CHAPTER VIII

EFFECT OF WITHANIA SOMNIFERA ON

THE CHEMICALLY INDUCED SKIN

CARCINOGENESIS
1. Introduction

Carcinogenesis is a multistage process which was first postulated by Bereublum (69). Carcinogenesis is a three-step process which involves initiation; promotion and progression. The first stage initiation results from a single application of carcinogen. Promotion involves cellular proliferation and selective clonal expansion, and in early stage it is reversible, but becomes irreversible with time (70).

Chemopreventive agents which come under various chemical classes have been shown to inhibit initiation, and to act as blocking and suppressing agents (71). Oxygen radicals are associated in the activation of carcinogen as well as in the promotion of an initiated cell. Scavengers of O₂ radicals have been shown to inhibit the cancer causation in animals and in human trials (72). Cancer prevention could be acheived by avoidance of cancer causing substances, and by using chemopreventive agents that can inhibit the metabolism of carcinogen or causing its detoxification (73). Immuno-stimulators which can destroy the cancer cells by augmenting the immune response, inhibition of signal transduction pathway which can either inhibit the conversion of normal cells into cancerous cells, or reduce its growth capability and destroy the cells by increasing the immunocompetent cells.

This chapter mainly deals with the effect of Withania somnifera on the development of chemically induced skin carcinogenesis.
II. Materials and Methods.

II.1. Animals

Swiss albino mice (6-8 weeks old; 25g b.wt.) were used in this study.

II.2. Chemicals

The following chemicals were used in this study. Glutathione (GSH); 5-5- Dithiobis-2-nitrobenzoic acid (DTNB), Nicotinamide Adenine Dinucleotide Phosphate (NADPH) and Dimethyl sulphoxide (DMSO). Carcinogens used were Dimethyl Benzanthracene (DMBA) and 20- Methyl cholanthrene. All other chemicals used were of analytical reagent grade (Chapter II).

II.3. Determination of the effect of Withania somnifera on DMBA induced carcinogenesis.

Two groups (12 nos/group) of Swiss albino mice were used for the study. Group-1 animals were treated with Withania extract (20mg/dose/animal, i. p) for 5 consecutive days. After the last dose of the drug treatment, all the animals in both the groups were treated with a single topical application of 7-12-Dimethyl Benzanthracene (470nm in 200µl acetone/mice). After two weeks of initiation, mice received topical application of croton oil (10%, 200µl) as promoter twice weekly for 6 weeks. Group 1 animals were continued with Withania (20mg/dose/animal, i. p.) prior to 30-40 minutes of croton oil application twice weekly for 10 weeks. Number of skin papillomas was recorded (Chapter II).
II.4 Determination of the effect of *Withania somnifera* on the enzymes in the skin and liver.

The animals were sacrificed after 6 months. The dorsal skin was removed, washed thrice in cold isotonic KCl, homogenized and centrifuged at 50,000 g for 15 "in cold. The supernatant was used for the estimation of GST (74), GSH (75), GPX (81); Catalases (82) and lipid peroxides (46). The above parameters were analysed using liver also as explained in chapter II.

II.5 Determination of the effect of *Withania somnifera* on 20 Methyl Cholanthrene induced fibrosarcoma formation.

Two groups of Swiss albino mice (25g, 15 animals/group) were used for this study. Hair was removed from the dorsal side of all animals and a single dose of 20 methyl cholan- threne (200μg in 0.1ml DMSO/mice) was injected subcutaneously on the dorsal side. Group I animals received Withania extract (20mg/dose/animal, i.p) on 5 days prior to carcinogen induction and continued twice weekly for 10 weeks and group II animals were kept as controls. The animals were observed for the onset of sarcoma and the survival rate was also recorded. After 15 weeks the animals were sacrificed, liver was taken and used for the estimation of lipid peroxides (46); GSH (75) as well as GST (74) levels as described in chapter II.

III. Results

III.1 Effect of *Withania somnifera* on papilloma formation.

Effect of *Withania somnifera* on DMBA induced papilloma formation is given in table

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VIII.1. In control group all the animals developed tumour on 120th day after tumour induction while in the Withania treated group only 4 animals developed papilloma on the same day. Only 6 animals (50%) developed papillomas at the end of the experimental period (180th day). In control group average number of papillomas per animal was 11 while in Withania treated group average number of papillomas per animal was only 6 on 180th day after carcinogen injection (Table VIII. 2).

III.2  Effect of *Withania somnifera* on enzyme levels of liver and skin of papilloma induced animals.

