4. RESULTS & DISCUSSION...
PHYLLANTHUS EMBLICA...
4.1.1 Experiment I

In these set of experiments, the effect of Phyllanthus emblica on benzo(a)pyrene induced forestomach tumorigenesis in male Swiss albino mice has been examined.

Results

Table 4.1 depicts the results obtained by dietary supplementation of Phyllanthus emblica on B(a)P induced forestomach tumorigenesis. No noticeable difference was seen in weight gain profile of animals treated with either dose of Phyllanthus emblica diet as well as in the positive control group of mice.

Tumor Burden

The mean number of papilloma/mouse (tumor burden) in the control group (B[a]P only) of animals was 7.182±1.328. In contrast, animals treated with 2.5% and 5% diet of Phyllanthus emblica, tumor burden reduced to 1.182±0.751 (p<0.001) and 0.818±0.751 (p<0.001) respectively. The percentage inhibition of tumor multiplication was 83.54 and 88.61 by the low and high doses of Phyllanthus emblica treatment respectively.

Tumor Incidences

100% of the control animals developed forestomach papillomas by B(a)P treatment. Relative to control tumor incidences were 81.82% and 63.63% in case of 2.5% and 5% dose of Phyllanthus emblica respectively. Total tumor incidence decreased was 18.18 % with the low dose and 36.37% with the high dose of Phyllanthus emblica. 5% diet of Phyllanthus emblica treatment (negative control group) did not result in any tumor.

4.1.2 Experiment II

In these set of experiments, modulation of hepatic xenobiotic metabolising phase I and phase II enzymes; antioxidants, LDH, Glyoxalase I and lipid peroxidation by Phyllanthus emblica has been examined.
Results

Body weights and relative liver weights of various groups of animals at the termination of experiment have been summarized in Table 4.2. There was no significant difference of body weight gain and liver/final body weight profile between control and modulator treated animals.

Phase I Enzymes

(Refer: Table 4.3 and Figure 4.1)

Cytochrome P450

Cytochrome P450 was reduced significantly by 0.49 fold (p<0.005) with 2.5% diet, 0.35 folds (p<0.005) with 5% diet and 0.41 folds (p<0.005) with 10% diet of *Phyllanthus emblica*.

Cytochrome b5

*Phyllanthus emblica* in diet resulted in a reduction of cytochrome b5 activity. The reduction was 0.83 folds (p<0.01) with 2.5% diet, 0.78 folds (p<0.005) with 5% diet and 0.78 folds (p<0.005) with 10% diet of *Phyllanthus emblica*.

Cytochrome P450R

No significant change was observed in the activity of cytochrome P450R.

Cytochrome b5R

No significant change was also observed in the activity of cytochrome b5R.

Phase II Enzymes

(Refer: Table 4.3 and Figure 4.2)

GST

Specific activity of GST showed significant increase with all the doses of *Phyllanthus emblica* used in our experiments. Relative to the level in untreated control animals, the activity of GST was enhanced by 2.19 folds (p<0.005), 2.13 folds (p<0.005) and 1.82 folds (p<0.005) with the 2.5%, 5% and 10% of *Phyllanthus emblica* diet respectively.
**DTD**

Specific activity of DTD showed significant increase with 2.5% and 5% dose of *Phyllanthus emblica* used in our experiments. Relative to the level in untreated control animals, the activity of DTD was enhanced by 1.44 folds (p<0.005) and 1.31 folds (p<0.05) with the 2.5% and 5% dose of *Phyllanthus emblica* diet respectively. No significant change was observed at the 10% dose of *Phyllanthus emblica*.

**Antioxidants**

(Refer: Table 4.4 and Figure 4.3)

**SOD**

Specific activity of SOD showed significant increase by all the doses of *Phyllanthus emblica* used in our experiments. Relative to the level in untreated control animals, the activity of SOD was enhanced by 1.11 folds (p<0.05), 1.18 (p<0.05) and 1.17 folds (p<0.05) with the 2.5%, 5% and 10% of *Phyllanthus emblica* diet respectively.

**CAT**

Relative to the level in untreated control animals, the activity of CAT was enhanced by 1.32 folds (p<0.01) with the 2.5% dose of *Phyllanthus emblica* diet. No significant change was observed at the 5% and 10% dose of *Phyllanthus emblica*.

**Reduced Glutathione**

The level of GSH was enhanced by all the doses of the *Phyllanthus emblica*. With 2.5% diet, GSH increased by 1.40 folds (p<0.005), 5% diet resulted in an increase of 1.44 folds (p<0.005) and the 10% diet showed an elevation of 1.56 folds (p<0.005).

**Other Parameters**

**Glyoxalase I**

The activity of Glyoxalase I was inhibited significantly by all the investigated doses of *Phyllanthus emblica*. With 2.5% diet there was an inhibition of 0.49 folds (p<0.01), by 5% diet the reduction was 0.68 folds (P<0.01) and the 10% diet revealed a decrease of 0.60 folds (p<0.01) (Table 4.4, Figure 4.3).
**LDH**

The activity of LDH was inhibited significantly by the 5% and 10% dose of *Phyllanthus emblica*. With 5% diet, the reduction was 0.76 folds (p<0.005) and the 10% diet revealed a decrease of 0.69 folds (p<0.005) (Table 4.4, Figure 4.2).

**Lipid peroxidation**

Lipid peroxidation measured as formation of malondialdehyde (MDA), showed significant inhibition by all the doses of *Phyllanthus emblica*. Lipid peroxidation was reduced by 0.69 folds (p<0.005) with 2.5% diet, 0.69 folds (p<0.005) with 5% diet and 0.56 folds (p<0.005) with 10% diet of *Phyllanthus emblica* as compared to the control group (Table 4.4, Figure 4.2).

**4.1.3 Experiment III**

The modulatory influence of *Phyllanthus emblica* fruit extract on Vitamin C content in the liver of Swiss albino mice was investigated.

**Results**

**Vitamin C**

Feeding of freshly prepared 100 µl juice of *Phyllanthus emblica* for 15 days resulted in a sharp increase in the vitamin C content of the liver by 3.31 folds (p<0.005) as compared to the untreated control (Table 4.5, Figure 4.4).

**Discussion**

*Phyllanthus emblica* fruit is probably the richest known natural source of vitamin C. It has been used in India since ancient times against asthma, bronchitis and biliousness. Dried amla fruit is used against haemorrhage, diarrhoea and dysentery. Fermented liquor prepared from the amla fruit is used in jaundice, dyspepsia and cough. It is also known for its cooling, refrigerant, diuretic and laxative effects.
The chemopreventive potentials of *Phyllanthus emblica* has not been evaluated before in B(a)P induced forestomach tumorigenesis. B(a)P is one of the best characterized prototype chemical that is suspected to be a potent human carcinogen. It is one of the component of cigarette smoke and burning of tar and petrol. Burning of anything that is organic in nature also gives rise to B(a)P. Therefore, it is important to search for substances that inhibit activation or activate the detoxification of B(a)P.

Findings of the present investigation reveals that *Phyllanthus emblica* is very potent inhibitor of B(a)P induced stomach tumorigenesis at peri-initiational level. It showed significant reduction in the total tumor incidence as well as tumor burden. *Phyllanthus emblica* fruit juice is non-toxic as even the high dose alone did not result into any adverse effect like body weight loss or tumor initiations.

Since, the modulatory influence of *Phyllanthus emblica* on the process of carcinogenesis is likely be due to alterations in the level of enzymes that catalyze biotransformation of endogenous as well as exogenous chemicals including carcinogens. The effect of *Phyllanthus emblica* on the modulation of phase I and phase II drug metabolising enzymes has been evaluated.

A number of enzymes required for oxidative metabolism of drugs/xenobiotics are termed as “mixed-function oxidases”. The key enzyme of these oxidases is cytochrome P450, which represents a major defense against chemical challenge from environment, constituting part of an adaptive response mounted by an organism following exposure to harmful agents (Henderson *et al.*, 2000). At the same time excessive P450 activity without adequate concomitant conjugating activity may be a risk factor for cancer. Because the intermediate metabolites are often highly reactive that can form DNA adducts or cause oxidative stress and cytotoxic effects (Lampe *et al.*, 2000). Disulfiram, One of the first cytochrome P450 inhibitors was shown to have chemopreventive activity (Wattenberg, 1979).

Many studies in human beings suggested that the formation of various DNA adducts are positively related to the expression and activity of associated CYP enzymes (Rojas *et al.*, 1998 and Mollerup *et al.*, 1999). In the present study all the three doses of *Phyllanthus emblica* significantly inhibited the cytochrome P450 as well cytochrome
b5 levels. This inhibitory effect of *Phyllanthus emblica* on phase I system lower the possibility of activation of B(a)P to great extent and help in preventing the forestomach carcinogenesis.

The products of phase I metabolism are acted upon by phase II enzymes. GST, one of the major phase II enzymes, catalyze the conjugation of variety of structurally diverse exogenous and endogenous components with the non-protein thiol glutathione. It has been proposed that GST may have evolved to provide protection against reactive products of oxidation metabolism (Mannervik *et al.*, 1978). GST is known to assist in the detoxification of many carcinogens; therefore the changes in its activity have great significance from view of chemical carcinogens (Sparnis *et al.*, 1998). Loss of GST is associated with several kinds of cancer including prostate, urothelial, lung and colorectal (Mates, 2000). In the present study, *Phyllanthus emblica* has significantly induced the activity of GST and probably suggestive of its usefulness in cancer prevention.

GSH content and its associated enzymes have been widely studied and shown to have important role in the protection against the free radicals as well as in drug detoxification (Meister and Anderson, 1983). The protective action of GSH is due to the thiol group of its cysteine with the formation of oxidized glutathione (Tzeon-Jye chiou, 2000). GSH is a natural antioxidant found in many dietary components, which can protect the cell during both initial and the promotional phases of carcinogenesis. GSH is involved in the conjugation of electrophiles, such as reactive metabolite of B(a)P. Such mechanism would decrease the levels of reactive electrophiles available to attack DNA, reducing the likelihood of DNA damage and possible induction of the carcinogenesis process. Our investigation revealed a sharp increase in the level of hepatic GSH with the treatment of *Phyllanthus emblica*.

Lipid peroxidation is a free radical process, which involves the oxidation of polyunsaturated fatty acids (PUFA) as component of cell membranes. The products of this process have been linked to spontaneous mutagenesis and carcinogenesis. Malondialdehyde (MDA), the major products of lipid peroxidation has been proved mutagenic in bacterial and mammalian cells and carcinogenic in rats. Elevated lipid
peroxidation is associated with antioxidant depletion due to generation of reactive oxygen species (ROS). Hence, a check on lipid peroxidation is essential for all the aerobic organisms. Our study showed *Phyllanthus emblica* as a potent inhibitor of lipid peroxidation.

The glyoxalase system is present in prokaryotes as well as in eukaryotes. Methylglyoxal and α-keto-aldehydes are substrates of the glyoxalase system, which are associated with anti-viral (Underwood and Weed, 1952) and antitumor activities (Szent-Gyorgi, 1965). It has been found that methylglyoxal and α-keto-aldehydes changes the electronic nature of proteins by binding and modifying the susceptible amino acid residue leading to the arrest of cell division (Szent-Gyorgyi, 1973). Conditions that destabilize these complexes and lower their concentrations can promote cell division. Thus, the increase of glyoxalase I activity is generally correlated with the cell proliferation. For example, glyoxalase I activity has been found to be higher in platelets of patients suffering with thrombocytosis with myeloproliferative disorders (Leocini, 1984). Twelve folds increase in Gly I RNA transcription level in colonic carcinoma was seen as compared to the colonic tissue of the same patient (Ranganathan et al., 1993). In the present study the *Phyllanthus emblica* juice diet resulted in significant decrease of glyoxalase I. Methylglyoxal as well as α-keto-aldehydes accumulated due to inhibition of glyoxalase I, would persist and arrest possible abnormal cell proliferation, which is one of the major properties of cancer cells.

Vitamin C has been shown to inhibit the intra-gastric formation of N-nitroso compounds. In animal studies, the inhibition of tumor formation has been observed when vitamin C is fed together with nitrite, amines, amides or with pre-formed carcinogens (Mirvish *et al.*, 1983; Mirvish, 1986). Supplementation with vitamin C has been shown to decrease the mutagenicity of gastric juice in humans (Singh and Gaby, 1992). The protection provided by *Phyllanthus emblica* against clastogenicity of the metal salt was found due to its vitamin C content (Ghosh *et al.*, 1992). The present study revealed that vitamin C content in the liver increased by three folds with
Phyllanthus emblica fruit extract treatment. Therefore, it could be speculated that vitamin C has a key role in the chemopreventive action of Phyllanthus emblica.

