.9 ml prepared (0.2 mM) sodium phosphate buffer (pH 8.0) to 0.1 ml of the supernatant and the absorbance was read at 412 nm using a Shimadzu UV-160 spectrophotometer. Glutathione was used as a standard to calculate μmole GSH/100 g tissue. The protein content was measured according to the method of Lowry et al. using bovine serum albumin (BSA) as a standard.

**Statistical analysis**—The data were subjected to Student's t test for comparison between the groups. The values are expressed as mean ± SE.
Oral treatment with *Brassica* seed extract at 800 mg/kg body wt revealed significant elevation in GST in lung and forestomach but not significant in the kidney and skin of mice. GST activity increased in forestomach at low dose of 400 mg/kg body wt in comparison to control group. However, in the BHA fed group there was significant induction in the GST activity in the lung, forestomach, kidney and skin (Table 1).

The DT-diaphorase activity showed significant enhancement in the skin and no significant difference in the lung and kidney at the dose of 800 mg/kg body wt for 15 days in comparison with control group. The lower dose of 400 mg/kg body wt of *Brassica* extract showed significant elevation in DT-diaphorase activity in forestomach only. In the BHA treated mice DT-diaphorase enzyme activity was found to be higher in lung and kidney and decreased significantly in the skin (Table 1).

The glutathione level was found to be significantly higher in all organs studied (lung, kidney, forestomach and skin) in the mice fed with *Brassica* seed extract at the dose level of 800 mg/kg body wt. Oral treatment with 400 mg/kg body wt of the *Brassica* extract was effective in inducing the glutathione level in the lung only. However, there was a significant increase in the glutathione level in lung, kidney and forestomach after animals were fed with BHA in diet for 15 days (Table 1).

The concept of chemoprevention was based in part on epidemiological observations that suggested that high intake of vegetables could be associated with reduced risk of cancer and notably cruciferous vegetables including broccoli, cabbage, cauliflower and brussels sprout seemed particularly beneficial in preventing carcinogenesis.

The active phytochemicals present in several members of the *Brassicaceae* family that led credence to chemoprevention include indole glucosinolates, aromatic isothiocyanates, dithiolthione and phenols.

These compounds have been reported to exert broad-based anticarcinogenic activity against a variety of chemical carcinogens at multiple target sites in animal models via induction of phase-II detoxification enzymes, such as DTD and GST. As a group, these inducible enzymes facilitate the metabolic detoxification of xenobiotics in mammals and can achieve chemopreventive activity by modification of carcinogen metabolism through increased carcinogen excretion and decreased carcinogen DNA interactions.

Table 1 — Modulatory influence of *Brassica compestris* extract on glutathione S-transferase (GST), DT-diaphorase (DTD) activities and glutathione levels (GSH) in mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Treatment</th>
<th>Organ</th>
<th>GST*</th>
<th>DTD**</th>
<th>GSH***</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Skin</td>
<td>Liver</td>
<td>Lung</td>
<td>Kidney</td>
</tr>
<tr>
<td>Group - I</td>
<td>Double distilled water &amp; <em>R. compestris</em> extract (400 mg/kg b.wt.)</td>
<td>0.039 ± 0.006</td>
<td>1.84 ± 0.18</td>
<td>0.38 ± 0.01</td>
<td>0.544 ± 0.036</td>
<td>0.47 ± 0.038</td>
</tr>
<tr>
<td>Group - II</td>
<td>BHA in diet (0.75%)</td>
<td>0.036 ± 0.002</td>
<td>2.38 ± 0.21</td>
<td>0.42 ± 0.02</td>
<td>0.607 ± 0.038</td>
<td>0.36 ± 0.28</td>
</tr>
<tr>
<td>Group - III</td>
<td><em>R. compestris</em> extract (800 mg/kg b.wt.)</td>
<td>0.041 ± 0.006</td>
<td>3.46 ± 0.12</td>
<td>0.43 ± 0.03</td>
<td>0.551 ± 0.019</td>
<td>0.57 ± 0.05</td>
</tr>
<tr>
<td>Group - IV</td>
<td>BHA in diet (0.75%)</td>
<td>0.073 ± 0.006</td>
<td>6.34 ± 0.28</td>
<td>0.68 ± 0.02</td>
<td>1.02 ± 0.053</td>
<td>0.85 ± 0.07</td>
</tr>
<tr>
<td>Group - V</td>
<td>Double distilled water</td>
<td>0.038 ± 0.003</td>
<td>0.070 ± 0.001</td>
<td>0.015 ± 0.007</td>
<td>0.091 ± 0.004</td>
<td>0.045 ± 0.003</td>
</tr>
<tr>
<td>Group - VI</td>
<td><em>B. compestris</em> extract (400 mg/kg b.wt.)</td>
<td>0.039 ± 0.001</td>
<td>0.068 ± 0.002</td>
<td>0.016 ± 0.005</td>
<td>0.097 ± 0.005</td>
<td>0.067 ± 0.007</td>
</tr>
<tr>
<td>Group - VII</td>
<td><em>B. compestris</em> extract (800 mg/kg b.wt.)</td>
<td>0.065 ± 0.003</td>
<td>0.194 ± 0.003</td>
<td>0.016 ± 0.001</td>
<td>0.090 ± 0.004</td>
<td>0.069 ± 0.006</td>
</tr>
<tr>
<td>Group - VIII</td>
<td>BHA in diet (0.75%)</td>
<td>0.018 ± 0.001</td>
<td>0.098 ± 0.004</td>
<td>0.031 ± 0.002</td>
<td>0.131 ± 0.007</td>
<td>0.059 ± 0.007</td>
</tr>
<tr>
<td>Group - IX</td>
<td>Double distilled water</td>
<td>0.062 ± 0.05</td>
<td>3.86 ± 0.16</td>
<td>1.12 ± 0.03</td>
<td>1.55 ± 0.03</td>
<td>0.68 ± 0.02</td>
</tr>
<tr>
<td>Group - X</td>
<td><em>B. compestris</em> extract (400 mg/kg b.wt.)</td>
<td>0.72 ± 0.06</td>
<td>4.44 ± 0.21</td>
<td>1.42 ± 0.06</td>
<td>1.46 ± 0.05</td>
<td>0.71 ± 0.03</td>
</tr>
<tr>
<td>Group - XI</td>
<td><em>B. compestris</em> extract (800 mg/kg b.wt.)</td>
<td>0.86 ± 0.06</td>
<td>6.38 ± 0.28</td>
<td>1.39 ± 0.05</td>
<td>2.33 ± 0.04</td>
<td>0.77 ± 0.04</td>
</tr>
<tr>
<td>Group - XII</td>
<td>BHA in diet (0.75%)</td>
<td>0.47 ± 0.04</td>
<td>6.88 ± 0.76</td>
<td>2.44 ± 0.04</td>
<td>2.42 ± 0.03</td>
<td>0.85 ± 0.05</td>
</tr>
</tbody>
</table>

