SUMMARY AND CONCLUSION
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At present cancer is the second major cause of death after heart disease. The efforts to improve treatment and to find cure for advanced stages of the disease have not declined the overall mortality for most forms of cancer in the past 25 years (WHO, 1997).

"Prevention is better than cure." This well known phrase has become increasingly popular in relation to the prevention of cancer. Chemoprevention represents a new strategy that involves administration of non toxic doses of synthetic chemical compounds or natural agents that either prevent the formation of carcinogens from precursor compounds, or inhibit the development of invasive cancer by blocking the DNA damage that initiates carcinogenesis, or arrest/reverse the progression of premalignant cells in which such damage has already occurred. Thus, certain precancerous changes can be reversed before the onset of irreversible stages of carcinogenesis.

The present study reports the chemopreventive potential of an ethanolic extract (95%) of mustard seed (*Brassica compestris* var sarason) fed orally in double distilled water on DMBA induced skin cancer in male swiss albino mice.

The study also reports the modulatory influence of an ethanolic extract (95%) of mustard seed (*Brassica compestris* var sarason) on mouse hepatic and extra hepatic carcinogen biotransformation enzyme activity, glutathione and lipid peroxidation level.
Materials and Methods:

Random-bred, male Swiss albino mice (7-8 weeks old) were used for this study. They were provided with standard mice feed (Hindustan Lever Ltd., India) and tap water ad libitum.

7. 12-dimethyl benz (a) anthracene (DMBA) and croton oil were procured from Sigma Chemicals Co., USA. DMBA was dissolved in acetone at a concentration of 100 μg/50 μl. Croton oil was diluted in acetone to give 1% dilution.

1-chloro-2-4-dinitrobenzene (CDNB). 5. 5-dithiobis nitrobenzoic acid (DTNB), reduced glutathione (GSH), oxidized glutathione, bovin serum albumen (BSA), reduced nicotinamide adenine dinucleotide (NADH). reduced nicotinamide adenine dinucleotide phosphate (NADPH). 2. 6-dichlorophenol-indophenol (DCPIP), 2-thiobarbituric acid (TBA) and 3(2)-tert-butyl-4-hydroxyanisole (BHA) were obtained from Sigma Chemical Co. (St. Louis MO, USA).

An ethanolic mustard seed extract of Brassica campestris var sarason was prepared by extracting the seed powder exhaustively by soxhalation with 95% of ethanol for 12 hour thrice (20 x 3 hrs.). The extract was filtered and concentrated under reduced pressure, where upon viscous brown mass was obtained. The extract was appropriately dissolved in double distilled water and given at two dose levels 400 mg/kg and 800 mg/kg b.w. for biotransformation enzymes study and one dose level 800 mg/kg b.w. for skin tumor model system study. The extract was given through gastric intubation.

For the induction of tumors, the two stage protocol consisting of initiation with a single topical application of DMBA followed by thrice weekly treatment with croton oil was employed.
EXPERIMENTAL PROTOCOL
an ethanolic *Brassica compestris* seed extract

- Clipping of Hair → Application of DMBA → Application of Crotol Oil → *
  - 15 days
  - 3 days
  - 2 weeks
  - 14 weeks

* - Crotol oil was applied for 14 weeks, thrice weekly, and oral treatment of *Brassica compestris* extract at the dose of 800 mg./kg body weight in double distilled water was given daily throughout the experimental protocol and at the termination of the experiment, the following PARAMETERS WERE ASSESSED:

PARAMETERS

- Tumor size
- Tumor weight
- Tumor incidence
- Cumulative number of papillomas
- Tumor mean
- Tumor burden
- Body weight
- Average latent period of tumor
- Tumor histology
### EXPERIMENTAL DESIGN