In our investigation, the phase I enzymes were inhibited significantly along with sharp increase of phase II drug detoxification enzyme by Phyllanthus emblica. Therefore, Phyllanthus emblica comes under duel acting category of chemopreventive agents. The protection afforded by Phyllanthus emblica diet might be due to selective inhibition of cytochrome P450 system and induction of GST activity. GSH content showed an elevation that correlates with the significant inhibition of lipid peroxidation by Phyllanthus emblica. Further, the treatment of Phyllanthus emblica resulted in decrease of glyoxalase I activity, which suggests that it has a potential to arrest abnormal cell proliferation. Thus, the decreased activity of cytochrome P450, cytochrome b5 and glyoxalase I, inhibition of lipid peroxidation and enhancement of GST activity and GSH as well as vitamin C contents signifies important chemopreventive action of Phyllanthus emblica and possible application of this modulator as an anti-cancer drug.
Table 4.1: Effect of Two Different Doses of Amla (*Phyllanthus emblica*) on Benzo(a)pyrene Induced Forestomach Tumorigenesis in Swiss Albino Mice

<table>
<thead>
<tr>
<th>Groups &amp; Treatment</th>
<th>Body weight (gms)</th>
<th>Total tumor incidence (%)</th>
<th>Tumor burden (tumor / mouse)</th>
<th>Percentage inhibition of tumor burden</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>28.4 ± 3.24</td>
<td>32.0 ± 3.51</td>
<td>100</td>
<td>7.182 ± 1.328</td>
</tr>
<tr>
<td>+ 2.5% diet of</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Phyllanthus emblica</em></td>
<td>29.9 ± 3.17</td>
<td>34.0 ± 3.5</td>
<td>81.82</td>
<td>1.182 ± 0.751&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>30.6 ± 1.99</td>
<td>35.0 ± 2.9</td>
<td>63.63</td>
<td>0.818 ± 0.751&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>+ 5% diet of</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Phyllanthus emblica</em></td>
<td>31.6 ± 2.01</td>
<td>36.7 ± 2.3</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of 15-20 animals
<sup>d</sup>(p < 0.001), represent significant changes against control
Table 4.2: Influence of *Phyllanthus emblica* on Weight Gain, Liver/Body Weight and Protein Levels

<table>
<thead>
<tr>
<th>Groups &amp; Treatment</th>
<th>Body weight (gm)</th>
<th>Liver wt.X100/ Final body wt.</th>
<th>Protein (mg / ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Microsome</td>
</tr>
<tr>
<td>Control</td>
<td>31.5 ± 1.07</td>
<td>31.62 ± 1.30</td>
<td>6.62 ± 1.19</td>
</tr>
<tr>
<td></td>
<td>(100)</td>
<td>(100)</td>
<td>(100)</td>
</tr>
<tr>
<td>2.5% diet of</td>
<td>30.1 ± 2.23</td>
<td>32.50 ± 0.926</td>
<td>6.32 ± 0.49</td>
</tr>
<tr>
<td><em>Phyllanthus</em></td>
<td>(95.63)</td>
<td>(102.78)</td>
<td>(95.33)</td>
</tr>
<tr>
<td><em>emblica</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5% diet of</td>
<td>31.1 ± 1.72</td>
<td>32.25 ± 1.03</td>
<td>6.42 ± 0.99</td>
</tr>
<tr>
<td><em>Phyllanthus</em></td>
<td>(98.8)</td>
<td>(101.99)</td>
<td>(96.92)</td>
</tr>
<tr>
<td><em>emblica</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% diet of</td>
<td>30.3 ± 1.59</td>
<td>31.875 ± 1.72</td>
<td>6.01 ± 0.80</td>
</tr>
<tr>
<td><em>Phyllanthus</em></td>
<td>(96.41)</td>
<td>(100.8)</td>
<td>(90.76)</td>
</tr>
<tr>
<td><em>emblica</em></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of 6 - 8 animals
Values in parentheses represent changes in parameters assessed relative to control group
Treatment duration: 15 days
**Table 4.3: Modulatory Influence of Different Doses of Amla (*Phyllanthus emblica*) Fruit Extract on the Hepatic Phase I and Phase II Drug Metabolizing Enzyme in Swiss Albino Mice**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cyt P450</th>
<th>Cyt b5</th>
<th>Cyt P450 R</th>
<th>Cyt b5 R</th>
<th>GST</th>
<th>DTD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.273±0.042 (100)</td>
<td>0.505 ±0.061 (100)</td>
<td>1.10±0.16 (100)</td>
<td>4.01±0.21 (100)</td>
<td>2.87±0.50 (100)</td>
<td>0.039±0.007 (100)</td>
</tr>
<tr>
<td>2.5% diet of</td>
<td>0.134±0.027&lt;sup&gt;c&lt;/sup&gt; (49.08)</td>
<td>0.419±0.034&lt;sup&gt;b&lt;/sup&gt; (82.97)</td>
<td>1.22±0.06 (110.51)</td>
<td>3.96±0.16 (110.51)</td>
<td>6.30±0.27&lt;sup&gt;c&lt;/sup&gt; (219.66)</td>
<td>0.056±0.006&lt;sup&gt;c&lt;/sup&gt; (143.59)</td>
</tr>
<tr>
<td><em>Phyllanthus emblica</em></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>5% diet of</td>
<td>0.095±0.028&lt;sup&gt;c&lt;/sup&gt; (34.80)</td>
<td>0.396±0.055&lt;sup&gt;c&lt;/sup&gt; (78.41)</td>
<td>1.21±0.08 (109.33)</td>
<td>3.79±0.12 (94.64)</td>
<td>6.12±0.18&lt;sup&gt;c&lt;/sup&gt; (213.32)</td>
<td>0.051±0.007&lt;sup&gt;a&lt;/sup&gt; (130.77)</td>
</tr>
<tr>
<td><em>Phyllanthus emblica</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% diet of</td>
<td>0.113±0.025&lt;sup&gt;c&lt;/sup&gt; (41.39)</td>
<td>0.394±0.062&lt;sup&gt;c&lt;/sup&gt; (78.02)</td>
<td>1.22±0.09 (110.33)</td>
<td>3.91±0.06 (97.53)</td>
<td>5.23±0.31&lt;sup&gt;c&lt;/sup&gt; (182.43)</td>
<td>0.048±0.008 (123.08)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of 6-8 animals.

Values in parentheses represent relative change in parameters assessed (i.e., levels of activity in livers of mice receiving test substance to activity in liver of control mice).

<sup>a</sup> (p < 0.05), <sup>b</sup> (p < 0.01) and <sup>c</sup> (p < 0.005) represent significant changes against control.

1 n mole/mg protein, 2 μmole of NADPH oxidized/min/mg protein, 3 μmole of NADH oxidized/min/mg protein 4 μmole CDNB-GSH conjugate formed/min/mg protein and 5 μmole of DCPIP reduced/min/mg protein.

Abbreviation: Cyt P450: cytochrome P450, Cyt b5: cytochrome b5, Cyt P450 R: cytochrome P450 reductase, Cyt b5 R: cytochrome b5 reductase, GST: glutathione S-transferase, DTD: DT-diaphorase.

Treatment duration: 15 days.
Figure 4.1: Modulatory Influence of Different Doses of Amla (Phyllanthus emblica) Fruit Extract on the Hepatic Phase I Drug Metabolizing Enzyme in Swiss Albino Mice

Values are expressed as mean ± SD of 6-8 animals.

Control - Animals were kept on normal diet
2.5% - Animals were kept on 2.5% diet of Amla (Phyllanthus emblica)
5% - Animals were kept on 5% diet of Amla (Phyllanthus emblica)
10% - Animals were kept on 10% diet of Amla (Phyllanthus emblica)

\(^b(p < 0.01)\) and \(^c(p < 0.005)\) represent significant changes against control.

Abbreviation: Cyt P450: cytochrome P450, Cyt b5: cytochrome b5, Cyt P450 R: cytochrome P450 reductase, Cyt b5 R: cytochrome b5 reductase.

Treatment duration: 15 days.
Figure 4.2: Modulatory Influence of Different Doses of Amla (Phyllanthus emblica) Fruit Extract on Hepatic Phase II Drug Metabolizing Enzyme, LDH and LP in Swiss Albino Mice

Values are expressed as mean ± SD of 6-8 animals.

Control - Animals were kept on normal diet
2.5% - Animals were kept on 2.5% diet of Amla (Phyllanthus emblica)
5% - Animals were kept on 5% diet of Amla (Phyllanthus emblica)
10% - Animals were kept on 10% diet of Amla (Phyllanthus emblica)

*(p < 0.05) and **(p < 0.005) represent significant changes against control.

Abbreviation: GST: glutathione S-transferase, DTD: DT-diaphorase
LDH: lactate dehydrogenase, LP: lipid peroxidation

Treatment duration: 15 days.
Table 4.4: Modulatory Influence of Different Doses of Amla (*Phyllanthus emblica*) Fruit Extract on Hepatic Antioxidants, LDH and Lipid peroxidation in Swiss Albino Mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (GSH/g tissue)</th>
<th>SOD (specific activity, units/mg protein)</th>
<th>CAT (units/mg protein)</th>
<th>LDH (units/mg protein)</th>
<th>Gly I (nmole/min/mg protein)</th>
<th>LP (nmole malondialdehyde formed/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.22±0.336</td>
<td>6.00±0.24</td>
<td>41.74±2.63</td>
<td>1.74±0.12</td>
<td>0.574±0.041</td>
<td>1.133±0.121</td>
</tr>
<tr>
<td>2.5% diet of <em>Phyllanthus emblica</em></td>
<td>4.50±0.510 (139.73)</td>
<td>6.67±0.49 (111.17)</td>
<td>54.95±3.87 (131.65)</td>
<td>1.62±0.07 (93.1)</td>
<td>0.282±0.052 (49.13)</td>
<td>0.784±0.075 (69.20)</td>
</tr>
<tr>
<td>5% diet of <em>Phyllanthus emblica</em></td>
<td>4.62±0.372 (143.60)</td>
<td>7.13±0.53 (118.83)</td>
<td>42.74±4.69 (102.40)</td>
<td>1.33±0.04 (76.44)</td>
<td>0.392±0.053 (68.29)</td>
<td>0.782±0.049 (69.02)</td>
</tr>
<tr>
<td>10% diet of <em>Phyllanthus emblica</em></td>
<td>5.01±0.314 (155.72)</td>
<td>7.04±0.09 (117.33)</td>
<td>45.14±2.80 (108.14)</td>
<td>1.20±0.04 (68.96)</td>
<td>0.346±0.032 (60.28)</td>
<td>0.638±0.069 (56.31)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of 6-8 animals

Values in parentheses represent relative change in parameters assessed (i.e., levels of activity in livers of mice receiving test substance to activity in liver of control mice)

* a (p < 0.05), b (p < 0.01) and c (p<0.005) represent significant changes against control

①nmole GSH/g tissue, ②specific activity expressed as μmole/mg protein, ③μmole H₂O₂ consumed/min/mg protein, ④μmole/mg protein, ⑤μmole of S-D-Lactoylglutathione/min/mg proteins ⑥nmole malondialdehyde formed/mg protein.


Treatment duration: 15 days
Figure 4.3: Modulatory Influence of Different Doses of Amla (Phyllanthus emblica) Fruit Extract on Hepatic Antioxidants and Glyoxalase I in Swiss Albino Mice

Values are expressed as mean ± SD of 6-8 animals.

Control - Animals were kept on normal diet
2.5% - Animals were kept on 2.5% diet of Amla (Phyllanthus emblica)
5% - Animals were kept on 5% diet of Amla (Phyllanthus emblica)
10% - Animals were kept on 10% diet of Amla (Phyllanthus emblica)

* (p < 0.05), † (p < 0.01) and ‡ (p < 0.005) represent significant changes against control.

Abbreviation: GSH: reduced glutathione, SOD: Superoxide dismutase, CAT: Catalase, Gly 1: Glyoxalase I.

Treatment duration: 15 days
Table 4.5: Vitamin C Level in the Liver of Swiss Albino Mice Treated with 100 μL Juice of *Phyllanthus emblica*

<table>
<thead>
<tr>
<th>Groups of Animals</th>
<th>Vit C Content (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.211 ± 0.0124 (100)</td>
</tr>
<tr>
<td>Treated</td>
<td>0.699 ± 0.0159&lt;sup&gt;c&lt;/sup&gt; (331.28)</td>
</tr>
</tbody>
</table>

Values in parentheses show the percentage change.
Each value represents the mean ± SD of 6-8 animals.
<sup>c</sup> (p < 0.005) represent significant changes against control.
Treatment duration: 15 days.
Figure 4.4: Vitamin C Level in the Liver of Swiss Albino Mice Treated with 100 μL Juice of *Phyllanthus Emblica*

Each value represents the mean ± SD of 6-8 animals.

( \( p < 0.005 \) ) represent significant changes against control.

Treatment duration: 15 days.
TERMINALIA CHEBULA...
4.2.1 Experiment I

In these set of experiments, the effect of *Terminalia chebula* on benzo(a)pyrene induced forestomach tumorigenesis in male Swiss albino mice has been examined.

Results

Table 4.6 depicts the chemopreventive effects of *Terminalia chebula* on B(a)P induced forestomach tumorigenesis. No difference was noticed in weight gain profile of animals treated with either doses of *Terminalia chebula* diet as well as in the positive control group of mice.

