5. Results
Part A: Effect of plants under investigation on tumorigenesis and xenobiotic metabolizing and antioxidant enzymes

The chemopreventive efficacy of certain plants like *Withania somnifera, Hippophae rhamnoides, Decalepis hamiltonii, Piper longum* and *Micrococcus paniculata* was studied in DMBA induced skin tumor and B(a)P induced stomach tumor model systems. In both the tumor model systems, apart from the carcinogen and modulator treated groups, an untreated group was maintained. The animals did not develop any spontaneous tumors. The effect of these plant modulators on the Phase I, Phase II enzymes and antioxidant parameters was also studied as these enzymes play an important role in xenobiotic metabolism and in reducing oxidative stress.

5.1 *Withania somnifera* (Ashwagandha)

5.1.1 Expt 1-Effect of *Withania somnifera* on DMBA induced skin papillomagenesis in Swiss albino mice

In DMBA induced skin papillomagenesis, *Withania somnifera* reduced both tumor incidence and tumor multiplicity at both dose levels of treatment. The tumor incidence in lower and higher dose treated animals was 67% and 56% respectively, whereas it was 100% in the control group of animals. In the lower and higher dose treated animals the mean number of tumors/animal was $1.13 \pm 1.55$ ($P < 0.05$) and $1.00 \pm 1.55$ ($P < 0.05$) respectively whereas it was $3.33 \pm 2.39$ in the control group animals (Fig 1, Picture 1).

5.1.2 Expt 2-Effect of *Withania somnifera* on B(a)P induced forestomach papillomagenesis in swiss albino mice

In B(a)P induced forestomach papillomagenesis the tumor incidence was decreased by 60% and 13% respectively in lower and higher dose treated animals where as there was no reduction in the control group. The mean number of tumors/animal was $1.08 \pm 0.99$ ($P < 0.001$) and $2.60 \pm 1.72$ ($P < 0.001$) in lower and higher dose treated animals respectively as compared to $11.0 \pm 2.79$ in the control group (Table 1, Picture 2).
Effect of *Withania somnifera* extract on DMBA induced skin papillomagenesis

**Figure 1.** Effect of two different doses of *Withania somnifera* (*Ashwagandha*) in diet on DMBA-induced skin papillomagenesis.

**Groups:** Control-DMBA + croton oil; LD- DMBA+ croton oil + 2.5% of *Withania* in diet; HD- DMBA+ croton oil + 5% of *Withania* in diet.

**Effective no:** 12-15 animals/group.
DMBA induced skin papillomagenesis in Swiss albino mice

C- control DMBA alone

LD- lower dose of *Withania somnifera* in diet + DMBA

HD- higher dose of *Withania somnifera* in diet + DMBA
Table 1. Effect of two different doses of *Withania somnifera* (Ashwagandha) on B(a)P-induced forestomach papillomagenesis in Swiss albino mice.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Group</th>
<th>Effective no.</th>
<th>% tumor incidence</th>
<th>No. of tumors/mouse</th>
<th>% tumor multiplicity</th>
<th>Total no. of tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>B(a)P only</td>
<td>11</td>
<td>100</td>
<td>11.0±2.79</td>
<td>100</td>
<td>121</td>
</tr>
<tr>
<td>2.</td>
<td>B(a)P+2.5% <em>Withania</em> in diet</td>
<td>20</td>
<td>40</td>
<td>1.08±0.99&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.8</td>
<td>13</td>
</tr>
<tr>
<td>3.</td>
<td>B(a)P+5% <em>Withania</em> in diet</td>
<td>15</td>
<td>87</td>
<td>2.6±1.72&lt;sup&gt;d&lt;/sup&gt;</td>
<td>23.6</td>
<td>39</td>
</tr>
</tbody>
</table>

Values are mean±S.D of 15-20 animals in each group.
Statistical significance – <sup>d</sup> (p = 0.001) when compared with the control group.
B(a)P induced forestomach papillomagenesis in Swiss albino mice

C- control B(a)P alone

LD- lower dose of *Withania somnifera* in diet + B(a)P

HD- higher dose of *Withania somnifera* in diet + B(a)P
5.1.3 Expt 3-Effect of *Withania somnifera* on xenobiotic metabolizing enzymes and antioxidant parameters in Swiss albino mice

All the phase I enzymes showed either a significant reduction or no change in both the dose levels of treatment. The lower dose treated animals showed a significant reduction in cyt b5 and cyt P450 levels. The induction was 0.87 (P < 0.005) and 0.86 (P < 0.05) folds in cyt P450 and cyt b5 in comparison with the control group. There were no significant changes in the higher dose of treatment. No significant changes were observed in cyt P450 and cyt b5 reductases at both dose levels of treatment.

In phase II enzymes the specific activities of GST and DTD were elevated in low dose treated animals by 1.26 (P < 0.005) and 1.67 (P < 0.005) folds respectively. The animals that received higher dose of treatment showed an elevation by 33% and 98%, in the activities of GST and DTD, the induction being 1.33 (P < 0.01) and 1.98 (P < 0.005) folds respectively. (Figure 2, Table 2).

Specific activities of SOD, CAT, GPx were significantly elevated at both dose levels of treatment and GSH was elevated in low dose treated animals in comparison with the control group. In the lower dose treated animals SOD, CAT, GPx and GSH showed an elevation by 1.48 (P < 0.01), 3.15 (P < 0.01), 1.14 (P < 0.01) and 1.31 (P < 0.05) folds respectively. In higher dose treated animals except for GSH an induction by 1.73 (P < 0.01), 2.99 (P < 0.01), 1.14 (P < 0.05) folds was observed in SOD, CAT and GPx respectively as compared to control group.

Levels of lipidperoxidation, as measured by malondialdehyde formation showed reduction by 0.73 (P < 0.01) folds only in lower dose treated animals as compared to the control group. There was a significant dose dependent reduction in LDH activity by 0.75 (P < 0.01) and 0.68 (P < 0.01) folds respectively as compared to control group (Figure 3, Table 3).

5.2 *Hippophae rhamnoides* (Seabuckthorn)

5.2.1 Expt 1-Effect of *Hippophae rhamnoides* on DMBA induced skin papillomagenesis in Swiss albino mice

*Hippophae rhamnoides* treated animals did not show any significant reduction in tumor multiplicity though the tumor incidence was 87% and 57% in both lower and
Effect of *Withania somnifera* in diet on the Phase I and Phase II metabolizing enzymes

![Graph showing the effect of two different doses of *Withania somnifera* (Ashwagandha) in diet on the levels of cytochrome P450 (Cyt P 450), cytochrome b5 (Cyt b5), and on the specific activities of NADPH-cytochrome P450 reductase (Cyt P 450 R), NADH-cytochrome b5 reductase (Cyt b5 R), glutathione S-transferase (GST) and DT-diaphorase (DTD) in the liver of mice. Error bars represent standard deviation.](image)

**Figure 2.** Effect of two different doses of *Withania somnifera* (Ashwagandha) in diet on the levels of cytochrome P450 (Cyt P 450), cytochrome b5 (Cyt b5), and on the specific activities of NADPH-cytochrome P450 reductase (Cyt P 450 R), NADH-cytochrome b5 reductase (Cyt b5 R), glutathione S-transferase (GST) and DT-diaphorase (DTD) in the liver of mice. Error bars represent standard deviation.