*P values: *< 0.1; **< 0.05; ***< 0.01; ****< 0.001*

*GST activity is expressed as µmole of GSH-CNB conjugate formed/min/mg protein.

**DTD activity is expressed as amount of enzyme required to reduce one µmole of DCPIP/min.

***GSH level was measured as µmole/100 g tissue.
Natural isothiocyanates derived from aromatic and aliphatic glucosinolates are effective chemopreventive agents that block chemical carcinogenesis and prevent several types of cancer in rodent models. Mechanistic studies have shown that isothiocyanates target mammalian phases I and II drug-metabolizing enzymes and their coding genes, resulting in decreased carcinogen-DNA interactions and an increase in carcinogen detoxification. Members of the family Brassicaceae contain relatively high glucosinolate content. Glucosinolate is usually broken down due to hydrolysis catalyzed by myrosinase. Some of this hydrolytic products mainly indoles, and isothiocyanates are able to influence phases I and II biotransformation enzyme activities, thereby possibly influencing inhibitory processes related to chemical carcinogenesis.

It has been reported that male Wistar rats fed with a mustard diet showed a decrease in aryl hydrocarbon hydroxylase (AHH), an increase in the glucuronosyl transferase (UDP) and glutathione-S-transferase (GST) and a decrease in the benzo(a)pyrene binding hepatic DNA.

Indole 3 carbinol (1-3 C) has been shown to be one of the major anti-cancer substances found in cruciferous vegetables. Frequent consumption of cruciferous vegetables is associated with reduced risk of cancer in many human epidemiological studies and animal experiments. I-3C and other glucosinolates (other indoles and isothiocyanates, such as sulfur-alphane) are antioxidants and potent stimulators of phase I and II detoxification enzymes in the liver and intestinal epithelial cells. Further, it has been shown that phenethyl isothiocyanate and allyl isothiocyanate induce apoptosis, DNA fragmentation and cell death of human leukemia HL 60 cells in vitro.

The findings of the present study reveal that the administration of alcoholic seed extract of Brassica campestris can significantly modulate the activities of the enzymes that are crucial for cancer protection. Oral treatment with Brassica extract revealed a significant increase in DT-diaphorase activity in skin and forestomach at high dose and in forestomach at low dose only. DT-diaphorase induction facilitated bio-reductive activation metabolism of quinones by two electron reduction to hydroxyquinone obliterating semiquinone and oxygen radical formation hydroquinone formed via two electron reduction by DT-diaphorase can be conjugated by glucuronic or sulfate and excreted. It may be speculated that increase in DT-diaphorase activity might provide in vivo protection to tissues whereas carcinogenicity or genotoxicity are mediated by electrophilic metabolites.

The present study also showed that Brassica seed extract produced substantial elevation in GST activity in lung and forestomach when animals were treated at high dose level only. The role of GST in catalyzing the reaction of a wide variety of electrophiles (reactive carcinogenic forms of chemical carcinogens) to GSH (one of the major endogenous antioxidants) has been well established and the formation of glutathione conjugates of xenobiotics has been associated with the cellular detoxification system. In addition, GST can bind covalently with a variety of carcinogens. So, the enhanced level of GST activity suggests further metabolic disposition of chemical carcinogens.

The present investigation also demonstrates that oral treatment with Brassica seed extract significantly elevated the level of reduced glutathione in all tissues studied when given at high dose and in lung only at low dose. The reduced glutathione functions to protect the cells against free radicals generated during oxidative metabolism as well as from cellular lethality following exposure to drugs or radiation and acting as an acceptor of the electrophilic molecules involved in cancer initiation. Thus, Brassica campestris seed extract renders the mice resistant to harmful oxygen species and chemical carcinogenic action.

In addition to our earlier report of Brassica extract protection against DMBA-induced skin papillomagenesis, the current data suggest that Brassica seed extract may be an effective chemopreventive agent in other organ sites where in phase-II detoxification enzymes and glutathione levels are induced.

References
2. Qiblawi S & Kumar A, Chemopreventive action by an extract from Brassica campestris (Var sarson) on 7, 12-dimethyl benz (a) anthracene induced skin papillomagenesis in mice, Planta Med, 63 (1999) 261.