**Short Term Study**

*Tumor Burden*

The mean number of papilloma/mouse (tumor burden) in the control group (B[a]P only) of animals was 6.867±1.06. In contrast, animals treated with 2.5% and 5% diet of *Terminalia chebula* for short term, tumor burden reduced to 3.538±1.56 (P<0.001) and 3.286±1.33 (P<0.001) respectively. The percentage inhibition of tumor multiplication was 48.88 and 52.15 by the lower and higher doses of *Terminalia chebula* treatment respectively.

*Tumor Incidences*

In the short-term treatment groups, total tumor incidences decreased by 18.75 % with the low dose and by 22.22% with the high dose of *Terminalia chebula*.

**Long Term Study**

*Tumor Burden*

In the long-term treatment the tumor burden was reduced to 3.30±0.82 (P<0.001) and 3.10±1.73 (p<0.001) by 2.5% and 5% diet of *Terminalia chebula* respectively. The percentage inhibition of tumor multiplication was 51.94 and 54.86 by the low and high doses of *Terminalia chebula* treatment respectively.
**Tumor Incidences**

The long-term treatment of *Terminalia chebula* reduced the total tumor incidence by 23.08% with the low dose and 23.08% with the high dose.

The long-term treatment of 5% diet of *Terminalia chebula* (negative control group) did not result in any tumor.

**4.2.2 Experiment II**

In these set of experiments, modulation of hepatic xenobiotic metabolising phase I and phase II enzymes; antioxidants, LDH and lipid peroxidation by *Terminalia chebula* has been examined.

**Results**

There was no significant difference in body weight gain and liver/final body weight profile between the control and treated with modulator.

**Phase I Enzymes**

(Refer: Table 4.7, Figure 4.5)

**Cytochrome P450**

Cytochrome P450 content increased significantly by 1.74 folds (p<0.01) with 2.5% diet and 2.09 folds (p<0.01) by 5% diet of *Terminalia chebula*.

**Cytochrome b5**

No significant change was observed in the content of cytochrome b5 with either dose of *Terminalia chebula*.

**Cytochrome P450R**

5% diet of *Terminalia chebula* significantly increased (p<0.05) the specific activity of cytochrome P450R by 1.13 folds. While no significant change was observed in the specific activity of cytochrome P450 with 2.5% dose of *Terminalia chebula*.

**Cytochrome b5R**

No significant change was observed in the specific activity of cytochrome b5R with 2.5% and 5% dose of *Terminalia chebula*.
Phase II Enzymes

(Refer: Table 4.7, Figure 4.5)

**GST**

Relative to the untreated control animals, the specific activity of GST was enhanced by 1.18 folds (P<0.01) with 2.5% and 1.25 folds (P<0.005) by 5% *Terminalia chebula* diet.

**DTD**

The specific activity of DTD was significantly enhanced by 1.38 folds (p<0.005) with the 2.5% dose of *Terminalia chebula*. No significant change was observed in the specific activity of DTD with 5% dose of *Terminalia chebula*.

**Antioxidants**

(Refer: Table 4.8, Figure 4.6)

**SOD**

The specific activity of SOD showed significant increase by both the doses of *Terminalia chebula* used in the present study. Relative to the untreated control animals, the activity of SOD was enhanced by 1.83 folds (p<0.05) and 1.92 folds (p<0.05) by 2.5% and 5% *Terminalia chebula* diet respectively.

**CAT**

The specific activity of CAT showed significant increase by both the doses of *Terminalia chebula* used in our experiment. Relative to the level in untreated control animals, the activity of CAT was enhanced by 1.98 folds (P<0.05) and 1.46 folds (p<0.05) by the 2.5% and 5% *Terminalia chebula* diet respectively.

**Reduced Glutathione**

The level of GSH was enhanced by both the doses of the *Terminalia chebula*. With 2.5% diet, GSH increased by 1.16 folds (P<0.05) and the 5% diet caused an elevation of 1.35 folds (P<0.05).
Other Parameters
(Refer: Table 4.8, Figure 4.6)

**LDH**

The activity of LDH was inhibited significantly by 0.72 folds (p<0.05) due to the treatment of diet containing 5% of *Terminalia chebula*. No significant change was observed in the specific activity of LDH with 2.5% dose of *Terminalia chebula*.

**Lipid peroxidation**

Lipid peroxidation measured as formation of malondialdehyde (MDA) production found to be significantly inhibited by both the doses of *Terminalia chebula*. Lipid peroxidation was reduced by 0.37 folds (p<0.001) with 2.5% diet and 0.28 folds (P<0.001) with 5% diet of *Terminalia chebula* as compared to the control group.

**Discussion**

An attempt has been made in the present study to examine the chemopreventive potentials of *Terminalia chebula* using experimental carcinogenesis as well as its modulatory action on detoxifying and antioxidant enzymes. Dietary administration of *Terminalia chebula* fruit powder exerted a strong chemopreventive effect against BP-induced forestomach in Swiss albino mice. Benzo(a)pyrene, employed for initiating stomach cancer, is widely distributed in the environment, in cigarette smoke, automobile exhaust etc. B(a)P, like many other poly aromatic hydrocarbons, also needs metabolic activation before it can exert carcinogenicity. The phase I enzymes (cytochrome P450, cytochrome b5, cytochrome P450R and cytochrome b5R) are involved in this activation process (Sun *et al.*, 1995; Fukuhara *et al.*, 1999; Bowes *et al.*, 1996 and Singh *et al.*, 1998). The modulation of these enzymes can have a significant effect on carcinogenicity and mutagenicity (Yang *et al.*, 1994 and Henderson *et al.*, 2000). B(a)P is initially converted in to 7,8 diol which is then converted to anti 7,8-dihydroxy 9,10-epoxy;7,8,9,10-tetrahydrobenzapyrene (anti-BPDE). The ultimate carcinogen, anti-BPDE is an inducer of DNA damage by virtue of its capacity in forming DNA adducts, predominantly at N² position of guanine to
form N²-BPDE-deguanosine adduct. But anti-BPDE readily gets inactivated by conjugating with GSH (Hesse et al., 1980).

As phase I enzymes play key role in the biotransformation of carcinogens, their modulation by 2.5% and 5% doses of *Terminalia chebula* was evaluated. *Terminalia chebula* significantly increased the specific activity of hepatic cytochrome P450 at both the dose levels. While cytochrome P450R increased significantly at the higher dose (5%) of *Terminalia chebula*. Many proven chemopreventive substances, e.g. indole 3-carbinol, are known to act via induction of cytochrome P450 (Manson et al., 1998).

Phase II enzymes, namely GST and DTD facilitate the detoxification of the carcinogens and their excretion. The main function of GST is to catalyze the conjugation of electrophilic xenobiotics/carcinogens to the endogenous nucleophile GSH, thus protecting the cellular components from toxic compounds (Awasthi et al., 1994). Reduced mutagenic response to 7,12-DMBA and AFB1 in Fisher 344 rat hepatocytes has been correlated to the induction of GST (Rogers et al., 1990). The chemopreventive action of *Terminalia chebula* against B(a)P induced carcinogenesis can be due to increased activity of GST. As B(a)P 4,5-epoxide and anti-BPDE have been shown to be good substrates of GST (Cooper et al., 1980; Ketterer, 1982). GST and glutathione detoxify the ultimate carcinogenic metabolite of B(a)P i.e. Benzo(a)-pyrene-7,8-diol-9,10-epoxide and may thus inhibit the deleterious effects of B(a)P (Ketterer, 1988).

DT-diaphorase, another phase II enzyme, is a flavoprotein and catalyzes the two-electron reduction of quinones, quinone imines, azo dyes and other nitrogen oxides (Ernster, 1987; Riley and Workman, 1992). Through redox cycling, quinones contribute to oxidative stress due to generation of reactive oxygen species (ROS). Thus, major metabolic function of this enzyme might be to reduce the formation of ROS from redox reactions. The induction of DTD, which mediates the two-electron reduction of quinones leading to the formation of hydroquinones, is important in attenuating the toxicity of quinone metabolite. Dithiolthiones and their analogues (Oltipraz), known to be chemopreventive against variety of chemical carcinogens, are
effective inducers of DTD (Begleiter et al., 1997). Thus, inducers of DTD can be assumed to play important roles in cancer chemoprevention. *Terminalia chebula* significantly increased the specific activity of DTD at the lower dose (2.5%). It is quite possible that its chemopreventive action might be linked with DTD functions.

An increasing body of evidence indicates that oxidative stress plays an important role in mutagenesis, which is intimately linked with the carcinogenesis (Ames et al., 1993). Hydroxal radical produced as a result of oxidative stress initiate a chain of reactions that lead to the process of lipid peroxidation and generating in turn the highly mutagenic singlet oxygen or bring out change in DNA directly, leading to carcinogenesis. Glutathione is known to be an effective quencher of this singlet oxygen (Mascio et al., 1990 and Devasagayam et al., 1991). Reduced glutathione is one of the major cellular defenses against ROS generated endogenously or the electrophilic metabolites of carcinogen generated during phase I biotransformation. The electrophilic functional groups are conjugated with glutathione to form amphiphilic thioester either spontaneously or through GSTs (Jakoby, 1980). *Terminalia chebula* significantly enhanced the GSH content at both the dose of cumin used providing probable protection against oxidative stress.

Superoxide dismutase and Catalase are important members of cellular defence against oxidative stress. SOD dismutates superoxide anions to hydrogen peroxide. Hydrogen peroxide, which is also considered to be mutagenic, is degraded to H₂O and oxygen by catalase. Metabolism of the carcinogens and the applications of the tumor promoters have been known to generate activated oxygen species like O²⁻ and H₂O₂. Since SOD-CAT system is effective in detoxifying these free radicals, the agents that cause enhancement in the activities of SOD-CAT system would be useful in protection against activated oxygen species. The activities of SOD and CAT were significantly enhanced by both the doses of *Terminalia chebula* and suggestive of its chemopreventive potential through metabolizing free radicals and their products.

Peroxidation, initiated in membranes by hydroxyl radical, is self-propagating and yields mutagenic species/products. *Terminalia chebula* significantly enhanced the
antioxidant defence and also the specific activity of GST, which might have contributed to a reduction in lipid peroxidation.

LDH, a known biochemical indicator of cell-damage is induced by several factors, including xenobiotic compounds and radiations (Reddy and Lokesh, 1996; Deters et al., 1998). The *Terminalia chebula* treatment resulted in decrease in LDH activity signifying its protective ability against the cellular damage.

In conclusion, the present study has clearly demonstrated the cancer preventive action of *Terminalia chebula* using chemically induced tumorigenesis in murine model system. The preventive potentials could be due to modulatory influence of *Terminalia chebula* on phase I and phase II enzymes involved in the carcinogen metabolism. *Terminalia chebula* also facilitates and/or enhances the activity of the natural antioxidant system, which in turn is likely to protect the cell against the oxidative stress involved in the multistage process of carcinogenesis. The *Terminalia chebula* at the given dose levels appears to be safe as it could not cause any adverse effects on the animals as evidenced by the level of lipid peroxidation, specific activity of LDH, food intake and general body weight gain (of the animals) during the course of the experiment. Therefore, it could be inferred that *Terminalia chebula* may have cancer chemopreventive effects in human population. *Terminalia chebula* seems to posses most of the characteristics of an ideal cancer chemopreventive agent i.e. orally effective, less or no toxicity, cost effectiveness and higher efficacy. It is suggested to promote the use of *Terminalia chebula*, which might reduce the risk of cancer incidences and cancer burden in human population.
Table 4.6: Effect of Different Doses of Haritaki (*Terminalia chebula*) on Benzo(a)Pyrene Induced Forestomach Tumorigenesis in Mice.

<table>
<thead>
<tr>
<th>Groups &amp; Treatment</th>
<th>Body weight (gms)</th>
<th>Total tumor incidence (%)</th>
<th>Tumor burden (tumor/mouse)</th>
<th>% Inhibition of tumor burden</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only Benzo(a)pyrene</td>
<td>25.67 ± 0.82</td>
<td>28.13 ± 0.74</td>
<td>100</td>
<td>6.867 ± 1.06</td>
</tr>
<tr>
<td>Benzo(a)pyrene + 2.5% <em>Terminalia chebula</em> diet</td>
<td>26.06 ± 0.93</td>
<td>29.25 ± 1.06</td>
<td>81.25</td>
<td>3.538 ± 1.56^d</td>
</tr>
<tr>
<td>Benzo(a)pyrene + 5% <em>Terminalia chebula</em> diet</td>
<td>25.39 ± 1.29</td>
<td>28.89 ± 1.32</td>
<td>77.78</td>
<td>3.286 ± 1.33^d</td>
</tr>
<tr>
<td>Benzo(a)pyrene + 2.5% <em>Terminalia chebula</em> long term diet</td>
<td>25.69 ± 1.18</td>
<td>28.92 ± 0.86</td>
<td>76.92</td>
<td>3.30 ± 0.82^d</td>
</tr>
<tr>
<td>Benzo(a)pyrene + 5% <em>Terminalia chebula</em> long term diet</td>
<td>25.15 ± 1.40</td>
<td>28.54 ± 1.27</td>
<td>76.92</td>
<td>3.10 ± 1.73^d</td>
</tr>
<tr>
<td>5% <em>Terminalia chebula</em> long term diet</td>
<td>24.50 ± 2.21</td>
<td>28.0 ± 1.12</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of 13-18 animals.