**Groups:** Co: control, LD-2.5% of *Withania* in diet; HD-5% of *Withania* in diet.  
a (p < 0.05), b (p < 0.01) and c (p < 0.005) indicate significant changes against control.

**Treatment duration:** 14 days.
Table 2. Modulatory influence of two different doses of *Withania somnifera* (Ashwagandha) in diet on mouse hepatic phase I and phase II drug metabolizing enzyme levels.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cyt P450 (1)</th>
<th>Cyt b5 (1)</th>
<th>Cyt P450 R (2)</th>
<th>Cyt b5 R (3)</th>
<th>GST (4)</th>
<th>DTD (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.592±0.091 (100)</td>
<td>0.370±0.031 (100)</td>
<td>0.313±0.043 (100)</td>
<td>3.57±0.153 (100)</td>
<td>1.49±0.146 (100)</td>
<td>0.007±0.0006 (100)</td>
</tr>
<tr>
<td>LD- 2.5% Withania</td>
<td>0.478±0.056a (87)</td>
<td>0.319±0.018c (86)</td>
<td>0.296±0.023 (94)</td>
<td>3.45±0.285 (97)</td>
<td>1.88±0.167c (126)</td>
<td>0.012±0.0017c (167)</td>
</tr>
<tr>
<td>HD- 5% Withania</td>
<td>0.674±0.038 (113)</td>
<td>0.353±0.043 (95)</td>
<td>0.342±0.028 (109)</td>
<td>3.63±0.215 (102)</td>
<td>1.98±0.265b (133)</td>
<td>0.014±0.0017c (198)</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 < 0.5), *b* (p < 0.01) and *c* (p < 0.005) represent significant changes against control.

1 nmole/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.

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

Treatment duration: 14 days.
Effect of *Withania somnifera* in diet on antioxidant parameters, lipid peroxidation and lactate dehydrogenase

![Graph showing effects](image)

**Figure 3.** Effect of two different doses of *Withania somnifera* (Ashwagandha) in diet, on the reduced glutathione content (GSH) and on the specific activities of glutathione peroxidase (GPX), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), malondialdehyde formation (LP) and on the specific activity of lactate dehydrogenase (LDH) in the liver of mice. Error bars represent standard deviation.

**Groups:** Co: control, LD- 2.5% of *Withania* in diet; HD- 5% of *Withania* in diet.

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

**Treatment duration:** 14 days.
Table 3. Modulatory influence of two different doses of *Withania somnifera* (Ashwagandha) in diet on mouse hepatic antioxidant related parameters, lipid peroxidation and lactate dehydrogenase.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (nmole GSH/g tissue)</th>
<th>SOD (specific activity expressed as μmole/mg protein)</th>
<th>CAT (μmole H₂O₂ consumed/min/mg protein)</th>
<th>GPx (nmole of NADPH consumed/min/mg protein)</th>
<th>GR (μmole malondialdehyde formed/mg protein)</th>
<th>LP (nmole/mg protein)</th>
<th>LDH (μmole/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24.3±2.63 (100)</td>
<td>10.4±0.94 (100)</td>
<td>29.8±6.56 (100)</td>
<td>25.5±0.723 (100)</td>
<td>35.2±2.74 (100)</td>
<td>1.213±0.11 (100)</td>
<td>2.47±0.27 (100)</td>
</tr>
<tr>
<td>LD- 2.5% <em>Withania</em></td>
<td>31.9±5.31 (131)</td>
<td>15.9±2.11 (148)</td>
<td>94.0±12.74 (315)</td>
<td>29.0±2.90 (114)</td>
<td>37.0±1.81 (110)</td>
<td>1.231±0.17 (101)</td>
<td>1.87±0.16 (75)</td>
</tr>
<tr>
<td>HD- 5% <em>Withania</em></td>
<td>33.7±8.84 (138)</td>
<td>18.0±1.76 (173)</td>
<td>89.2±19.15 (299)</td>
<td>29.0±2.23 (114)</td>
<td>38.5±1.98 (113)</td>
<td>0.884±0.09 (73)</td>
<td>1.68±0.27 (68)</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) and b (p < 0.01) 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 nmole of NADPH consumed/min/mg protein, 5 nmole malondialdehyde formed/mg protein and 6 μmole/mg protein.


Treatment duration: 14 days
higher dose treated animals respectively as compared to 100% incidence in the control group. The mean number of papillomas/animal was 2.79 ± 2.36 and 1.92 ± 2.75 in lower and higher dose treated animals respectively where as it was 2.92 ± 2.87 in the control group (Figure 4).

5.2.2 Expt 2-Effect of *Hippophae rhamnoides* on B(a)P induced forestomach papillomagenesis in swiss albino mice

A significant attenuation in B(a)P induced tumor incidence by 6.25% in lower dose treated animals was seen as compared to the control animals. The mean number of tumors/animal was 2.313 ± 1.58 (P < 0.001) and 1.71 ± 1.05 (P < 0.001) in lower and higher dose treated animals respectively as compared to 6.25 ± 2.44 in the control group (Table 4).

5.2.3 Expt 3-Effect of *Hippophae rhamnoides* on xenobiotic metabolizing enzymes and antioxidant parameters in Swiss albino mice

*Hippophae rhamnoides* did not induce any significant levels of phase I enzymes except for the activity of cyt b5 reductase. *Hippophae rhamnoides* at its lower dose of treatment showed an induction of 1.13, 1.00, 1.00 and 0.78 (P < 0.005) folds in the activities of cyt P450, cyt b5, cyt P450 reductase and cyt b5 reductase respectively. The higher dose of treatment showed an induction of 1.14, 0.86, 0.97 and 0.67 (P < 0.005) folds in cyt P450, cyt b5, cyt P450 reductase and cyt b5 reductase levels in comparison with the control.

A significant dose dependent increase in the activities of GST and DTD were seen at both lower and higher doses of treatment. The lower dose showed an elevation by 1.27 (P < 0.01) and 1.76 (P < 0.005) folds in GST and DTD levels respectively. Similarly higher dose of treatment increased the activities of GST and DTD by 1.50 (P < 0.01) and 1.93 (P < 0.005) folds respectively. (Figure 5, Table 5)

All the antioxidant enzymes were induced significantly at both dose levels except CAT at higher dose of treatment. An induction by 1.52 (P < 0.005), 1.48 (P < 0.005), 1.59 (P < 0.005) and 2.06 (P < 0.01) folds was seen in the activities of SOD, CAT, GPx and GR in the lower dose of treatment. In the higher dose treated animals SOD, GPx and GR were induced by 1.77 (P < 0.01), 1.75 (P < 0.05) and 1.83 (P <
**Effect of *Hippophae rhamnoides* extract on DMBA induced skin papillomagenesis**

**Figure 4.** Effect of two different doses of *Hippophae rhamnoides* (Seabuckthorn) extract on DMBA-induced skin papillomagenesis.