<table>
<thead>
<tr>
<th>Group</th>
<th>TREATMENT &amp; DOSE</th>
<th>REMARKS</th>
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<tbody>
<tr>
<td>SKIN TUMOR MODEL SYSTEM</td>
<td></td>
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<tr>
<td>a 1a</td>
<td>Double distilled water: -</td>
<td>Control: 16 weeks duration inclusive of initiation.</td>
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<tr>
<td></td>
<td>DMBA \ Croton oil: + 100 µg/50µl acetone</td>
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<tr>
<td></td>
<td>+ 100 µl of 1% of croton oil in acetone</td>
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<td></td>
<td>Brassica compestris extract: -</td>
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<td></td>
<td>BHA: -</td>
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<td>b</td>
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<tr>
<td>CARCINOGEN BIOTRANSFORMATION ENZYME SYSTEM</td>
<td></td>
<td></td>
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<tr>
<td>a 2a</td>
<td>Double distilled water: + 0.05 ml/animal/day</td>
<td>Control - 15 days</td>
</tr>
<tr>
<td></td>
<td>DMBA \ Croton oil: -</td>
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<tr>
<td></td>
<td>Brassica compestris extract: -</td>
<td></td>
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<tr>
<td></td>
<td>BHA: -</td>
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<tr>
<td>b</td>
<td>Double distilled water: -</td>
<td>Experimental - 15 days</td>
</tr>
<tr>
<td></td>
<td>DMBA \ Croton oil: -</td>
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<tr>
<td></td>
<td>Brassica compestris extract: + 400 mg/kg b.w.</td>
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<tr>
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<td>BHA: -</td>
<td></td>
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<td>c</td>
<td>Double distilled water: -</td>
<td>Experimental - 15 days</td>
</tr>
<tr>
<td></td>
<td>DMBA \ Croton oil: -</td>
<td></td>
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<tr>
<td></td>
<td>Brassica compestris extract + 800 mg/kg b.w.</td>
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<tr>
<td></td>
<td>BHA: -</td>
<td></td>
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<tr>
<td>d</td>
<td>Double distilled water: -</td>
<td>Positive Control - 15 days</td>
</tr>
<tr>
<td></td>
<td>DMBA \ Croton oil: -</td>
<td></td>
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<tr>
<td></td>
<td>Brassica compestris extract: + 0.75% in diet</td>
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<td></td>
<td>BHA: -</td>
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a - Parameters studied after 14 weeks of promotion were tumor incidence, tumor size, tumor burden, tumor weight, cumulative numbers of papillomas, average latent period.

b - Parameters studied were hepatic carcinogen biotransformation enzyme i.e. cytochrome P-450, cytochrome h-5, GST activity, GR activity, -SH content, DT-D, Gpx, SOD, Catalase and extra hepatic - GST Activity, DT-D Activity, and SHI Content.
During the 16 weeks of experiments, the mice were weighed weekly and also at the time of autopsy. They were carefully examined once a week for the presence of skin papillomas. The number of papillomas on each affected mouse was recorded. Skin papillomas were defined as lesions with a diameter greater than 1mm that were present for at least two consecutive observations.

Cytochrome p-450 content was determined spectrophotometrically in microsomal suspension by the method of Omura and Sato (1964) by recording the difference in absorbance between 450 nm and 490 nm using an absorption coefficient of 91 cm$^2$/nmol.

Cytochrome b5 was determined spectrophotometrically in microsomal suspension by the method of Omura and Sato (1964) by recording the difference in absorbance between 424 nm and 490 nm using an absorption coefficient of 185 cm/nmol respectively.

The cytosolic GST activity was determined spectrophotometrically according to the procedure of Habig et al. (1974).

The cytosolic GR activity was also determined spectrophotometrically according to Cardilberg and Mannervik (1975).

The cytosolic GPX activity was determined spectrophotometrically according to Paglia and Valentine (1967).

The superoxide dismutase (SOD) activity was estimated spectrophotometrically as described by Marklund et al., 1974.

The catalase (CAT) activity was estimated spectrophotometrically as described by Aebi (1984).

The cytosolic DT - diaphorase (DT-D) was determined spectrophotometrically according to Ernster et al. (1962).
The acid soluble-SH group level was determined by the method of Moron et al. (1979) using Shimadzu UV 160 spectrophotometer.

The lipid peroxidation (LPO) level in the microsomal suspension was estimated at the number of n moles of MDA formed by the method of Pryor (1976).

The protein content was determined by the method of Lowry (1951) using bovin serum albumen as a standard.