^d (p < 0.001), represent significant changes against control
Table 4.7: Modulatory Influence of Different Doses of Haritaki (*Terminalia chebula*) Fruit Extract on Hepatic Phase I and Phase II Drug Metabolizing Enzyme in Swiss Albino Mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cyt P450</th>
<th>Cyt b5</th>
<th>Cyt P450 R</th>
<th>Cyt b5 R</th>
<th>GST</th>
<th>DTD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.314±0.072 (100)</td>
<td>0.480±0.060 (100)</td>
<td>0.769±0.030 (100)</td>
<td>5.176±0.40 (100)</td>
<td>1.08±0.11 (100)</td>
<td>0.024±0.002 (100)</td>
</tr>
<tr>
<td>2.5% dose of <em>Terminalia chebula</em></td>
<td>0.546±0.07b (173.88)</td>
<td>0.465±0.062 (96.87)</td>
<td>0.872±0.030 (113.39)</td>
<td>5.701±0.60 (110.14)</td>
<td>1.28±0.09b (118.52)</td>
<td>0.033±0.002c (137.5)</td>
</tr>
<tr>
<td>5% dose of <em>Terminalia chebula</em></td>
<td>0.658±0.048b (209.55)</td>
<td>0.504±0.042 (105)</td>
<td>0.899±0.047a (116.90)</td>
<td>5.322±0.53 (102.82)</td>
<td>1.35±0.08c (125)</td>
<td>0.027±0.004 (112.5)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of 6-8 animals.

Values in parentheses represent relative change in parameters assessed (i.e., levels of activity in livers of mice receiving test substance to activity in liver of control mice).

*(p < 0.05), *(p < 0.01) and *(p < 0.005) represent significant changes against control.

*nmole/mg protein, µmole of NADPH oxidized/min/mg protein, µmole of NADH oxidized/min/mg protein, µmole CDNB-GSH conjugate formed/min/mg protein and µmole of DCPIP reduced/min/mg protein.

Abbreviation: Cyt P450: cytochrome P450, Cyt b5: cytochrome b5, Cyt P450 R: cytochrome P450 reductase, Cyt b5 R: cytochrome b5 reductase, GST: glutathione S-transferase, DTD: DT-diaphorase.

Treatment duration: 15 days.
Figure 4.5: Modulatory Influence of Different Doses of Haritaki (Terminalia chebula) Fruit Extract on the Hepatic Phase I and Phase II Drug Metabolizing Enzyme in Swiss Albino Mice

Values are expressed as mean ± SD of 6-8 animals.

Control - Animals were kept on normal diet
2.5% - Animals were kept on 2.5% diet of Haritaki (Terminalia chebula)
5% - Animals were kept on 5% diet of Haritaki (Terminalia chebula)

\(^a\) (p < 0.05), \(^b\) (p < 0.01) and \(^c\) (p < 0.005) represent significant changes against control.

Abbreviation: Cyt P450: cytochrome P450, Cyt b5: cytochrome b5, Cyt P450 R: cytochrome P450 reductase, Cyt b5 R: cytochrome b5 reductase, GST: glutathione S-transferase, DTD: DT-diaphorase.

Treatment duration: 15 days.
Table 4.8: Modulatory Influence of Different Doses of Haritaki (*Terminalia chebula*) Fruit Extract on Hepatic Antioxidants, Lipid Peroxidation and LDH in Swiss Albino Mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (nmole/g tissue)</th>
<th>SOD (specific activity)</th>
<th>CAT (μmole H₂O₂ consumed/min/mg protein)</th>
<th>LDH (μmole/mg protein)</th>
<th>LP (nmole/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vehicle</td>
<td>50.90±3.97 (100)</td>
<td>4.05±0.25 (100)</td>
<td>26.62±1.71 (100)</td>
<td>1.72±0.13 (100)</td>
<td>1.45±0.07 (100)</td>
</tr>
<tr>
<td>2.5% dose of <em>Terminalia chebula</em></td>
<td>58.86±4.11 (115.64)</td>
<td>7.40±0.42 (182.72)</td>
<td>52.81±0.77 (198.38)</td>
<td>1.64±0.16 (95.35)</td>
<td>0.53±0.05 (36.55)</td>
</tr>
<tr>
<td>5% dose of <em>Terminalia chebula</em></td>
<td>68.91±4.16 (135.38)</td>
<td>7.76±0.47 (191.60)</td>
<td>38.84±0.93 (145.9)</td>
<td>1.24±0.12 (72.09)</td>
<td>0.41±0.03 (28.28)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of 6-8 animals

Values in parentheses represent relative change in parameters assessed (i.e., levels of activity in livers of mice receiving test substance to activity in liver of control mice)

* (p < 0.05) and ** (p<0.001) represent significant changes against control

1 nmole GSH/g tissue, 2 specific activity expressed as μmole/mg protein, 3 μmole H₂O₂ consumed/min/mg protein, 4 μmole/mg protein, 5 nmole malondialdehyde formed/mg protein.

Abbreviation: GSH: reduced glutathione, SOD: superoxide dismutase, CAT: catalase, LDH: lactate dehydrogenase, LP: lipid peroxidation

Treatment duration: 15 days
Figure 4.6: Modulatory Influence of Different Doses of Haritaki (Terminalia chebula) Fruit Extract on Hepatic Antioxidants, Lipid Peroxidation and LDH in Swiss Albino Mice

Values are expressed as mean ± SD of 6-8 animals.

Control - Animals were kept on normal diet
2.5% - Animals were kept on 2.5% diet of Haritaki (Terminalia chebula)
5% - Animals were kept on 5% diet of Haritaki (Terminalia chebula)

a (p < 0.05) and d (p<0.005) represent significant changes against control

Abbreviation: GSH: reduced glutathione, SOD: Superoxide dismutase, CAT: Catalase, LDH: Lactate dehydrogenase, LP: Lipid peroxidation

Treatment duration: 15 days
TERMINALIA BELERICA...
4.3.1 Experiment I

In these set of experiments, the effect of *Terminalia belerica* on benzo(a)pyrene induced forestomach tumorigenesis in male Swiss albino mice has been examined.

**Results**

Table 4.9 depicts the modulatory effect of *Terminalia belerica* supplementation in diet on B(a)P induced forestomach tumorigenesis. No difference was noticed in weight gain profile of animals treated with either doses of *Terminalia belerica* diet (2.5 and 5%) as well as in the positive control group of mice.

**Short Term Study**

*Tumor Burden*

The mean number of papilloma/mouse (tumor burden) in the control group (B[a]P only) of animals was 6.867±1.06. In contrast, animals treated with 2.5% and 5% diet of *Terminalia belerica* for short terms, tumor burden reduced to 4.533±2.264 (P<0.05) and 3.330±1.15 (P<0.001) respectively. The percentage inhibition of tumor multiplication was 33.99 and 51.51 by the low and high doses of *Terminalia belerica* treatment respectively.

*Tumor Incidences*

In short term treatment groups, total tumor incidence decreased by 6.25 % with the low dose and by 20% with the high dose of *Terminalia belerica*.

**Long Term Study**

*Tumor Burden*

In long-term studies the tumor burden was reduced to 4.385±1.32 (P<0.001) and 3.20±1.32 (p<0.001) by 2.5% and 5% diet of *Terminalia belerica* respectively. The percentage inhibition of tumor multiplication was 36.14 and 53.40 by the low and high doses of *Terminalia belerica* treatment respectively.
**Tumor Incidences**

The long-term treatment of *Terminalia belerica* reduced the total tumor incidence by 18.75% with the low dose and 28.57% with the high dose.

The long-term treatment of 5% diet of *Terminalia belerica* only (negative control group) did not result in any tumor.

**4.3.2 Experiment II**

In these set of experiments, modulation of hepatic xenobiotic metabolising phase I and phase II enzymes; antioxidants, LDH and lipid peroxidation by *Terminalia belerica* has been examined.

**Results**

There was no significant difference in body weight gain and liver/final body weight profile between the control and modulator treated animals.

**Phase I Enzymes**

(Refer: Table 4.10 and Figure 4.7)

**Cytochrome P450**

Cytochrome P450 content increased significantly by 2.24 folds (p<0.05) with 2.5% diet and 2.40 folds (p<0.05) with 5% diet of *Terminalia belerica*.

**Cytochrome b5**

No significant change was observed in the content of cytochrome b5 with either dose of *Terminalia belerica*.

**Cytochrome P450R**

No significant change was observed in the specific activity of cytochrome P450R with either dose of *Terminalia belerica*. 

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**Cytochrome b5R**

5% diet of *Terminalia belerica* significantly decreased (p<0.05) the specific activity of cytochrome b5R by 0.86 folds. While no significant change was observed in the specific activity of cytochrome b5R with 2.5% dose of *Terminalia belerica*.

**Phase II Enzymes**

(Refer: Table 4.10 and Figure 4.7)

**GST**

Relative to the level in untreated control animals, the specific activity of GST was enhanced by 1.20 folds (P<0.005) with 5% *Terminalia belerica* diet. While no significant change was observed in the specific activity of GST with 2.5% dose of *Terminalia belerica*.

**DTD**

No significant change was observed in the specific activity of DTD with either dose of *Terminalia belerica*.

**Antioxidants**

(Refer: Table 4.11 and Figure 4.8)

**SOD**

Specific activity of SOD showed significant increase by both the doses of *Terminalia belerica* used in our experiment. Relative to the level in untreated control animals, the activity of SOD was enhanced by 1.11 folds (P<0.05) and 1.56 folds (p<0.001) with the 2.5% and 5% *Terminalia belerica* diet respectively.

**CAT**

Specific activity of CAT showed significant increase by both the doses of *Terminalia belerica* used in our experiment. Relative to the level in untreated control animals, the activity of CAT was enhanced by 1.19 folds (P<0.05) and 1.20 folds (p<0.05) with the 2.5% and 5% *Terminalia belerica* diet respectively.
Reduced Glutathione

The level of GSH, the non-enzymatic antioxidant, was enhanced by both the doses of the Terminalia belerica. With 2.5% diet, GSH increased by 1.19 folds (P<0.05) and the 5% diet showed an elevation of 1.32 folds (P<0.005).

Other Parameters

(Refer: Table 4.11 and Figure 4.8)

LDH

The activity of LDH was inhibited significantly by both the doses of Terminalia belerica. With by 2.5% diet the reduction was 0.61 folds (P<0.05) and the 5% diet revealed a decrease of 0.57 folds (p<0.05).

Lipid peroxidation

Lipid peroxidation measured as formation of malondialdehyde (MDA) production showed significant inhibition by both the doses of Terminalia belerica. Lipid peroxidation was reduced by 0.92 folds (P<0.05) with 2.5% diet and 0.79 folds (P<0.005) with 5% diet of Terminalia belerica as compared to the control group.

Discussion

Terminalia belerica (Indian name bahera) is a 60-foot tall tree widely distributed in India. It is one of the constituents of the famous Indian combination drug “Triphala”- a composite mixture of Terminalia belerica, Terminalia chebula and Emblica officinalis. It has been used in traditional system of medicine for the treatment of many malaises. Terminalia belerica has an impressive past references of being anti-mutagenic (Padam et al., 1996) and anti-diabetic (Sabu et al., 2002). Terminalia belerica also has hypo-lipidemic activity (Shaila et al, 1998) and anti-microbial properties (Ahmed et al, 1998). The fruit extracts of Terminalia belerica reported to
have an inhibitory effects on human immunodeficiency virus (HIV) reverse transcriptase. (el Makkawy et al, 1995). The bioactivity-guided fractionation of the extract of *Terminalia belerica* fruit led to the isolation of two new lignans named termilignan and thannilignan, together with 7-hydroxy-3',4'(methylenedioxy) flavin and anoligan B. Both the compounds demonstrated anti-HIV-1, anti-malarial, and anti-fungal activity *in vitro* (Valsaraj et al., 1997).

Results of the present work with this popular plant of "Ayurvedic Medicine" showed potent inhibitory effect on stomach tumorigenesis at peri-initiational stages. The potentialities of *Terminalia belerica* to modulate phase I and phase II enzymes as well as anti-oxidative parameters has been investigated. The results revealed that *Terminalia belerica* fruit extract significantly increased only the level of cytochrome P450 and activity of cytochrome P450R. *Terminalia belerica* was not able to modulate the other phase I enzyme. In the case of phase II enzymes, only GST activity was elevated significantly by the higher dose of *Terminalia belerica*. There was no change in the activity of DTD. However, it was interesting that remarkable changes were observed in the activities of enzymes involved in antioxidant functions. The specific activities of SOD, catalase and GSH content were increased significantly by both the experimental doses of *Terminalia belerica*. It is also important that inhibited the activity of LDH and levels of lipid peroxidation at the same time. One of the major mechanisms of reducing and combating cancer risk is considered to be a selective elevation of antioxidants, which can tackle the reactive oxygen species (ROS) and the resulting oxidative stress. As mentioned earlier, reactive oxygen species have been linked with various events of mutagenesis and carcinogenesis. Thus, the increase in various antioxidant levels might help in preventing the free radical dependent process of carcinogenesis. This possibility is supported by significant decrease in the level of lipid peroxidation and LDH activity caused due to treatment of *Terminalia belerica*.