**Groups:** Control-DMBA + croton oil; LD- DMBA+ croton oil + 150 mg/kg body wt of *Hippophae* HD- DMBA+ croton oil + 300 mg/kg body wt of *Hippophae*.

**Effective no:** 12-15 animals/group.
Table 4. Effect of two different doses of *Hippophae rhamnoides* (Seabuckthorn) extract on B(a)P-induced forestomach papillomagenesis in Swiss albino mice.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Group</th>
<th>Effective no.</th>
<th>% tumor incidence</th>
<th>No. of tumors/mouse</th>
<th>% tumor multiplicity</th>
<th>Total no. of tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>B(a)P only</td>
<td>17</td>
<td>100</td>
<td>6.25±2.44</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2.</td>
<td>B(a)P+150 mg/kg body wt of <em>Hippophae</em> extract</td>
<td>16</td>
<td>94</td>
<td>2.31±1.58&lt;sup&gt;d&lt;/sup&gt;</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>3.</td>
<td>B(a)P+300 mg/kg body wt of <em>Hippophae</em> extract</td>
<td>17</td>
<td>100</td>
<td>1.71±1.05&lt;sup&gt;d&lt;/sup&gt;</td>
<td>27</td>
<td>29</td>
</tr>
</tbody>
</table>

Values are mean±S.D of 15-20 animals in each group.

Statistical significance — <sup>d</sup> (p = 0.001) when compared with the control group.
Effect of *Hippophae rhamnoides* hydroalcoholic extract on the Phase I and Phase II metabolizing enzymes

![Graph showing the effect of two different doses of *Hippophae rhamnoides* hydroalcoholic extract on the levels of cytochrome P 450 (Cyt P 450), cytochrome b5 (Cyt b5), and on the specific activities of NADPH-cytochrome P450 reductase (Cyt P 450 R), NADH-cytochrome b5 reductase (Cyt b5 R), glutathione S-transferase (GST) and DT-diaphorase (DTD) in the liver of mice. Error bars represent standard deviation.](image)

**Figure 5**: Effect of two different doses of *Hippophae rhamnoides* (Seabuckthorn) hydroalcoholic extract, on the levels of cytochrome P 450 (Cyt P 450), cytochrome b5 (Cyt b5), and on the specific activities of NADPH-cytochrome P450 reductase (Cyt P 450 R), NADH-cytochrome b5 reductase (Cyt b5 R), glutathione S-transferase (GST) and DT-diaphorase (DTD) in the liver of mice. Error bars represent standard deviation.

**Groups**: Co: control, LD-150 mg/kg body wt of *Hippophae* extract; HD- 300 mg/kg body wt of *Hippophae* extract.

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

**Treatment duration**: 14 days.
Table 5. Modulatory influence of two different doses of *Hippophae rhamnoides* (Seabuckthorn) hydroalcoholic extract on mouse hepatic phase I and phase II drug metabolizing enzyme levels.

<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>0.630 ±0.106 (100)</td>
<td>0.222±0.047 (100)</td>
<td>0.427±0.057 (100)</td>
<td>2.80±0.36 (100)</td>
<td>1.19±0.050 (100)</td>
<td>0.023±0.002 (100)</td>
</tr>
<tr>
<td>LD- 150 mg/kg body wt Hippophae</td>
<td>0.710±0.080 (113)</td>
<td>0.222±0.031 (100)</td>
<td>0.428±0.042 (100)</td>
<td>2.20±0.164 (78)</td>
<td>1.51±0.152 b (127)</td>
<td>0.041±0.007 c (176)</td>
</tr>
<tr>
<td>HD- 300 mg/kg body wt Hippophae</td>
<td>0.720±0.072 (114)</td>
<td>0.191±0.024 (86)</td>
<td>0.414±0.027 (97)</td>
<td>1.79±0.179 e (67)</td>
<td>1.77±0.144 e (150)</td>
<td>0.045±0.002 e (193)</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 < 0.5), b (p < 0.01) and c (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.

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

Treatment duration: 14 days.
0.005) folds respectively as compared to control. There was an increase by 1.26 (P < 0.01) and 1.32 (P < 0.01) folds in the level of GSH in lower and higher doses of treatment respectively as compared to control.

A dose dependent decrease in the lipid peroxidation level by 0.71 (P < 0.01) and 0.66 (P < 0.005) folds was observed in lower and higher dose treated animals respectively as compared to control group. Only the low dose treated animals showed a significant reduction by 0.79 (P < 0.05) folds in the activity of LDH as compared to control. (Figure 6, Table 6)

5.3 Decalepis hamiltonii (Makaliberu)

5.3.1 Expt 1-Effect of Decalepis hamiltonii on DMBA induced skin papillomagenesis in Swiss albino mice

Lower dose of Decalepis hamiltonii treatment was found to be very effective in decreasing the tumor incidence by 47% where as higher dose decreased tumor incidence by 8% as compared to no decrease of tumor incidence in the control group. The mean number of papillomas/animal was 1.53 ± 1.81 (P < 0.05) in lower dose treated mice and 1.923 ± 1.26 (P < 0.05) in high dose treated mice whereas it was 5.00 ± 4.22 in the control mice. (Figure 7)

5.3.2 Expt 2-Effect of Decalepis hamiltonii on B(a)P induced forestomach papillomagenesis in swiss albino mice

There was a significant reduction in tumor multiplicity or the number of tumors/animal by 54% only in the lower dose of Decalepis hamiltonii treatment. The tumor incidence was 100% in both control and higher dose treated mice but 12.5% in lower dose treated animals. The mean number of papillomas/animal was 2.69 ± 2.02 (P < 0.001) in the lower dose of treatment, 4.67 ± 3.11 in the higher dose of treatment and it was 5.84 ± 2.14 in the control group. (Table 7)
Effect of *Hippophae rhamnoides* extract on antioxidant parameters, lipid peroxidation and lactate dehydrogenase

**Figure 6.** Effect of two different doses of *Hippophae rhamnoides* (Seabuckthorn) extract, on the reduced glutathione content (GSH) and on the specific activities of glutathione peroxidase (GPX), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), malondialdehyde formation (LP) and on the specific activity of lactate dehydrogenase (LDH) in the liver of mice. Error bars represent standard deviation.

**Groups:** Co: control LD- 150 mg/kg body wt of *Hippophae* extract; HD- 300 mg/kg body wt of *Hippophae* extract.  
 a (p < 0.05), b (p < 0.01) and c (p < 0.005) indicate significant changes against control.