**Observations**:

1. An ethanolic mustard seed extract of *Brassica campestris* var sarason administered orally in double distilled water at doses of 400, 800 and 1000 mg/kg body weight for 7 days did not show any external toxicity symptoms.

2. The control group fed orally with 0.05 ml double distilled water for 15 days gave the following results:
   
   (a) The cytochrome p450 content was 0.40±0.03
   
   (b) The cytochrome b-5 content was recorded as 0.21±0.03
   
   (c) The glutathione-S-Transferase activity was observed to be 2.00±0.27
   
   (d) The DT-diaphorase activity was observed to be 0.075±0.001
   
   (e) The superoxide dismutase activity was recorded as 2.38±0.60
   
   (f) The catalase activity was found to be 73.59±2.15
   
   (g) The glutathione peroxidase activity found 18.74±1.28.
   
   (h) The acid sulphydryl group content was 4.06±0.14

3. The group treated with mustard seed extract of *Brassica campestris* var sarason orally at the dose of 400 mg/kg body weight for 15 days
did not show any significant changes in any of the hepatic parameters as compared to the control group. However the following changes were observed in the extra hepatic parameters.

(a) In the forestomach DT-diaphorase activity increased significantly (0.067±0.007) (0.05) (P < 0.05).
(b) In the lung the acid soluble sulphhydril content increased significantly (1.42±0.064) (P < 0.001).

4. Animals fed with mustard seed extract orally in double distilled water at the dose of 800 mg/kg body weight for 15 days showed the following changes in the hepatic parameters as compared to the control.

(a) The cytochrome p 450 content increased significantly (0.52±0.04) (P < 0.05).
(b) These cytochrome b-5 content enhanced significantly (0.30±0.026) (P < 0.05).
(c) The glutathione-S-Transferase activity increased significantly (3.68±0.18) (P < 0.1).
(d) No significant change in the glutathione reductase activity were recorded (47.97±0.85).
(e) No significant change (16.71±1.03) in the glutathione peroxidase activity recorded.
(f) The DT-diaphorase activity elevated significantly (0.086±0.004) (P < 0.05).
(g) The superoxide dismutase activity increased significantly (4.71±0.44) (P < 0.01).
(h) The catalase activity enhanced significantly (96.77±2.68) (P < 0.001).
(i) The acid soluble sulphhydril content was significantly increased
(6.44±0.34) (p < 0.001).

(j) There was no significant change in the lipid peroxidation level
(7.16±0.65).

5. Treatment of mustard seed extract at the dose of 800 mg/kg body
weight for 15 days showed the following changes in the extra hepatic
parameters as compared to the control group.

(a) In the skin, glutathione-S-Transferase activity showed no
significant change (0.041±0.006).

(b) In the skin, DT-diphorase activity increased significantly
(0.065±0.003).

(c) In the lung, GST activity elevated significantly (0.43±0.02) (P
< 0.05).

(d) In the fore stomach GST activity increased significantly
(1.56±0.27) (P < 0.1).

(e) In the lung, DT-diaphorase activity increased significantly
(0.016±0.001) (P < 0.001).

(f) In the fore stomach, DT-diaphorase activity enhanced
significantly (0.069 ± 0.008) (P < 0.05).

(g) In the skin, the acid soluble sulphhydril content increased
significantly (0.86±0.06) (P < 0.01).

(h) In the lung, the acid sulphhydril content increased significantly
(1.42 ± 0.064) (P < 0.001).

(i) In the kidney, the acid sulphhydril content increased significantly
(2.33±0.044) (p < 0.001).
6. The BHA fed group (0.75% in diet) for 15 days, serving as a positive control showed the following changes in the hepatic parameters.

(a) The cytochrome p-450 content elevated significantly (0.64±0.03) (P < 0.001).

(b) The cytochrome b-5 content increased significantly (0.30±0.014) (P < 0.01).

(c) The glutathione-S-Transferase activity enhanced significantly (6.54±0.48) (P < 0.1).

(d) The DT-diaphorase activity increased significantly 0.101±0.004 (P < 0.001).