Therefore, it could be speculated that *Terminalia belerica* probably inhibits the B(a)P induced forestomach tumor mainly through the selective elevation of hepatic antioxidants, phase I and phase II enzymes.
Table 4.9: Effect of Different Doses of *Terminalia belerica* on Benzo(a)pyrene Induced Forestomach Tumorigenesis in Swiss Albino Mice

<table>
<thead>
<tr>
<th>Groups &amp; Treatment</th>
<th>Body weight (gms)</th>
<th>Total tumor incidence (%)</th>
<th>Tumor burden (tumor/mouse)</th>
<th>% Inhibition of tumor burden</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td></td>
</tr>
<tr>
<td>only Benzo(a)pyrene</td>
<td>23.5 ± 2.22</td>
<td>26.75 ± 1.76</td>
<td>100</td>
<td>6.867 ± 1.06</td>
</tr>
<tr>
<td>Benzo(a)pyrene + 2.5% <em>Terminalia belerica</em> diet</td>
<td>23.58 ± 2.54</td>
<td>27.92 ± 1.83</td>
<td>93.75</td>
<td>4.533 ± 2.264(^a)</td>
</tr>
<tr>
<td>Benzo(a)pyrene + 5% <em>Terminalia belerica</em> diet</td>
<td>23.08 ± 2.91</td>
<td>26.55 ± 2.09</td>
<td>80.00</td>
<td>3.330 ± 1.15(^d)</td>
</tr>
<tr>
<td>Benzo(a)pyrene + 2.5% <em>Terminalia belerica</em> long term diet</td>
<td>24.67 ± 2.30</td>
<td>27.90 ± 1.29</td>
<td>81.25</td>
<td>4.385 ± 1.32(^d)</td>
</tr>
<tr>
<td>Benzo(a)pyrene + 5% <em>Terminalia belerica</em> long term diet</td>
<td>23.17 ± 2.41</td>
<td>27.67 ± 1.66</td>
<td>71.43</td>
<td>3.200 ± 1.32(^d)</td>
</tr>
<tr>
<td>5% <em>Terminalia belerica</em> long term diet</td>
<td>24.50 ± 2.25</td>
<td>28.54 ± 1.10</td>
<td>------</td>
<td>------</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of 12-15 animals.

\(^a\)(p < 0.05), represent significant changes against control

\(^d\)(p < 0.001), represent significant changes against control
Table 4.10: Modulatory Influence of Different Doses of Bahera (*Terminalia belerica*) Fruit Extract on Hepatic Phase I and Phase II Drug Metabolizing Enzyme in Swiss Albino Mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cyt P450</th>
<th>Cyt b5</th>
<th>Cyt P450 R</th>
<th>Cyt b5 R</th>
<th>GST</th>
<th>DTD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.275±0.520</td>
<td>0.565±0.095</td>
<td>0.855±0.038</td>
<td>6.10±0.45</td>
<td>1.28±0.07</td>
<td>0.036±0.006</td>
</tr>
<tr>
<td>2.5% dose of <em>Terminalia belerica</em></td>
<td>0.615±0.095 (223.64)</td>
<td>0.552±0.107 (97.70)</td>
<td>0.884±0.047 (103.39)</td>
<td>5.84±0.21 (95.74)</td>
<td>1.37±0.13 (107.03)</td>
<td>0.032±0.004 (88.88)</td>
</tr>
<tr>
<td>5% dose of <em>Terminalia belerica</em></td>
<td>0.658±0.016 (239.27)</td>
<td>0.492±0.068 (87.08)</td>
<td>0.966±0.099 (112.98)</td>
<td>5.26±0.27* (86.23)</td>
<td>1.53±0.10* (119.53)</td>
<td>0.039±0.006 (108.33)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of 6-8 animals.

Values in parentheses represent relative change in parameters assessed (i.e., levels of activity in livers of mice receiving test substance to activity in liver of control mice).

* (p < 0.05) and †(p < 0.005) represent significant changes against control.

Trademark: Cyt P450: cytochrome P450, Cyt b5: cytochrome b5, Cyt P450 R: cytochrome P450 reductase, Cyt b5 R: cytochrome b5 reductase, GST: glutathione S-transferase, DTD: DT-diaphorase.

Treatment duration: 15 days.
Figure 4.7: Modulatory Influence of Different Doses of Bahera (Terminalia belerica) Fruit Extract on the Hepatic Phase I and Phase II Drug Metabolizing Enzyme in Swiss Albino Mice

Values are expressed as mean ± SD of 6-8 animals.

Control - Animals were kept on normal diet
2.5% - Animals were kept on 2.5% diet of Bahera (Terminalia belerica)
5% - Animals were kept on 5% diet of Bahera (Terminalia belerica)

a (p < 0.05) and c (p < 0.005) represent significant changes against control.

Abbreviation: Cyt P450: cytochrome P450, Cyt b5: cytochrome b5,
Cyt P450 R: cytochrome P450 reductase, Cyt b5 R: cytochrome b5 reductase,
GST: glutathione S-transferase, DTD: DT-diaphorase.

Treatment duration: 15 days.
Table 4.11: Modulatory Influence of Different Doses of Bahera (*Terminalia belerica*) Fruit Extract on Hepatic Antioxidant Related Parameters, Lipid Peroxidation and LDH in Swiss Albino Mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (nmol GSH/g tissue)</th>
<th>SOD (μmol H₂O₂ consumed/min/mg protein)</th>
<th>CAT (μmol H₂O₂ consumed/min/mg protein)</th>
<th>LDH (μmol/mg protein)</th>
<th>LP (nmol malondialdehyde formed/μmol protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vehicle</td>
<td>52.42±2.27 (100)</td>
<td>12.45±1.17 (100)</td>
<td>30.45±3.88 (100)</td>
<td>2.64±0.13 (100)</td>
<td>1.40±0.07 (100)</td>
</tr>
<tr>
<td>2.5% dose of Bahera</td>
<td>62.23±2.54 (118.71)</td>
<td>13.78±1.11 (110.68)</td>
<td>36.30±3.46 (119.21)</td>
<td>1.62±0.24 (61.36)</td>
<td>1.29±0.05 (92.14)</td>
</tr>
<tr>
<td>5% dose of Bahera</td>
<td>70.37±3.50 (132.24)</td>
<td>19.39±1.52 (155.74)</td>
<td>36.68±3.39 (120.46)</td>
<td>1.51±0.21 (57.20)</td>
<td>1.10±0.07 (78.57)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of 6-8 animals

Values in parentheses represent relative change in parameters assessed (i.e., levels of activity in livers of mice receiving test substance to activity in liver of control mice)

a (p < 0.05), b (p < 0.01) and d (p < 0.005) represent significant changes against control

Abbreviation: GSH: reduced glutathione, SOD: superoxide dismutase, CAT: catalase, LDH: lactate dehydrogenase, LP: lipid peroxidation

Treatment duration: 15 days
Figure 4.8: Modulatory Influence of Different Doses of Bahera (*Terminalia belerica*) Fruit Extract on Hepatic Antioxidants, Lipid Peroxidation and LDH in Swiss Albino Mice.

Values are expressed as mean ± SD of 6-8 animals.

Control - Animals were kept on normal diet
2.5% - Animals were kept on 2.5% diet of Bahera (*Terminalia belerica*)
5% - Animals were kept on 5% diet of Bahera (*Terminalia belerica*)

* (p < 0.05), † (p < 0.01) and ‡ (p < 0.005) represent significant changes against control.

Abbreviation: GSH: reduced glutathione, SOD: superoxide dismutase, CAT: catalase, LDH: lactate dehydrogenase, LP: lipid peroxidation

Treatment duration: 15 days
TRIPHALA...
4.4.1 Experiment I

In these set of experiments, the effect of Triphala on benzo(a)pyrene induced forestomach tumorigenesis in male Swiss albino mice has been examined.

Results

Table 4.12 depicts the effect of Triphala mixed diet on B(a)P induced forestomach tumorigenesis male Swiss albino mice. No difference was noticed in weight gain profile of animals treated with either doses of Triphala diet as well as in the positive control group of mice.

Short Term Study

Tumor Burden

The mean number of papilloma/mouse (tumor burden) in the control group (B[a]P only) of animals was 7.273±1.16. In contrast, animals treated with 2.5% and 5% diet of Triphala for short terms, tumor burden reduced to 3.00±0.82 (p<0.005) and 2.33±1.03 (P<0.001) respectively. The percentage inhibition of tumor multiplication was 58.75 and 67.96 by the low and high doses of Triphala treatment respectively.

Tumor Incidences

In short term treatment groups, total tumor incidence decreased by 22.23 % with both the doses of Triphala (2.5% and 5%) compared to the B(a)P control.

Long Term Study

Tumor Burden

In long-term studies the tumor burden was reduced to 2.17±0.75 (p<0.001) and 2.00±0.71 (p<0.001) by 2.5% and 5% diet of Triphala respectively. The percentage inhibition of tumor multiplication was 70.16 and 72.50 by the low and high doses of Triphala treatment respectively.

Tumor Incidences

The long-term treatment of Triphala reduced the total tumor incidence by 33.34% with the low dose and 37.5% with the high dose.
The long-term treatment of 5% diet of Triphala only (negative control group) did not result in any tumor.

Discussion

Triphala is a mixture of fruit powder of Phyllanthus emblica, Terminalia belerica and Terminalia chebula. It has been proven to be a strong antimutagenic agent. (Kaur et al., 2002) and also reported to have radioprotective and antidiabetic effects (Jagetia et al., 2002; Sabu and Kuttan, 2002). The present study attempts to investigate the cancer chemoprevention efficacy of two different doses (2.5% and 5%) of Triphala against B(a)P induced forestomach tumorigenesis. The findings of the present work revealed significant inhibition of stomach tumor burden and incidence by both the doses of Triphala.

Triphala is rich in polyphenols (Kaur et al., 2002) and antioxidants (Sabu and Kuttam, 2002), which might have contributed to prevent carcinogenesis through the scavenging of free radicals and their products.

Triphala was shown to have antimutagenic properties, which might also have some role in providing protection against DNA damage caused by chemical carcinogens. Triphala inhibited lipid peroxidation. Malondialdehyde (MDA) as one of the major aldehyde products of lipid peroxidation is proven to be mutagenic and react with DNA to form the adducts. These properties of Triphala might have helped to prevent the carcinogenesis.

Thus, Triphala might have exerted its preventive effect against carcinogen induced forestomach tumorigenesis by elevating the level of antioxidant and inhibiting the lipid peroxidation through scavenging of the reactive oxygen species (ROS) and other carcinogenic electrophiles. It may be noted that Triphala mixture was not as effective as amla alone. However, the chemopreventive effect of mixture was better than other two ingredients namely Terminalia chebula and Terminalia belerica in the forestomach tumor model system.
Table 4.12: Effect of Different Doses of Triphala on Benzo(a)pyrene Induced Forestomach Tumorigenesis in Swiss Albino Mice.

<table>
<thead>
<tr>
<th>Groups &amp; Treatment</th>
<th>Body weight (gms)</th>
<th>Total tumor incidence (%)</th>
<th>Tumor burden (tumor/mouse)</th>
<th>% Inhibition of tumor burden</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td></td>
</tr>
<tr>
<td>only Benzo(a)pyrene</td>
<td>23.0 ± 2.22</td>
<td>27.75 ± 1.76</td>
<td>100</td>
<td>7.273±1.16</td>
</tr>
<tr>
<td>Benzo(a)pyrene + 2.5% Triphala</td>
<td>23.58 ± 2.54</td>
<td>27.92 ± 1.83</td>
<td>77.77</td>
<td>3.00±0.82&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Benzo(a)pyrene + 5% Triphala</td>
<td>24.08 ± 2.91</td>
<td>28.75 ± 2.09</td>
<td>77.77</td>
<td>2.33±1.03&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Benzo(a)pyrene + 2.5% Triphala long term diet</td>
<td>24.67 ± 2.31</td>
<td>27.50 ± 1.09</td>
<td>66.66</td>
<td>2.17±0.75&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Benzo(a)pyrene + 5% Triphala long term diet</td>
<td>23.17 ± 2.41</td>
<td>27.67 ± 1.61</td>
<td>62.5</td>
<td>2.00±0.71&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>5% Triphala long term diet</td>
<td>24.50 ± 2.21</td>
<td>28.0 ± 1.12</td>
<td>---------------------------</td>
<td>-----------------------------</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of 12-15 animals.

<sup>c</sup>(p <0.005) and <sup>d</sup>(p < 0.001) represent significant changes against control.
BRASSICA SP...
4.5.1 Experiment I

In these set of experiments, the effect of mustard seeds (*Brassica sp.*) on benzo(a)pyrene induced forestomach tumorigenesis in male Swiss albino mice has been examined.