**Treatment duration:** 14 days.
Table 6. Modulatory influence of two different doses of *Hippophae rhamnoides* in diet on mouse hepatic antioxidant related parameters, lipid peroxidation and lactate dehydrogenase.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (nmol GSH/g tissue)</th>
<th>SOD (U/mg protein)</th>
<th>CAT (U/mg protein)</th>
<th>GPx (nmol H₂O₂ consumed/min/mg protein)</th>
<th>GR (nmol NADPH consumed/min/mg protein)</th>
<th>LP (nmol mg protein)</th>
<th>LDH (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21.3±1.47 (100)</td>
<td>6.14±0.85 (100)</td>
<td>7.35±0.50 (100)</td>
<td>116.6±13.2 (100)</td>
<td>24.0±2.11 (100)</td>
<td>0.87±0.10 (100)</td>
<td>1.09±0.15 (100)</td>
</tr>
<tr>
<td>LD- 2.5% <em>Withania</em></td>
<td>26.8±2.08b (126)</td>
<td>9.36±0.77c (152)</td>
<td>10.9±0.53a (148)</td>
<td>185.8±29.4c (159)</td>
<td>49.4±4.04b (206)</td>
<td>0.62±0.06b (71)</td>
<td>0.86±0.12a (79)</td>
</tr>
<tr>
<td>HD- 5% <em>Withania</em></td>
<td>28.0±1.25b (132)</td>
<td>10.8±84b (177)</td>
<td>7.54±0.94 (102)</td>
<td>204.2±26.3e (175)</td>
<td>43.9±6.51b (183)</td>
<td>0.58±0.03b (66)</td>
<td>0.93±0.08 (85)</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) and b (p < 0.01) represent significant changes against control.


Treatment duration: 14 days
Effect of *Decalepis hamiltonii* extract on DMBA induced skin papillomagenesis

**Figure 7.** Effect of two different doses of *Decalepis hamiltonii* extract on DMBA induced skin papillomagenesis.

**Groups:** Control-DMBA + croton oil; LD- DMBA+ croton oil + 150 mg/kg body wt of *Decalepis* extract; HD- DMBA+ croton oil + 300 mg/kg body wt of *Decalepis* extract.

**Effective no:** 12-15 animals/group.
Table 7. Effect of two different doses of *Decalepis hamiltonii* (*Makaliberu*) hydroalcoholic extract on B(a)P-induced forestomach papillomagenesis in Swiss albino mice.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Group</th>
<th>Effective no.</th>
<th>% tumor incidence</th>
<th>No. of tumors/mouse</th>
<th>% tumor multiplicity</th>
<th>Total no. of tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>B(a)P only</td>
<td>19</td>
<td>100</td>
<td>5.84±2.14</td>
<td>100</td>
<td>111</td>
</tr>
<tr>
<td>2.</td>
<td>B(a)P+150 mg/kg body wt of <em>Decalepis</em> extract</td>
<td>16</td>
<td>87.5</td>
<td>2.69±2.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>46</td>
<td>43</td>
</tr>
<tr>
<td>3.</td>
<td>B(a)P+300 mg/kg body wt of <em>Decalepis</em> extract</td>
<td>16</td>
<td>100</td>
<td>4.67±3.11</td>
<td>80</td>
<td>73</td>
</tr>
</tbody>
</table>

Values are mean±S.D of 15-20 animals in each group.

Statistical significance – <sup>d</sup> (p = 0.001) when compared with the control group.
5.3.3 Expt 3-Effect of *Decalepis hamiltonii* on xenobiotic metabolizing enzymes and antioxidant parameters in Swiss albino mice

Among the Phase I enzymes cyt P450 showed a decrease by 0.74 ($P < 0.005$) and 0.73 ($P < 0.005$) folds in lower and higher dose of treatment respectively. An induction by 1.13, 1.05 folds was seen in the levels of cyt b5 in lower and higher dose of treatment as compared to control. An induction in the activity of both reductases cyt P450 reductase and cyt b5 reductase by 1.12 ($P < 0.05$) and 1.14 ($P < 0.01$) folds respectively was observed in higher dose of treatment. There was no significant change in the activities of cyt P450 reductase and cyt b5 reductase in lower dose of treatment, the induction being 1.06, 1.07 respectively as compared to control.

A significant elevation in GST and DTD levels were seen, the induction being 1.56 ($P < 0.01$) and 1.71 ($P < 0.01$) folds in the lower dose of treatment respectively. Higher dose induced the activities of both GST and DTD by 1.21 ($P < 0.05$) and 1.95 ($P < 0.01$) folds respectively as compared to control. (Figure 8, Table 8)

SOD, CAT, GPx and GR showed an increase in the activity by 1.24 ($P < 0.01$), 1.24, 1.27 ($P < 0.05$) and 1.27 ($P < 0.005$) in the lower dose of treatment where as the higher dose induced 0.99, 1.42 ($P < 0.05$), 1.14, 1.53 ($P < 0.005$) respectively as compared to control. A significant induction by 1.41 ($P < 0.01$) and 1.17 ($P < 0.01$) folds was seen in level of GSH at lower and higher doses of treatment respectively.

An attenuation by 0.79 ($P < 0.05$) folds was observed in the lipid peroxidation levels of the higher dose treated animals. Both the dose levels showed a decrease in LDH activity by 0.87 ($P < 0.05$) folds in lower dose and 0.86 ($P < 0.05$) in higher dose of treatment as compared to control. (Figure 9, Table 9)

5.4 *Piper longum* (Pipli)

5.4.1 Expt 1-Effect of *Piper longum* on DMBA induced skin papillomagenesis in Swiss albino mice

There was a significant decrease in the DMBA induced papillomagenesis only in the lower dose of *Piper longum* treatment. The tumor incidence was 58% and 72% in lower and higher dose treated animals where as it was 100% in the control group. The mean number of tumors/animal was 5.92 ± 3.87 in the control group whereas it
Effect of *Decalepis hamiltonii* in diet on the Phase I and Phase II metabolizing enzymes

**Figure 8.** Effect of two different doses of *Decalepis hamiltonii* in diet on the levels of cytochrome P 450 (Cyt P 450), cytochrome b5 (Cyt b5), and on the specific activities of NADPH-cytochrome P450 reductase (Cyt P 450 R), NADH-cytochrome b5 reductase (Cyt b5 R), glutathione S-transferase (GST) and DT-diaphorase (DTD) in the liver of mice. Error bars represent standard deviation.