(e) No significant change in the superoxide dismutase activity recorded 2.35±0.41.

(f) The catalase activity decreased significantly (34.12±1.33) (P < 0.001).

(g) The glutathione reductase activity elevated significantly (74.01±2.76) (P < 0.001).

(h) The glutathione peroxidase activity increased significantly (21.76±0.98) (P < 0.1).

(i) The acid soluble-sulphydryl content significantly increased (7.75±0.62) (P < 0.001)

(j) There was no significant change in the lipid peroxidation level 7.76 ± 0.37.

7. The group treated with BHA (0.75% in diet) showed the following changes in the extra hepatic parameters.

(a) In the skin. GST activity elevated significantly (0.073±0.006) (P < 0.001).
(b) DT-diphorase activity decreased significantly in the skin
(0.018±0.001) (P < 0.001).

c) In the lung, DT-diaphorase enhanced significantly (0.68±0.02)
(P < 0.001).

d) GST activity increased significantly in the fore stomach
(0.85±0.079) (P < 0.001).

e) DT-diaphorase activity increased significantly in the kidney
(0.131 ± 0.007) (P < 0.001).

(f) In the kidney GST activity increased significantly (1.021 ±
0.053) (P < 0.001).

(g) In the skin the acid sulphydryl group content significantly
decreased (0.47 ± 0.04) (P < 0.05).

(h) The acid sulphydryl group increased significantly in the lung
2.44 ± 0.042 (P < 0.001).

(i) The acid sulphydryl group enhanced significantly in the kidney
to 2.42±0.039 (P < 0.001).

Skin Tumor Model System

8. The control group of the skin tumor model system showed the
following results:

(a) The tumor incidence was 100%

(b) The tumor burden was 8.9.

(c) The average weight of tumor was 123.8 mg.

(d) The cumulative number of papillomas was 71.

(e) The average latent period was recorded as 7.8±0.17 weeks.

(f) The tumor mean per mouse was 8.9.
9. Mustard seed extract administered orally at the dose of 800 mg/kg body weight showed the following changes as compared to the control group.

(a) The tumor incidence decreased significantly to 50% (p < 0.001).
(b) The tumor burden decreased significantly to 2.3.
(c) The average tumor weight was significantly reduced 5.27 mg (p < 0.001).
(d) The cumulative number of papillomas decreased significantly 9.
(e) The average latent period increased significantly 11.3±0.40 (p < 0.005).
(f) The tumor mean per mouse decreased significantly 1.13±0.48 (p < 0.001).

Conclusion and Recommendations:

1. An ethanolic mustard seed extract of *Brassica compestris* var sarason at the dose of 800 mg/kg, body weight showed significant results but was not effective at lower doses.

2. An ethanolic mustard seed extract of *Brassica compestris* var sarason dissolved in double distilled water exhibits a chemopreventive action on DMBA induced skin papillomagenesis in Swiss albino mice when fed continuously at all stages of tumorigenesis.

3. Mustard seed extract treatment (when given orally) has a modulatory influence on mouse hepatic and extra hepatic biotransformation enzyme activities and glutathione level.
Alterations in these parameters can change the host response to xenobiotics owing to their ability to introduce a polar group in a xenobiotic compound, thereby providing a means by which a subsequent conjugation reaction can take place resulting in inhibition of carcinogenesis. Since a Cyt P-450 dependent mixed function oxidase, participates in generation of ultimate carcinogenic moiety in target tissue, concomitant elevation of phase II enzyme activity (GST, D-Td, SOD, Catalase) would result in the detoxification of ultimate carcinogenic moiety.

4. A continuous treatment of mustard seed extract not only lowers the carcinogenic ability of DMBA but also modulate the effects of promoter i.e. croton oil and the protective effect is reflected in the decreased values of tumor burden, tumor incidence, tumor mean and the cumulative number of papillomas as well as increase in the latency period.

The present study suggest the potential anti tumor activities of a crude 95% ethanolic extract of the mustard seed of *Brassica compestris* var sarason. Because mustard is already a part of the dietary habits, it's application in human cancer chemoprevention warrants more attention in the management of the global cancer burden in high risk population.