Results

Table 4.13 depicts the result obtained by mustard seeds mixed diet on B(a)P induced forestomach tumorigenesis. No difference was noticed in weight gain profile of animals treated with either doses of mustard seeds diet as well as in the positive control group of mice.

**Tumor Burden**

The mean number of papilloma/mouse (tumor burden) in the control group (B[a]P only) of animals was $7.08 \pm 2.47$. In contrast, in animals treated with 2.5% and 5% diet of mustard seeds tumor burden reduced to $1.36 \pm 1.12$ ($p<0.001$) and $1.18 \pm 0.87$ ($p<0.001$) respectively. The percentage inhibition of tumor multiplication was 80.74 and 83.31 by the 2.5% and 5% dose of mustard seeds treatment respectively.

**Tumor Incidences**

100% of the control animals developed forestomach papillomas by B(a)P treatment. Relative to control tumor incidences were 72.73% with both 2.5% and 5% dose of mustard seeds. Thus, total tumor incidence decreased by 27.27 % with both the doses of mustard seeds.

5% diet of mustard seeds only (negative control group) did not result in any tumor.

Discussion

Epidemiological and experimental studies have provided sufficient data, which suggest that cancer is a multifactorial, multi-staged, multi-mechanistic complex process and multifaceted disease in which several environmental and host factors play
an important role. There is a long interval between initiation and ultimate development of tumor. This period can be targeted to prevent progression of cancer by applying suitable chemopreventive agent(s). These agents are likely to inhibit the development of invasive cancer by either blocking the DNA damage that initiate carcinogenesis or arresting the progression of premalignant cells.

In the cancer preventive strategy, the role of many dietary components has been widely accepted. Among the various components of diet, epidemiological studies have demonstrated an inverse association between various cancers such as colorectal, lung, prostate etc and intake of cruciferous vegetables (Potter, 1996; Voorips et al., 2000; Cohen et al., 2000 and Verhoeven et al., 1996).

It has been reported that male wistar rats fed with mustard seed powder diet showed a decrease in the B(a)P binding of hepatic DNA (Rajpurohit and Krishnaswamy, 1994). It has also been demonstrated that mustard seeds are the potent antagonist of the ultimate metabolites of B(a)P (Polasa et al., 1994). Thus, the feeding of mustard seed mixed diet when started at peri-initiational stages of forestomach tumorigenesis may have lowered the carcinogenicity of B(a)P, resulting in decrease tumor incidences and tumor burden.

The modulation of the carcinogen metabolism is considered as one of the most effective and well-established strategy for protection of cells against the toxic and neoplastic effects of chemical carcinogen (Talalay et al., 1995). Diets augmented with cruciferous vegetables have been found to significantly altering the carcinogen metabolizing enzymes both in liver as well as in the intestine, accelerating the disposal of chemical carcinogens and thereby, destroying their ability to damage DNA (Whitty and Bjeldanes, 1987 and Guo et al., 1992). Specifically, the degradation products from cruciferous vegetables are believed to inhibit phase I activating enzymes, and induce phase II detoxification enzymes (Zhang and Talalay, 1994 and Hecht, 1999). Therefore, the chemopreventive potentials of mustard are may be due to its modulation of carcinogen metabolism.

It is concluded that mustard seeds effectively reduce the forestomach tumorigenesis in murine model system. Therefore, it could be speculated that mustard seeds may also
have cancer chemopreventive effects on human population. Through selectively promoting the use of mustard seeds in diet especially in high-risk group, the cancer incidences and burden could be reduced significantly.
Table 4.13: Effect of Different Doses of Mustard Seeds (*Brassica sp.*) on Benzo(a)pyrene Induced Forestomach Tumorigenesis in Swiss Albino Mice

<table>
<thead>
<tr>
<th>Groups &amp; Treatment</th>
<th>Body weight (gms)</th>
<th>Total tumor incidence (%)</th>
<th>Tumor burden (tumor/mouse)</th>
<th>% Inhibition of tumor burden</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzo(a)pyrene only</td>
<td>27.60 ± 1.73</td>
<td>29.90 ± 1.64</td>
<td>100</td>
<td>7.08 ± 2.47</td>
</tr>
<tr>
<td>Benzo(a)pyrene + 2.5% mustard seed diet</td>
<td>31.10 ± 1.82</td>
<td>34.90 ± 3.51</td>
<td>72.73</td>
<td>1.36 ± 1.12&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Benzo(a)pyrene + 5% mustard seed diet</td>
<td>30.20 ± 2.02</td>
<td>35.10 ± 3.30</td>
<td>72.73</td>
<td>1.18 ± 0.87&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>5% mustard seed diet only</td>
<td>28.26 ± 1.72</td>
<td>31.10 ± 1.42</td>
<td>------</td>
<td>------</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of 12-15 animals.

<sup>d</sup>(p < 0.001), represent significant changes against control.
CUMINUM CYMINUM...
4.6.1 Experiment I

In these set of experiments, the effect of cumin seeds (*Cuminum cyminum*) on benzo(a)pyrene induced forestomach tumorigenesis in male Swiss albino mice has been examined.

**Results**

Table 4.14 depicts the result obtained by cumin seeds supplemented diet on B(a)P induced forestomach tumorigenesis. No difference was noticed in weight gain profile of animals treated with either doses of cumin seeds diet as well as in the positive control group of mice.

**Tumor Burden**

The mean number of papilloma/mouse (tumor burden) in the control group (B[a]P only) of animals was 7.33±2.10. In contrast, animals treated with 2.5% and 5% diet of cumin seeds, tumor burden reduced to 3.10±0.57 (p<0.001) and 3.11±0.60 (p<0.001) respectively. The percentage inhibition of tumor multiplication was 57.71 and 57.57 by the 2.5% and 5% of cumin seeds treatment respectively.

**Tumor Incidences**

100% of the control animals developed forestomach papillomas by B(a)P treatment. Relative to control tumor incidences were 71.43% and 64.29% in case of 2.5% and 5% dose of cumin seeds respectively. Total tumor incidence decreased 28.57% with the low dose and 35.7% with the high dose of cumin seeds. 5% diet of cumin seeds only (negative control group) did not result in any tumor.

**Discussion**

Cumin is one of the most extensively used condiments in the world. It has a peculiar, strong and heavy odour and is widely used in ayurvedic medicine for the treatment of various disorders like dyspepsia, diarrhoea and jaundice. The chemical entities, which primarily establish its characteristically pungent flavor, are found in the oil of cumin. The chief constituent of the oil is cumaldehyde, C_{10}H_{12}O (p-isopropylbenzaldehyde).
An attempt has been made in the present work to examine the chemopreventive potentials of cumin seeds using experimental carcinogenesis. Dietary administration of cumin exerted a strong chemopreventive effect against BP-induced forestomach tumorigenesis in Swiss albino mice. Earlier studies in our laboratory have shown chemo-modulatory influence of cumin on xenobiotic metabolising system. Cumin was reported to be a bifunctional chemopreventive agent. Cumin seed treatment significantly increased the activity of phase I, phase II enzymes and antioxidant enzymes/contents (Dhanalakshmi, 2000).

The present study has clearly demonstrated the cancer preventive action of cumin seeds using chemically induced tumorigenesis in murine model system. The preventive potentials could be due to modulatory influence of cumin seeds on phase I and phase II enzymes involved in the carcinogen metabolism. Cumin also facilitates and/or enhances the activity of the natural antioxidant system, which in turn is likely to protect the cell against the oxidative stress involved in the multistage process of carcinogenesis.

Cumin in diet at the given dose levels appears to be safe as it could not cause any adverse effects on the animals as evident by the food intake and general body weight gain (of the animals) during the course of the experiment. Therefore, it could be inferred that cumin seeds may have cancer chemopreventive effects in human population also.
Table 4.14: Effect of Different Doses of Cumin Seeds (*Cuminum cyminum*) on Benzo(a)pyrene Induced Forestomach Tumorigenesis in Swiss Albino Mice

<table>
<thead>
<tr>
<th>Groups &amp; Treatment</th>
<th>Body weight (gms)</th>
<th>Total tumor incidence (%)</th>
<th>Tumor burden (papilloma per mouse)</th>
<th>Percentage inhibition of tumor burden</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>32.1 ± 2.69</td>
<td>33.2 ± 2.52</td>
<td>100</td>
<td>7.33 ± 2.10</td>
</tr>
<tr>
<td>Benzo(a)pyrene + 2.5% diet of cumin seeds</td>
<td>30.5 ± 2.25</td>
<td>32.7 ± 2.68</td>
<td>71.43</td>
<td>3.10 ± 0.57&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Benzo(a)pyrene + 5% diet of cumin seeds</td>
<td>30.8 ± 2.66</td>
<td>35.0 ± 2.9</td>
<td>64.29</td>
<td>3.11 ± 0.60&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>5% diet of cumin seeds</td>
<td>31.2 ± 2.01</td>
<td>36.7 ± 2.2</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of 12-15 animals.

<sup>d</sup> (p < 0.001), represent significant changes against control.
MOMORDICA CHARANTIA...
4.7.1 Experiment I

In these set of experiments, the effect of Karela (Momordica charantia) on benzo(a)pyrene induced forestomach tumorigenesis in male Swiss albino mice has been examined.

Results

Table 4.15 depicts the result obtained by Momordica charantia fruit juice supplementation in diet on B(a)P induced forestomach tumorigenesis. No difference was noticed in weight gain profile of animals treated with either doses of Momordica charantia diet as well as in the positive control group of mice.

Short Term Study

Tumor Burden

The mean number of papilloma/mouse (tumor burden) in the control group (B[a]P only) of animals was 7.50±1.62. In contrast, animals treated with 2.5% and 5% diet of Momordica charantia for short terms, tumor burden reduced to 1.33±0.98 (p<0.001) and 2.00±1.68 (p<0.001) respectively. The percentage inhibition of tumor multiplication was 82.23 and 76.97 by the low and high doses of Momordica charantia treatment respectively.

Tumor Incidences

In short term treatment groups, total tumor incidence decreased by 16.67% with the low dose and by 9.1% with the high dose of Momordica charantia.

Long Term Study

Tumor Burden

In long-term studies the tumor burden was reduced to 1.73±0.90 (p<0.001) and 1.77±1.59 (p<0.001) by 2.5% and 5% diet of Momordica charantia respectively. The percentage inhibition of tumor multiplication was 73.33 and 76.41 by the low and high doses of Momordica charantia treatment respectively.
**Tumor Incidences**

The long-term treatment of *Momordica charantia* reduced the total tumor incidence by 23.08% with the low dose and 30.77% with the high dose.

The long-term treatment of 5% diet of *Momordica charantia* only (negative control group) did not result in any tumor.

**Discussion**

The tumorigenesis process occurs by discrete stepwise changes and involves specific quantifiable events, which provides us with the opportunity of interfering with these processes in order to prevent tumor development. Thus one of the major approaches to chemoprevention is the use of agents known to block or reverse specific pathways. The present study was designed to evaluate the effect of *Momordica charantia* on carcinogen induced forestomach tumorigenesis at peri-initiational stages.

*Momordica charantia* (Karela) is commonly used as an antidiabetic and antihyperglycemic agent. The mature fruits of *Momordica charantia* are used externally for the rapid healing of wounds and internally for the treatment of peptic ulcers in Turkish folk medicine. It showed significant and dose-dependent anti-ulcerogenic activity against ethanol-induced ulcerogenesis model in rats (Gurbuz *et al.*, 2000). Also, it inhibits the bacterial mutagenesis and aberrant crypt focus formation in the rat colon (Chiampanichayakul *et al.*, 2001). It has proved its efficacy against mammary tumorigenesis (Nagasawa *et al.*, 2002). The peel, pulp, seed as well as the whole fruit aqueous extract of *Momordica charantia* effectively prevented the carcinogen induced mouse skin papilloma. It has been proposed that the anti-carcinogenic potential of *Momordica charantia* is may be due to the modulation of biotransformation system as it has increased the level of glutathione S transferase and the reduced glutathione level significantly (Ganguly *et al.*, 2000).

*Helicobacter pylori* infection has recently been identified as an important cause of stomach cancer, as a result of the consistent association between the infection and stomach cancer, especially in prospective cohort studies (Forman, 1991). Fruit extract...
of *Momordica charantia* possesses anti-helicobacter pylori activity (Yesilada *et al.*, 1999).

Due to pharmacological safety there has been an increased interest in phytochemicals that may exhibit anticancer activity. The search for new chemopreventive and antitumor agents that are more effective and are less toxic has kindled great interest in phytochemicals. This modulator, *Momordica charantia* is already a popular dietary constituent in many countries around the world. It has been used as a herbal medicine for centuries in India and in several southeastern countries. This study clearly indicates the chemopreventive efficacy of *Momordica charantia* against fore-stomach tumorigenesis, with no toxic effects at long-term dietary supplementation. *Momordica charantia* has also proven its cancer preventive efficacy in other cancer-models. Above-mentioned characteristics make *Momordica charantia* a strong candidate for developing anticancer drugs. Though, further investigations are needed to clearly understand its mechanism of action(s).