**Groups:** Co: control, LD- 150 mg/ kg body wt of *Decalepis* extract; HD- 300 mg/kg body wt of *Decalepis* extract.

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

**Treatment duration:** 14 days.
Table 8. Modulatory influence of two different doses of Decalepis hamiltonii hydroalcoholic extract on mouse hepatic phase I and phase II drug metabolizing enzyme levels.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cyt P450</th>
<th>Cyt b5</th>
<th>Cyt P50 R</th>
<th>Cyt b5 R</th>
<th>GST</th>
<th>DTD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(nmole/mg protein)</td>
<td>(nmole of NADPH oxidized/min/mg protein)</td>
<td>(nmole of NADH oxidized/min/mg protein)</td>
<td>(nmole CDNB-GSH conjugate formed/min/mg protein)</td>
<td>(nmole of DCPIP reduced/min/mg protein)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.537±0.049 (100)</td>
<td>0.182±0.022 (100)</td>
<td>0.170±0.00 (100)</td>
<td>0.684±0.08 (100)</td>
<td>1.23±0.15 (100)</td>
<td>0.021±0.0013 (100)</td>
</tr>
<tr>
<td>LD-150 mg/kg body wt Decalepis</td>
<td>0.396±0.027 (74)</td>
<td>0.206±0.018 (113)</td>
<td>0.180±0.01 (106)</td>
<td>0.730±0.07 (107)</td>
<td>1.92±0.22 (156)</td>
<td>0.036±0.0036 (171)</td>
</tr>
<tr>
<td>HD-300mg/kg body wt Decalepis</td>
<td>0.392±0.033 (73)</td>
<td>0.192±0.044 (105)</td>
<td>0.190±0.02 (112)</td>
<td>0.962±0.14 (141)</td>
<td>1.49±0.11 (121)</td>
<td>0.041±0.005 (195)</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).

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

\(\text{CD nmole/mg protein, } \text{® J..Lmole of NADPH oxidized/min/mg protein, } \text{® J..Lmole of NADH oxidized/min/mg protein, } \text{® J..Lmole of CDNB-GSH conjugate formed/min/mg protein and } \text{® J..Lmole of DCPIP reduced/min/mg protein.}


Treatment duration: 14 days.
Effect of *Decalepis hamiltonii* extract on antioxidant parameters, lipid peroxidation and lactate dehydrogenase

![Graph showing % change in GSH, SOD, CAT, GPx, GR, LDH, and LP]

**Figure 9.** Effect of two different doses of *Decalepis hamiltonii* extract, on the reduced glutathione content (GSH) and on the specific activities of glutathione peroxidase (GPX), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), malondialdehyde formation (LP) and on the specific activity of lactate dehydrogenase (LDH) in the liver of mice. Error bars represent standard deviation.

**Groups:** Co: control, LD- 150 mg/kg body wt of *Decalepis* extract; HD- 300 mg/kg body wt of *Decalepis* extract.

- a (p < 0.05), b (p < 0.01) and c (p < 0.005) indicate significant changes against control.

**Treatment duration:** 14 days.
Table 9. Modulatory influence of two different doses of *Decalepis hamiltonii* hydroalcoholic extract on mouse hepatic antioxidant related parameters, lipid peroxidation and lactate dehydrogenase.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (nmol GSH/g tissue)</th>
<th>SOD (specific activity)</th>
<th>CAT (nmol H2O2 consumed/min/mg protein)</th>
<th>GPx (nmole of NADPH consumed/min/mg protein)</th>
<th>GR (nmole of NADPH consumed/min/mg protein)</th>
<th>LP (nmole of malondialdehyde formed/mg protein)</th>
<th>LDH (nmole/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>47.76±4.66 (100)</td>
<td>9.94±1.10 (100)</td>
<td>51.85±8.31 (100)</td>
<td>27.46±2.95 (100)</td>
<td>29.97±3.14 (100)</td>
<td>1.92±0.28 (100)</td>
<td>2.44±0.14 (100)</td>
</tr>
<tr>
<td>LD-150 Decalepis mg/kg b.wt</td>
<td>67.51±4.52b (141)</td>
<td>12.33±1.56b (124)</td>
<td>64.57±10.34 (124)</td>
<td>34.79±4.33a (127)</td>
<td>38.13±2.76c (127)</td>
<td>1.87±0.17 (97)</td>
<td>2.12±0.21a (87)</td>
</tr>
<tr>
<td>HD-300 Decalepis mg/kg b.wt</td>
<td>55.89±3.41b (117)</td>
<td>9.81±0.85a (99)</td>
<td>73.63±10.14a (142)</td>
<td>31.36±2.37 (114)</td>
<td>45.89±2.59a (153)</td>
<td>1.53±0.16a (79)</td>
<td>2.09±0.17a (86)</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.

| 1 nmole GSH/g tissue, 2 specific activity expressed as μmole/mg protein, 3 μmole H2O2 consumed/min/mg protein, 4 nmole of NADPH consumed/min/mg protein, 5 nmole malondialdehyde formed/mg protein and 6 μmole/mg protein. |


Treatment duration: 14 days.
was $1.75 \pm 2.42$ ($P < 0.05$) and $3.31 \pm 2.96$ in the lower and higher dose treated animals respectively. (Figure 10).

5.4.2 Expt 2-Effect of *Piper longum* on B(a)P induced forestomach papillomagenesis in swiss albino mice

In B(a)P induced forestomach tumorigenesis there was no decrease in tumor incidence lower dose treated animals but it was 5.9% in higher dose of treatment. Tumor multiplicity was 73% and 66% in lower and higher dose treated animals as compared to 100% tumor multiplicity in control. The mean number of tumors/mouse was $4.44 \pm 1.50$ ($P < 0.01$) in lower dose of treatment and $4.00 \pm 2.42$ ($P < 0.01$) in higher dose of treatment and it was $6.06 \pm 1.98$ in the control group (Table 10).

5.4.3 Expt 3-Effect of *Piper longum* on xenobiotic metabolizing enzymes and antioxidant parameters in Swiss albino mice

There were no significant changes in the activities of Phase I enzymes of the animals treated with *Piper longum*. There was an induction of 1.06, 1.10, 0.93 and 0.84 ($P < 0.005$) folds in the activities of cyt P450, cyt b5, cyt P450 reductase and cyt b5 reductase levels respectively in lower dose of treatment. An induction of 1.12, 1.18, 1.18 ($P < 0.01$) and 1.06 folds was seen in the levels of cytochrome P450, b5, P450 reductase and b5 reductase of the higher dose treated animals as compared to control.

A significant dose dependent elevation was seen in the levels of GST and DTD. The activities of GST and DTD were increased by 1.32 ($P < 0.01$) and 1.58 ($P < 0.005$) folds respectively in the lower dose treated animals and elevated by 1.58 ($P < 0.005$) and 1.93 ($P < 0.001$) folds respectively in the higher dose of treatment as compared to control group. (Figure 11, Table 11)

A significant elevation was seen in the activities of CAT and GR where as only the higher dose treated animals showed an induction in GPx activity. A very slight decrease though not significant was observed in SOD activity. The induction was 0.95, 2.61 ($P < 0.01$), 1.13 and 1.34 ($P < 0.05$) folds in the activities of SOD, CAT, GPx and GR. Higher dose treated animals showed an elevation by 0.99, 2.51 ($P < 0.001$), 1.20 ($P < 0.05$) and 1.31 ($P < 0.05$) folds in the levels of SOD, CAT, GPx
Effect of *Piper longum* in diet on DMBA induced skin papillomagenesis

![Diagram showing the effect of different doses of *Piper longum* in diet on DMBA-induced skin papillomagenesis.](image)

**Figure 10.** Effect of two different doses of *Piper longum* (*Pipli*) in diet on DMBA-induced skin *papillomagenesis*.