A comparative account of chemopreventive efficacy of all the modulaters investigated in the present study is given in figures 4.9, 4.10, 4.11 and 4.12.
Table 4.15: Effect of Different Doses of *Momordica charantia* on Benzo(a)pyrene Induced Forestomach Tumorigenesis in Swiss Albino Mice

<table>
<thead>
<tr>
<th>Groups &amp; Treatment</th>
<th>Body weight (gms)</th>
<th>Total tumor incidence (%)</th>
<th>Tumor burden (tumor/mouse)</th>
<th>% Inhibition of tumor burden</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td></td>
</tr>
<tr>
<td>only Benzo(a)pyrene</td>
<td>22.0 ± 2.22</td>
<td>25.75 ± 1.76</td>
<td>100</td>
<td>7.50 ± 1.62</td>
</tr>
<tr>
<td>Benzo(a)pyrene + 2.5% <em>Momordica charantia</em> diet</td>
<td>21.58 ± 2.54</td>
<td>25.92 ± 1.83</td>
<td>83.33</td>
<td>1.33 ± 0.98</td>
</tr>
<tr>
<td>Benzo(a)pyrene + 5% <em>Momordica charantia</em> diet</td>
<td>22.67 ± 2.31</td>
<td>27.50 ± 1.09</td>
<td>76.92</td>
<td>1.73 ± 0.90</td>
</tr>
<tr>
<td>Benzo(a)pyrene + 2.5% <em>Momordica charantia</em> long term diet</td>
<td>21.17 ± 2.41</td>
<td>27.67 ± 1.61</td>
<td>69.23</td>
<td>1.77 ± 1.59</td>
</tr>
<tr>
<td>5% <em>Momordica charantia</em> long term diet</td>
<td>22.50 ± 2.21</td>
<td>28.0 ± 1.12</td>
<td>100</td>
<td>6.00 ± 1.82</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of 12-15 animals.

\( d (p < 0.001) \), represent significant changes against control
Figure 4.9: Relative Inhibition of Tumor Burden by Short Term Treatment of all the Modulators Investigated.

2.5% - Low dose of the modulator in diet.
5% - High dose of the modulator in diet.
Figure 4.10: Relative Inhibition of Tumor Incidences by Short Term Treatment of Modulators Investigated.

2.5% - Low dose of modulator in diet.
5% - High dose of modulator in diet.
Figure 4.11: Relative Inhibition of Tumor Burden by Long Term Treatment of Modulators Investigated.

2.5% - Low dose of modulator in diet.
5% - High dose of modulator in diet.
**Figure 4.12:** Relative Inhibition of Tumor Incidences by Long Term Treatment of Modulators Investigated.

2.5% - Low dose of modulator in diet.
5% - High dose of modulator in diet.
OXIDATIVE STRESS IN TUMOR AND DISTANT NORMAL ORGANS...
4.8.1 Experiment I

The oxidative stress in the forestomach tumor tissue in the Swiss albino mice was evaluated.

Results

Results are shown in table 4.16 and figure 4.13.

**GSH**

Compare to control group, the level of GSH increased significantly by 2.12 folds \( (p<0.05) \) in the forestomach tissue with tumors.

**GST**

Compare to control group, the specific activity of GST decreased significantly by 0.60 folds \( (p<0.05) \) in the forestomach tissue with tumors.

**DTD**

Compare to control group, the specific activity of DTD decreased significantly by 0.64 folds \( (p<0.01) \) in the forestomach tissue with tumors.

**SOD**

No significant change was observed in the specific activity of SOD in the forestomach tissue with tumors.

**CAT**

Compare to control group, the specific activity of CAT decreased significantly by 0.65 folds \( (p<0.05) \) in the forestomach tissue with tumors.

**LDH**

Compare to control group, the specific activity of LDH increased significantly by 1.38 folds \( (p<0.005) \) in the forestomach tissue with tumors.
4.8.2 Experiment II

The oxidative stress in distant normal organs due to forestomach tumor burden in the Swiss albino mice was investigated.

Results

Results are shown in table 4.17 and figure 4.14.

Spleen

\textit{GSH}

Compare to control group, the level of GSH increased significantly by 1.23 folds (p<0.05) in the normal spleen of animals with forestomach tumor.

\textit{GST}

Compare to control group, the specific activity of GST decreased significantly by 0.84 folds (p<0.05) in the normal spleen of animals with forestomach tumor.

\textit{DTD}

No significant change was observed in the specific activity of DTD in the normal spleen of animals with forestomach tumor.

\textit{SOD}

Compare to control group, the specific activity of SOD increased significantly by 1.13 folds (p<0.05) in the normal spleen of animals with forestomach tumor.

\textit{CAT}

No significant change was observed in the specific activity of CAT in the normal spleen of animals with forestomach tumor.

\textit{LDH}

No significant change was observed in the specific activity of LDH in the normal spleen of animals with forestomach tumor.
Kidney

**GSH**
Compare to control group, the level of GSH increased significantly by 2.02 folds (p<0.05) in the normal kidney of animals with forestomach tumor.

**GST**
Compare to control group, the specific activity of GST decreased significantly by 0.87 folds (p<0.05) in the normal kidney of animals with forestomach tumor.

**DTD**
No significant change was observed in the specific activity of DTD in the normal kidney of animals with forestomach tumor.

**SOD**
No significant change was observed in the specific activity of SOD in the normal kidney of animals with forestomach tumor.

**CAT**
No significant change was observed in the specific activity of CAT in the normal kidney of animals with forestomach tumor.

**LDH**
No significant change was observed in the specific activity of LDH in the normal kidney of animals with forestomach tumor.

Heart

**GSH**
Compare to control group, the level of GSH increased significantly by 1.29 folds (p<0.05) in the normal heart of animals with forestomach tumor.

**GST**
Compare to control group, the specific activity of GST decreased significantly by 0.90 folds (p<0.05) in the normal heart of animals with forestomach tumor.
**DTD**

No significant change was observed in the specific activity of DTD in the normal heart of animals with forestomach tumor.

**SOD**

No significant change was observed in the specific activity of SOD in the normal heart of animals with forestomach tumor.

**CAT**

No significant change was observed in the specific activity of CAT in the normal heart of animals with forestomach tumor.

**LDH**

Compare to control group, the specific activity of LDH increased significantly by 1.32 folds (p<0.001) in the normal normal heart of animals with forestomach tumor.

### 4.8.3 Experiment III

The modulation of drug detoxification system of normal liver as a distant organ due to forestomach tumor burden in the Swiss albino mice was examined.

**Results**

**Phase I Enzymes**

Results are shown in table 4.18 and figure 4.15.

**Cytochrome P450**

Compare to control group, the cytochrome P450 content decreased significantly by 0.72 folds (p<0.005) in the normal hepatic tissue of animals with forestomach tumor.

**Cytochrome b5**

Compare to control group, the cytochrome b5 content decreased significantly by 0.63 folds (p<0.05) in the normal hepatic tissue of animals with forestomach tumor.
**Cytochrome P450R**

Compare to control group, the specific activity of cytochrome P450R decreased significantly by 0.62 folds (p<0.001) in the normal hepatic tissue of animals with forestomach tumor.

**Cytochrome b5R**

No significant change was observed in the specific activity of cytochrome b5R in the normal hepatic tissue of animals with forestomach tumor.

**Phase II Enzyme**

Results are shown in table 4.19 and figure 4.15.

**GST**

Compare to control group, the specific activity of GST decreased significantly by 0.62 folds (p<0.05) in the normal hepatic tissue of animals with forestomach tumor.

**DTD**

No significant change was observed in the specific activity of DTD in the normal hepatic tissue of animals with forestomach tumor.

**Antioxidant Parameters**

Results are shown in table 4.19 and figure 4.15.

**SOD**

Compare to control group, the specific activity of SOD increased significantly by 1.29 folds (p<0.05) in the normal hepatic tissue of animals with forestomach tumor.

**CAT**

Compare to control group, the specific activity of CAT increased significantly by 1.22 folds (p<0.05) in the normal hepatic tissue of animals with forestomach tumor.

**Reduced Glutathione**

Compare to control group, the levels of GSH decreased significantly by 0.86 folds (p<0.05) in the normal hepatic tissue of animals with forestomach tumor.
Other Parameters

Results are shown in table 4.19 and figure 4.15.

LDH

No significant change was observed in the specific activity of LDH in the normal hepatic tissue of animals with forestomach tumor.

Lipid peroxidation

Compare to control group, the levels of lipid peroxidation increased significantly by 115.22 folds (p<0.05) in the normal hepatic tissue of animals with forestomach tumor.

Discussion

Cancer cells are known to experience persistent oxidative stress (Toyokuni et al., 1995). The oxidative stresses suggested to be closely associated with the characteristics of cancer such as activation of proto-oncogenes, and transcriptional factors, genomic instability, chemotherapy resistance, invasion and metastasis. It is now well established now that reactive oxygen species (ROS) are responsible for the oxidative stress and might have resulted from the suppression of antioxidant defense system. Inspite of these facts, studies on metabolism of ROS have not been extensively persued (Toyokuni et al., 1995; Agrawal et al., 2001a, 2001b). Therefore, the present work was undertaken to examine the activities of antioxidant enzymes in cancer tissue using forestomach tumors.

Chemotherapy and radiotherapy are important tools to cure cancer. The radio sensitivity of normal tissue particularly organs away from the tumor sites are suggested to limit the therapeutic gain (Brock et al., 1995). Because of this fact, in radiation therapy practice the radiation dose is chosen primarily on the basis of maximum dose-tolerated by the normal tissue rather than local tumor control. Similarly, in chemotherapy anti-cancer drugs are required to differentially kill the malignant cells causing minimum damage to surrounding normal tissue as well as distant normal organs.
It may be mentioned that function of distant normal organs of tumor bearing animals are likely to be adversely affected as a consequence of the disease. It is an accepted fact that free radicals play an important role in cause and complications of various diseases. The same might be true for the distant organs of tumor bearing animals as in such events an antioxidant defense system may be severely impaired in the normal tissues/organs away from the tumor. Impairment in the form of lowered antioxidant status might have serious implications and affects on the therapeutic gain. Therefore, the measurements of antioxidant enzymes have been examined in different normal tissues as distant organs in forestomach tumor bearing Swiss albino mice.

Results shown in table 4.16 reflect the specific activities of enzymes involved in free radical metabolism, LDH as well as level of GSH in the forestomach cancer tissue of Swiss albino mice. The specific activities GST, DTD and CAT were found to decrease significantly. GST detoxifies both endogenous and xenobiotic alkylating agents and catalyses many antioxidants processes of thiols (Choudhary et al., 1997, 1999; Dixon et al., 1998). DTD has the ability, apart from catalyzing two-electron transfer and decreasing the electrophilic character of quinones, to provide protection against free radical damage (Beyer et al., 1996). Catalase is known to provide the protection against peroxide (H$_2$O$_2$). The decrease levels of these enzymes will cause an accumulation of free radicals and enhance the extent of oxidative stress in stomach tumor tissue. Although, SOD is not adversely affected, it will not be useful in such situation in lowering the oxidative stress as H$_2$O$_2$ generated from dismutation of O$_2^-$, is expected to contribute to the oxidative stress. Our results clearly showed that forestomach tumor is persistently oxidatively stressed. The elevated levels of lipid peroxidation is probably support this possibility. In such event, cancer cells should have mechanisms other than antioxidant enzymes to resist this oxidative stress. Since, the oxidative stress is usually insufficient to cause cell death, from the present results it is also tempting to speculate that the extent of oxidative stress is such that it might be necessary for maintenance of the malignant state.

The increase in the levels of GSH in forestomach cancer tissue is an important observation. GSH is suggested to synthesize in the tumors (Perry et al., 1993; Toyokuni et al., 1995). Therefore, increased levels of GSH found in the present work
could be due to its synthesis in stomach tumor in Swiss albino mice. It is important that GSH is known to protect against electrophiles free radical induced damage and oxidative stress. It has a redox potential of around (-) 230 mV, which makes it behave as an antioxidant. The antioxidant enzymes work co-operatively; any changes in one of them may break this equilibrium and cause cell damage and death. As in the present system except SOD antioxidant enzymes are adversely affected, it is unlikely that GSH alone can compensate for the functions carried out by antioxidant enzymes. Thus, forestomach tumor is oxidatively stressed. However, role of oxidative stress in tumor needs to be understood, as it does not induce damage and death in malignant tissue.

Antioxidant status of normal tissue/organ away from the tumor is likely to determine the radiation and chemotherapy to a great extent. Therefore, we have examined the antioxidant enzymes such as GST, DTD, SOD and CAT in the spleen, kidney, heart and liver as a representative of distant organs in forestomach tumor bearing animals.

In the liver, the specific activities of SOD and CAT were increased. On the contrary, DTD showed no response and GST was inhibited in the liver. Interestingly the levels of GSH and lipid peroxidation were increased simultaneously. There was not significant change in the activity of LDH. The increased levels of lipid peroxidation is suggestive of oxidative stressed experienced by the liver of tumor bearing animals. Enhancement of activity of SOD, CAT and levels of GSH might be in response to the oxidative stress in the liver. Increase in peroxidation but no change in LDH seems to be interesting. It appears that certain degree of membrane damage is required to release LDH in the cytosol.

The cytochrome P450 system in the liver plays vital role in metabolism. Apart from this, the cytochrome P450 has been shown to protect the cells against oxidative damage (Morichetti et al., 1989), have peroxidase activity (O’ Brien and Rahimtula 1980 and Kappeli, 1986) and partially replaces catalase in protecting the cells (Morichetti et al., 1989). Therefore, it will be interesting to examine the cytochrome P450 system in the normal liver of tumor bearing animals. The content of cytochrome P 450 and cytochrome b5 as well as activities of cytochrome P 450R and cytochrome
b5 R in the normal liver were significantly inhibited. These results suggest that the drug metabolising function of the liver is severely disturbed. It may have serious implications for the chemotherapy. In addition, the altered cytochrome P 450 system would also contribute to oxidative stress.

The findings of the present study also showed that organs distant from the tumor in tumor bearing animals had altered antioxidant enzymes and GSH levels. The mode and magnitude of change depend on the type of tissue. All the three extra hepatic organs showed decreased levels of GST and increased levels of GSH. No change was noticed in the DTD activity. Similar response was also seen with catalase. SOD showed enhancement in the spleen but not in kidney and heart. LDH was enhanced in the heart but not in other tissues. The decreased activity of GST was accompanied by the increased LDH activity in the spleen. Although SOD and GSH were enhanced probably were not able to control the damage in the spleen. In case of kidney, the specific activities of GST, DTD and SOD were inhibited and were possibly lower the antioxidant status and in turn contributed to damage to tissue. However, no increase in the activity of the LDH was seen in the kidney under this condition. Increase in LDH was noticed in the heart, which might be caused mainly due to inhibition of GST as DTD, SOD and catalase did not show any alteration in their activities. GSH was increased significantly. However, it seems that the increase was not sufficient to control the damage.

It may be concluded that tumor tissue experienced persistent oxidative stress, which probably affect the antioxidant defense system in the distant normal organ. The changed antioxidant status of distant organs is likely to have serious implications in radiotherapy as well as chemotherapy of cancer.
### Table 4.16: Levels of Enzymes Involved in Antioxidant Functions, LDH and GSH in Tumor Bearing Fore stomach of Swiss Albino Mice

<table>
<thead>
<tr>
<th>Enzymes/GSH</th>
<th>Specific activities/content in forestomach tissue</th>
<th>Normal animals</th>
<th>Tumor bearing animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specific activities/content in forestomach tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD(^{\theta})</td>
<td>0.612±0.003 (100)</td>
<td>0.655±0.002 (106.92)</td>
<td></td>
</tr>
<tr>
<td>CAT(^{\theta})</td>
<td>0.29±0.01 (100)</td>
<td>0.19±0.01(^{a}) (65.52)</td>
<td></td>
</tr>
<tr>
<td>DTD(^{\theta})</td>
<td>0.047±0.004 (100)</td>
<td>0.030±0.03(^{d}) (63.83)</td>
<td></td>
</tr>
<tr>
<td>GST(^{\theta})</td>
<td>3.56±0.27 (100)</td>
<td>0.135±0.01(^{a}) (60.27)</td>
<td></td>
</tr>
<tr>
<td>LDH(^{\theta})</td>
<td>0.47±0.07 (100)</td>
<td>0.65±0.03(^{c}) (138.30)</td>
<td></td>
</tr>
<tr>
<td>GSH(^{\theta})</td>
<td>3.56±0.27 (100)</td>
<td>7.57±0.47(^{a}) (212.32)</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of 6-8 animals.

Values in parentheses represent relative change in parameters assessed (i.e., levels of activity in extrahepatic organs of mice receiving test substance to activity in extra hepatic organs of control mice).

\(^{a}\) (p < 0.05), \(^{c}\) (p < 0.005) and \(^{d}\) (p < 0.001) represent significant changes against control.

\(^{\theta}\) Specific activity expressed as μmole/mg protein, \(^{\vartheta}\) μmole H₂O₂ consumed/min/mg protein, \(^{\varnothing}\) μmole of DCP/IP reduced/min/mg protein,
\(^{\circ}\) μmole CDNB-GSH conjugate formed/min/mg protein, \(^{\circ}\) μmole/mg protein,
\(^{\circ}\) nmole GSH/g tissue


Treatment duration: 15 days
Figure 4.13: Levels of Enzymes Involved in Antioxidant Functions, LDH and GSH in Tumor Bearing Forestomach of Swiss Albino Mice.

\(^a(p < 0.05), ^c(p < 0.005)\) and \(^d(p < 0.001)\) represent significant changes against control.


Treatment duration: 15 days
Table 4.17: The Specific Activities of Antioxidant Enzymes, LDH and GSH content in Spleen, Kidney and Heart of Swiss Albino Mice Bearing Forestomach Tumors

<table>
<thead>
<tr>
<th>Enzymes/GSH</th>
<th>Specific activities/content</th>
<th>Tissue of non-tumor bearing animals</th>
<th>Tissue of tumor bearing animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Spleen</td>
<td>Kidney</td>
</tr>
<tr>
<td>GST©</td>
<td>0.65±0.03 (100)</td>
<td>0.55±0.05* (83.72)</td>
<td></td>
</tr>
<tr>
<td>DTD©</td>
<td>0.07±0.02 (100)</td>
<td>0.07±0.02 (94.44)</td>
<td></td>
</tr>
<tr>
<td>SOD©</td>
<td>2.45±0.08 (100)</td>
<td>2.07±0.15* (113.12)</td>
<td></td>
</tr>
<tr>
<td>CAT©</td>
<td>1.85±0.10 (100)</td>
<td>1.91±0.02 (103.24)</td>
<td></td>
</tr>
<tr>
<td>LDH©</td>
<td>0.31±0.01 (100)</td>
<td>0.27±0.05 (119.1)</td>
<td></td>
</tr>
<tr>
<td>GSH®</td>
<td>1.74±0.18 (100)</td>
<td>2.14±0.19* (122.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GST©</td>
<td>1.67±0.02 (100)</td>
<td>1.46±0.02* (82.27)</td>
<td></td>
</tr>
<tr>
<td>DTD©</td>
<td>0.07±0.02 (100)</td>
<td>0.05±0.02 (79.41)</td>
<td></td>
</tr>
<tr>
<td>SOD©</td>
<td>1.07±0.18 (100)</td>
<td>0.98±0.06 (90.70)</td>
<td></td>
</tr>
<tr>
<td>CAT©</td>
<td>2.41±0.10 (100)</td>
<td>2.90±0.90 (120.33)</td>
<td></td>
</tr>
<tr>
<td>LDH©</td>
<td>1.12±0.07 (100)</td>
<td>1.17±0.11 (104.46)</td>
<td></td>
</tr>
<tr>
<td>GSH®</td>
<td>1.75±0.13 (100)</td>
<td>3.53±0.27* (201.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GST©</td>
<td>1.08±0.09 (100)</td>
<td>0.90±0.06* (83.07)</td>
<td></td>
</tr>
<tr>
<td>DTD©</td>
<td>0.07±0.03 (100)</td>
<td>0.07±0.02 (94.37)</td>
<td></td>
</tr>
<tr>
<td>SOD©</td>
<td>1.25±0.05 (100)</td>
<td>1.36±0.17 (109.2)</td>
<td></td>
</tr>
<tr>
<td>CAT©</td>
<td>0.74±0.01 (100)</td>
<td>0.76±0.05 (102.7)</td>
<td></td>
</tr>
<tr>
<td>LDH©</td>
<td>1.33±0.09 (100)</td>
<td>1.76±0.10* (132.33)</td>
<td></td>
</tr>
<tr>
<td>GSH®</td>
<td>1.36±0.14 (100)</td>
<td>1.75±0.15* (128.80)</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of 6-8 animals. Values in parentheses represent relative change in parameters assessed (i.e., levels of activity in livers of mice receiving test substance to activity in liver of control mice). *(p < 0.05) and **(p<0.001) represent significant changes against control.

Figure 4.14: The Specific Activities of Antioxidant Enzymes, LDH and GSH Content in Normal Spleen, Kidney and Heart of Swiss Albino Mice Bearing Forestomach Tumor.

*p < 0.05* and *d < 0.001* represent significant changes against control.

Table 4.18: The Cytochrome P450 System in the Normal Liver of Swiss Albino Mice Bearing Forestomach Tumor

<table>
<thead>
<tr>
<th>Enzymes/GSH</th>
<th>Specific activities/content in forestomach tissue</th>
<th>Normal animals</th>
<th>Tumor bearing animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyt P450Ø</td>
<td>0.575 ±0.08 (100)</td>
<td>0.412 ±0.04c (71.65)</td>
<td></td>
</tr>
<tr>
<td>Cyt b5Ø</td>
<td>0.275±0.05 (100)</td>
<td>0.173±0.03a (62.91)</td>
<td></td>
</tr>
<tr>
<td>Cyt P450RØ</td>
<td>0.853 ±0.02 (100)</td>
<td>0.530±0.04d (62.13)</td>
<td></td>
</tr>
<tr>
<td>Cyt b5RØ</td>
<td>3.794 ±0.32 (100)</td>
<td>3.67±0.37 (96.63)</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of 6-8 animals.

Values in parentheses represent relative change in parameters assessed (i.e., levels of activity in livers of mice receiving test substance to activity in liver of control mice)

a (p < 0.05), c (p < 0.005) and d (p<0.001) represent significant changes against control.

Ønmole/mg protein, Øµmole of NADPH oxidized/min/mg protein, Øµmole of NADH oxidized/min/mg protein

Abbreviation: Cyt P450: cytochrome P450, Cyt b5: cytochrome b5, Cyt P450 R: cytochrome P450 reductase, Cyt b5 R: cytochrome b5 reductase

Treatment duration: 15 days.
**Table 4.19:** The Specific Activities of Antioxidant Enzymes, LDH, GSH Content and Peroxidative Damage in the Normal Liver of Swiss Albino Mice, Bearing Forestomach Tumor

<table>
<thead>
<tr>
<th>Enzymes/ GSH/LP</th>
<th>Specific activities/content in liver tissue</th>
<th>Normal animals</th>
<th>Tumor bearing animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td>SOD(\textcircled{1})</td>
<td>13.21 ±0.61 (100)</td>
<td>17.09±0.29(\textcircled{a}) (129.37)</td>
<td></td>
</tr>
<tr>
<td>CAT(\textcircled{2})</td>
<td>9.16±0.39 (100)</td>
<td>11.14±0.62(\textcircled{a}) (121.61)</td>
<td></td>
</tr>
<tr>
<td>DTD(\textcircled{3})</td>
<td>0.747±0.07 (100)</td>
<td>0.760±0.041 (101.74)</td>
<td></td>
</tr>
<tr>
<td>GST(\textcircled{4})</td>
<td>0.188±0.01 (100)</td>
<td>0.155±0.01(\textcircled{a}) (82.45)</td>
<td></td>
</tr>
<tr>
<td>LDH(\textcircled{5})</td>
<td>2.40±0.12 (100)</td>
<td>2.31±0.13 (96.25)</td>
<td></td>
</tr>
<tr>
<td>GSH(\textcircled{6})</td>
<td>55.35±3.81 (100)</td>
<td>47.63±1.38(\textcircled{a}) (86.05)</td>
<td></td>
</tr>
<tr>
<td>LP(\textcircled{7})</td>
<td>1.38±0.08 (100)</td>
<td>1.59±0.06(\textcircled{a}) (115.22)</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of 6-8 animals.

Values in parentheses represent relative change in parameters assessed (i.e., levels of activity in extrahepatic organs of mice receiving test substance to activity in extra hepatic organs of control mice).

\(\textcircled{a}\) \((p < 0.05)\) represent significant changes against control.

\(\textcircled{1}\) Specific activity expressed as \(\mu\)mole/mg protein, \(\textcircled{2}\) \(\mu\)mole \(H_2O_2\) consumed/ min/mg protein, \(\textcircled{3}\) \(\mu\)mole of DCPIP reduced/ min/mg protein, \(\textcircled{4}\) \(\mu\)mole CDNB-GSH conjugate formed/min/mg protein, \(\textcircled{5}\) \(\mu\)mole GSH/g tissue, \(\textcircled{6}\) nmole GSH/g tissue, \(\textcircled{7}\) nmole malondialdehyde formed/mg protein.

Abbreviations: GSH: reduced glutathione, GST: glutathione S-transferase, DTD: DT-diaphorase, SOD: Superoxide dismutase, CAT: Catalase and LDH: Lactate dehydrogenase, LP: lipid peroxidation

Treatment duration: 15 days
Figure 4.15: The Specific Activities of Phase I and Phase II, LDH and Antioxidant Enzymes, GSH Content and Peroxidative Damage in the Normal Liver of Swiss Albino Mice Bearing Forestomach Tumors.

*(p < 0.05)* and *(p < 0.001)* represent significant changes against control.


Treatment duration: 15 days
Pic 4.1: Stomach with normal epithelium (100×)

Pic 4.2: Stomach with papillomas (100×)
**Pic 4.3:** Stomach with normal epithelium (200×)

**Pic 4.4:** Stomach with papillomas (200×)