**Groups:** Control-DMBA + croton oil; LD- DMBA+ croton oil + 1.125% of *Pipli* in diet; HD- DMBA+ croton oil + 2.5% of *Pipli* in diet.

**Effective no:** 12-15 animals/group.
Table 10. Effect of two different doses of *Piper longum* (Pipli) in diet on B(a)P-induced forestomach papillomagenesis in Swiss albino mice.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Group</th>
<th>Effective no.</th>
<th>% tumor incidence</th>
<th>No. of tumors/mouse</th>
<th>% tumor multiplicity</th>
<th>Total no. of tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>B(a)P only</td>
<td>18</td>
<td>100</td>
<td>6.06±1.98</td>
<td>100</td>
<td>109</td>
</tr>
<tr>
<td>2.</td>
<td>B(a)P+1.125% Pipli in diet</td>
<td>18</td>
<td>100</td>
<td>4.44±1.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73</td>
<td>80</td>
</tr>
<tr>
<td>3.</td>
<td>B(a)P+2.5% Pipli in diet</td>
<td>17</td>
<td>94</td>
<td>4.00±2.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66</td>
<td>68</td>
</tr>
</tbody>
</table>

Values are mean±S.D of 15-20 animals in each group.

Statistical significance – <sup>b</sup> (p =0.01) when compared with the control group.
Effect of *Piper longum* in diet on the Phase I and Phase II metabolizing enzymes

*Figure 11.* Effect of two different doses of *Piper longum* (Pipli) in diet on the levels of cytochrome P450 (Cyt P 450), cytochrome b5 (Cyt b5), and on the specific activities of NADPH-cytochrome P450 reductase (Cyt P 450 R), NADH-cytochrome b5 reductase (Cyt b5 R), glutathione S-transferase (GST) and DT-diaphorase (DTD) in the liver of mice. Error bars represent standard deviation.

**Groups:** Co: control, LD- 1.125% of Pipli in diet; HD- 2.25% of Pipli in diet. b (p < 0.01) and c (p < 0.005) indicate significant changes against control.

**Treatment duration:** 14 days.
Table 11. Modulatory influence of two different doses of *Piper longum* (Pipli) in diet on mouse hepatic phase I and phase II drug metabolizing enzyme levels.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cyt P450 1</th>
<th>Cyt b5 1</th>
<th>Cyt P450 R 2</th>
<th>Cyt b5 R 3</th>
<th>GST 4</th>
<th>DTD 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.592±0.091 (100)</td>
<td>0.370±0.031 (100)</td>
<td>0.352±0.026 (100)</td>
<td>4.02±0.371 (100)</td>
<td>1.34±0.135 (100)</td>
<td>0.007±0.001 (100)</td>
</tr>
<tr>
<td>LD- 1.125% Pipli</td>
<td>0.628±0.105 (106)</td>
<td>0.406±0.026 (110)</td>
<td>0.328±0.024 (93)</td>
<td>3.38±0.261c (84)</td>
<td>1.77±0.164b (132)</td>
<td>0.011±0.002c (158)</td>
</tr>
<tr>
<td>HD-2.5% Pipli</td>
<td>0.662±0.086 (112)</td>
<td>0.436±0.028 (118)</td>
<td>0.415±0.044b (118)</td>
<td>4.26±0.286 (106)</td>
<td>2.13±0.278b (158)</td>
<td>0.013±0.003c (193)</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).

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

1 nmole/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.

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

Treatment duration: 14 days.
and GR respectively as compared to control. Only higher dose of treatment increased GSH levels by 1.49 (P < 0.01) folds as compared to control group.

No significant attenuation was seen in lipid peroxidation levels. The lower dose decreased lipid peroxidation and LDH activity by 0.94 and 0.68 (P < 0.005) folds and higher dose did not show any significant decrease as compared to control. (Figure 12, Table 12)

5.5 *Micrococcus paniculata* (*Aselu or asola*)

5.5.1 Expt 1-Effect of *Micrococcus paniculata* on DMBA induced skin papillomagenesis in Swiss albino mice

*Micrococcus paniculata* treatment showed a tumor incidence of 67% and 57% in the animals given lower and higher dose of treatment respectively as compared to 100% incidence of tumors in the control. The mean number of tumors/animal was 2.93 ± 2.95 and 2.48 ± 0.66 (P < 0.05) in lower and higher dose of treatment respectively whereas the mean number of papillomas/animal was 5.00 ± 4.22 in the control group. (Figure 13)

5.5.2 Expt 2-Effect of *Micrococcus paniculata* on B(a)P induced forestomach papillomagenesis in swiss albino mice

Only higher dose was effective in decreasing the tumor mutiplicity. The decrease in tumor incidence was 12.5% and 26.7% in lower and higher dose treated animals respectively as compared to no decrease in the control group. The tumor burden was 100% in the control and it was 73%, 50% in lower and higher doses of treatment respectively. The number of papillomas/animal was 3.07 ± 1.86, 2.25 ± 1.53 and 1.53 ± 1.81 (P < 0.05) in control, lower and higher dose treated animals respectively. (Table 13)

5.5.3 Expt 3-Effect of *Micrococcus paniculata* on xenobiotic metabolizing enzymes and antioxidant parameters in Swiss albino mice

A significant reduction in the activities of cyt b5 and cyt P450 reductases were observed at both dose levels of treatment. The lower doses showed an attenuation in the activities of cyt b5 and cyt P450 reductase by 0.55 (P < 0.01) and 0.84 (P < 0.05)
Effect of *Piper longum* in diet on antioxidant parameters, lipid peroxidation and lactate dehydrogenase

Figure 12. Effect of two different doses of *Piper longum* (Pipli) in diet, on the reduced glutathione content (GSH) and on the specific activities of glutathione peroxidase (GPX), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), malondialdehyde formation (LP) and on the specific activity of lactate dehydrogenase (LDH) in the liver of mice. Error bars represent standard deviation.

**Groups:** Co: control, LD- 1.125% of Pipli in diet; HD- 2.25% of Pipli in diet. 

a (p < 0.05) and b (p < 0.01) indicate significant changes against control.

**Treatment duration:** 14 days.
Table 12. Modulatory influence of two different doses of *Piper longum* (Pipli) in diet on mouse hepatic antioxidant related parameters, lipid peroxidation and lactate dehydrogenase.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (nmol GSH/g tissue)</th>
<th>SOD (nMol H2O2 consumed/min/mg protein)</th>
<th>CAT (specific activity expressed as μMol/mg protein)</th>
<th>GPx (nmol of NADPH consumed/min/mg protein)</th>
<th>GR (nMol of NADPH consumed/min/mg protein)</th>
<th>LP (nmol of malondialdehyde formed/mg protein)</th>
<th>LDH (μMol/ mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24.32±2.63 (100)</td>
<td>7.81±0.93 (100)</td>
<td>29.8±6.56 (100)</td>
<td>31.2±4.83 (100)</td>
<td>41.5±2.69 (100)</td>
<td>1.08±0.16 (100)</td>
<td>2.47±0.27 (100)</td>
</tr>
<tr>
<td>LD-1.125% Pipli</td>
<td>26.63±2.29 (109)</td>
<td>7.39±1.15 (95)</td>
<td>77.8±6.05b (261)</td>
<td>35.2±2.01 (113)</td>
<td>55.9±10.3a (134)</td>
<td>1.01±0.18 (94)</td>
<td>1.69±0.26c (68)</td>
</tr>
<tr>
<td>HD-2.5% Pipli</td>
<td>36.44±1.41b (149)</td>
<td>7.75±1.16b (99)</td>
<td>74.9±5.54b (251)</td>
<td>37.4±1.99b (120)</td>
<td>54.6±8.80a (131)</td>
<td>1.18±0.18 (109)</td>
<td>2.33±0.17 (94)</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.

1 nmol GSH/g tissue, 2 specific activity expressed as μMol/mg protein, 3 μMol H2O2 consumed/min/mg protein, 4 nmol of NADPH consumed/min/mg protein, 5 nmol malondialdehyde formed/mg protein and 6 μMol/mg protein.


Treatment duration: 14 days
Effect of *Micrococcus paniculata* extract on DMBA induced skin papillomagenesis

**Figure 13.** Effect of two different doses of *Micrococcus paniculata* (Aselu) extract on DMBA-induced skin papillomagenesis.

**Groups:** Control-DMBA + croton oil; LD- DMBA+ croton oil + 100 mg/kg body wt of Aselu HD- DMBA+ croton oil + 200 mg/kg body wt of Aselu.

**Effective no:** 12-15 animals/group.
Table 13. Effect of two different doses of *Micrococcus paniculata* (Aselu) hydroalcoholic extract on B(a)P-induced forestomach papillomagenesis in Swiss albino mice.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Group</th>
<th>Effective no.</th>
<th>% tumor incidence</th>
<th>No. of tumors/mouse</th>
<th>% tumor multiplicity</th>
<th>Total no. of tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>B(a)P only</td>
<td>15</td>
<td>100</td>
<td>3.07±1.86</td>
<td>100</td>
<td>43</td>
</tr>
<tr>
<td>2.</td>
<td>B(a)P+100 mg/kg body wt of Aselu extract</td>
<td>16</td>
<td>87.5</td>
<td>2.25±1.53</td>
<td>73</td>
<td>36</td>
</tr>
<tr>
<td>3.</td>
<td>B(a)P+200 mg/kg body wt of Aselu extract</td>
<td>15</td>
<td>73</td>
<td>1.53±1.81^a</td>
<td>50</td>
<td>23</td>
</tr>
</tbody>
</table>

Values are mean±S.D of 15-20 animals in each group.

Statistical significance – ^a (p < 0.05) when compared with the control group.
folds respectively where as higher doses decreased by 0.56 (P < 0.01) and 0.76 (P < 0.005) folds. Cyt P450 showed an increase by 1.49 (P < 0.05) folds only in the higher dose of treatment. Both the lower and higher dose levels increased cyt b5 reductase activity by 2.06 (P < 0.01) and 1.17 (P < 0.01) respectively as compared to control.

Lower dose was effective in increasing the activities of both GST and DTD by 1.43 (P < 0.005) and 1.49 (P < 0.01) folds respectively as compared to control. An induction of 1.27 (P < 0.05) and 1.23 (P < 0.05) was seen in the levels of GST and DTD respectively in the higher dose of treatment. (Figure 14, Table 14)

There was no significant elevation in the levels of antioxidant enzymes except for CAT which showed an elevation by 1.74 (P< 0.01) and 1.29 (P < 0.01) folds in lower and higher dose of treatment respectively as compared to control. GSH levels showed an increase by 1.59 (P < 0.01) and 1.96 (P < 0.01) folds in lower and higher dose of treatment respectively.

The levels of lipid peroxidation were 0.55 (P < 0.01) and 0.58 (P < 0.01) in lower and higher doses of treatment respectively as compared to control. LDH activity was also decreased by 0.75 (P < 0.05) and 0.70 (P < 0.01) folds in lower and higher dose of treatment respectively as compared to control. (Figure 15, Table 15)

5.6 Histopathology:

Plate I-  (a) Forestomach with normal epithelium 100x
    (b) Forestomach with papillomas 200x
Plate II-  (a) Skin with normal epithelium 100x
    (b) Skin with papilloma 200x

Part B: Effect of Hippophae rhamnoides on transcription factors- NF-kB and IRF-1

Among the plants investigated for their chemopreventive potential Hippophae rhamnoides was selected to study its effect on the transcription factors NF-kB and IRF-1. Hippophae rhamnoides showed a significant attenuation in the B(a)P induced forestomach papillomagenesis in Swiss albino mice. It showed a significant induction of Phase II enzymes (GST and DTD), antioxidant enzymes (SOD, CAT, GPx and
Effect of Micrococcus paniculata hydroalcoholic extract on the Phase I and Phase II metabolizing enzymes

Figure 14. Effect of two different doses of Micrococcus paniculata (Aselu) hydroalcoholic extract, on the levels of cytochrome P 450 (Cyt P 450), cytochrome b5 (Cyt b5), and on the specific activities of NADPH-cytochrome P450 reductase (Cyt P 450 R), NADH-cytochrome b5 reductase (Cyt b5 R), glutathione S-transferase (GST) and DT-diaphorase (DTD) in the liver of mice. Error bars represent standard deviation.

Groups: Co: control LD-100 mg/ kg body wt of Aselu extract; HD- 200 mg/kg body wt of Aselu extract.

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

Treatment duration: 14 days.
Table 14. Modulatory influence of two different doses of *Micrococcus paniculata* (Aselu) hydroalcoholic extract on mouse hepatic phase I and phase II drug metabolizing enzyme levels.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cyt P450 (1)</th>
<th>Cyt b5 (1)</th>
<th>Cyt P50 R (2)</th>
<th>Cyt b5 R (3)</th>
<th>GST (4)</th>
<th>DTD (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.188±0.030</td>
<td>0.394±0.041</td>
<td>0.356±0.043</td>
<td>2.10±0.40</td>
<td>2.44±0.44</td>
<td>0.021±0.0030</td>
</tr>
<tr>
<td>LD-100 mg/kg b.wt Aselu</td>
<td>0.234±0.062</td>
<td>0.216±0.053²</td>
<td>0.296±0.029³</td>
<td>4.33±0.54²</td>
<td>3.48±0.34³</td>
<td>0.032±0.0018²</td>
</tr>
<tr>
<td>HD-200 mg/kg b.wt Aselu</td>
<td>0.280±0.062²</td>
<td>0.220±0.021²</td>
<td>0.268±0.015³</td>
<td>3.71±0.28²</td>
<td>3.09±0.20³</td>
<td>0.026±0.0024²</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).

²(p < 0.05), ³(p < 0.01) and ⁴(p < 0.005) represent significant changes against control.

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


Treatment duration: 14 days.
Effect of *Micrococcus paniculata* extract on antioxidant parameters, lipid peroxidation and lactate dehydrogenase

**Figure 15.** Effect of two different doses of *Micrococcus paniculata* (Aselu) extract, on the reduced glutathione content (GSH) and on the specific activities of glutathione peroxidase (GPX), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), malondialdehyde formation (LP) and on the specific activity of lactate dehydrogenase (LDH) in the liver of mice. Error bars represent standard deviation.

**Groups:** Co: control, LD- 100 mg/kg body wt of Aselu extract; HD- 200 mg/kg body wt of Aselu extract.

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

**Treatment duration:** 14 days.
Table 15. Modulatory influence of two different doses of *Micrococcus paniculata* (Aselu) hydroalcoholic extract on mouse hepatic antioxidant related parameters, lipid peroxidation and lactate dehydrogenase.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (1)</th>
<th>SOD (2)</th>
<th>CAT (3)</th>
<th>GPx (4)</th>
<th>GR (5)</th>
<th>LP (6)</th>
<th>LDH (7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21.58±4.52 (100)</td>
<td>13.16±1.09 (100)</td>
<td>87.80±13.17 (100)</td>
<td>20.24±4.28 (100)</td>
<td>65.84±9.67 (100)</td>
<td>1.65±0.26 (100)</td>
<td>5.59±0.88 (100)</td>
</tr>
<tr>
<td>LD-100 mg/kg b.wt Aselu</td>
<td>34.38±5.63b (159)</td>
<td>15.02±1.10 (114)</td>
<td>153.2±16.58b (174)</td>
<td>24.31±3.45 (120)</td>
<td>60.44±5.66 (92)</td>
<td>0.902±0.16b (55)</td>
<td>4.19±0.74a (75)</td>
</tr>
<tr>
<td>HD-200 mg/kg b.wt Aselu</td>
<td>42.38±7.83b (196)</td>
<td>12.69±1.42 (96)</td>
<td>112.2±5.445b (129)</td>
<td>21.43±0.58 (106)</td>
<td>56.35±5.07 (86)</td>
<td>0.962±0.14b (58)</td>
<td>3.93±0.32b (70)</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) and \(^b\) (p < 0.01) 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) nmole of NADPH consumed/min/mg protein, (5) nmole malondialdehyde formed/mg protein and (6) μmole/mg protein.


Treatment duration: 14 days.
Plate 1

(a) Forestomach with normal epithelium (100x)

(b) Forestomach with papillomas (200x)
Plate II

(a) Skin with normal epithelium (100x)

(c) Skin with papillomas (200x)
GR) and decreased lipid peroxidation levels. Moreover, it is rich in vitamins like A, C and E and therefore we speculated that such plant products may be acting through cellular regulatory mechanisms either directly or indirectly to bring out the chemopreventive effects. As an example, we therefore investigated if such plant products may influence activities of certain important transcription factors like Nuclear Factor kappa B (NF-kB) and Interferon Regulatory Factor-1 (IRF-1) to further investigate these effects. NF-kB was opted because it has been shown to be upregulated as a result of oxidative stress (Pinkus et al., 1996) and there are several reports showing its down regulation by chemopreventive agents (Yamamoto et al., 1999). IRF-1 on the other hand has been shown to be an antioncogenic factor (Taniguchi et al., 1997).

5.7 Expt 1-DNA binding of NF kappaB:

Nuclear extracts of liver were subsequently checked for the expression of NF kappa B, 20 fmole of $^{32}$P labeled 45 bp NF kappa B oligonucleotide was used for binding reactions having 20 μg of nuclear extracts, 2 μg of calf thymus DNA was used as carrier DNA. The nuclear extracts were prepared from the liver of mice belonging to different groups. C- untreated control group, B-benzo(a)pyrene treated group, BH-benzo(a)pyrene + Hippophae treated group and H-only Hippophae treated group. NF kappaB specific 45 bp oligonucleotide detected three complexes and their pixel intensities were quantified (Figure 1).

Complex 1, complex 2 and complex 3 may correspond to $P^{65}/P^{65}$, $P^{50}/P^{65}$ or $P^{65}/P^{65}$ and complex 3 was a higher complex of the two proteins. There was a distinct decrease in all the three complexes of B(a)P treated mice. There was restoration of these complexes in B(a)P + Hippophae treated mice in comparison with B(a)P treated group. Hippophae alone treated group showed a significant increase in all the three complexes. Complex 1 showed a marked increase in Hippophae alone treated group. The effect of B(a)P was abrogated and resulted in restortion of all the three complexes. The plausible reasons may be- (a) Treatments were done in vivo condition wherein the earlier reports were in vitro condition.(b) Our results deviate from the earlier reports due to the fact that the duration of experimentation is longer, i.e, 14 days.

(I) EMSA showing NF-κB-DNA complex in the liver of mice. Treatments: control (C), Benzo(a)pyrene (B), Benzo(a)pyrene + Hippophae (BH) and Hippophae (H). F-Free Oligo

(II) Quantitation of NF-κB-DNA complexes 1-3.
5.8 Expt 2-DNA binding activity of IRF-1:

50 fmole of $^{32}$P labeled 24 bp (GAAAGT)$_4$ IRF-1/IRF-2 specific oligonucleotide was used for binding reactions having 20 µg of nuclear extracts, 2 µg of calf thymus DNA was used as carrier DNA. The nuclear extracts were prepared from the liver of mice pertaining to four different groups. C- untreated control group, B-benzo(a)pyrene treated group, BH-benzo(a)pyrene + Hippophae treated group and H-only Hippophae treated group. Five complexes were observed viz- complex 1, 2, 3, 4 and 5 (Figure-2) where- complexes 1, 2 and 3 may correspond to IRF-1 higher complexes and complex 4 corresponds to IRF-2 monomer and complex 5 corresponds to IRF-1 monomer. There was a decrease in complexes 1 and 2 in the nuclear extracts of mice treated with B(a)P. Further there was a significant increase in complexes 1 and 2 in extracts of Hippophae (H) treated mice. As the Hippophae (H) treatment caused as increase in the higher DNA-protein complexes, it is possible that these higher complexes of IRF-1 may be involved in the activation of some antioxidant and Phase II enzymes.
Expression of DNA binding activity of IRF-1 in the mouse liver and its induction by Hippophae treatment

(I) EMSA showing IRF-1-DNA complex in the liver of mice.

Treatments: control (C), Benzo(a)pyrene (B), Benzo(a)pyrene + Hippophae (BH) and Hippophae (H). F-Free Oligo

(II) Quantitation of IRF-1-DNA complexes 1-5