Effect of *Withania somnifera* on enzyme levels of liver and skin of papilloma induced animals is given in table VIII.3 and 4.

a. Reduced Glutathione (GSH)

GSH content in liver and skin was drastically lowered in the control group compared to the normal (6.33 nanomoles/mg protein and 12.73 nanomoles/mg protein in liver and skin respectively). Administration of Withania extract could significantly enhance the GSH content in liver (6.33 nanomoles/mg protein) as well as in skin (8.66 nanomoles/ mg protein).

b. Glutathione - S - transferase (GST)

GST content in liver (186 n moles of CDNB/min/mg protein) and skin (301 n moles of CDNB/ min/mg protein) was drastically lowered in control group. Withania administration could significantly enhance the GST level to 323 n moles of CDNB/min/mg protein in liver and
625 n moles of CDNB/min/mg protein in skin. Normal animals showed the levels of GST of 362 n moles of CDNB/min/mg protein in liver and 749 n moles of CDNB/min/mg protein in skin.

c. Glutathione peroxidase - (GPX)

Administration of Withania extract could significantly enhance the GPX level in liver (188.09 n moles of NADPH/Min/mg protein) compared to the control group (77.89 n moles of NADPH/min/mg protein). *Withania somnifera* administration could significantly enhance the GPX in skin (82.9 n moles of NADPH/min/mg protein) compared to control (72.2 n moles of NADPH/min/mg protein).

d. Catalase

Administration of Withania could enhance the catalase level in liver (58.22 nanomoles of H$_2$O$_2$/min/mg protein) compared to the control group which showed only 28.32 nanomoles of H$_2$O$_2$/min/mg protein.

e. Lipid Peroxides

Administration of Withania could lower the level of lipid peroxide of liver (168 nanomoles of MDA/mg protein) and skin (226 nanomoles of MDA/mg protein) which was drastically elevated in the liver (282 n moles of MDA/mg protein) and skin (226 n moles of MDA/mg protein) of control group.
III.3 Effect of *Withania somnifera* on the sarcoma development.

The effect of *Withania somnifera* on the sarcoma development is given in table VIII. 5. The animals in the control group started developing sarcoma after 40 days of carcinogen administration and by 80 days all the animals developed sarcoma whereas in the Withania treated group only 3 animals developed sarcoma after 15 weeks of carcinogen administration.

III.4 Effect of *Withania somnifera* on the survival of animals.

There was only 40% of survival in the control group of animals at the end of the experiment schedule (15 weeks) while in Withania treated group there was 100% survival rate (Table VIII 7).

III.5 Effect of *Withania somnifera* on lipid peroxidation.

The elevated levels of lipid peroxide in the liver of the control group (198 nanomoles of MDA/mg protein) was significantly (P<0.01) lowered to 152 nanomoles of MDA/mg protein in the presence of Withania (Table VIII 6).

III.6 Effect of *Withania somnifera* on reduced glutathione level.

Withania treated group showed significant (P<0.01) enhancement in the liver GSH level (7.7 nanomoles/mg protein) compared to the control (3.96 nanomoles/mg protein) sarcoma bearing animals (Table VIII 6).
III.7 Effect of *Withania somnifera* on glutathione-s transferase level in the liver.

Withania administration could significantly enhance the GST level (451 nanomoles/min/mg protein) compared to control (205 nanomoles/min/mg protein (P<0.001) (Table VIII). 

IV. Discussion

Present study indicates that Withania could act as an anticarcinogen in DMBA induced papilloma formation as well as 20-Methyl cholangrene induced fibrosarcoma formation both in terms of incidence and survival of animals. There was a significant enhancement in the level of antioxidant enzymes such as GSH, GST, Catalases, and Glutathione peroxidases and inhibited lipid peroxides in Withania treated group compared to control tumour bearing animals. As this extract was found to be immunostimulatory, other possibilities such as stimulation of immune response and subsequent removal of cancer cells need to be considered. Mechanism of action of Withania extract in the inhibition of chemically induced carcinogenesis may be the antioxidant activity. Another possible mechanism of action may by the stimulation of phase II enzymes and inhibition of phase I enzyme may be responsible for its anticarcinogenic activity.
Table VIII - 1

Effect of Withania somnifera on Papilloma Formation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days: 40</th>
<th>80</th>
<th>120</th>
<th>160</th>
<th>180</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12/12</td>
</tr>
<tr>
<td>Treated</td>
<td>Nil</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>6</td>
<td>6/12</td>
</tr>
</tbody>
</table>

Swiss albino mice were treated with Dimethyl Benzantracene (DBMA) (470 nm) and 10% croton oil twice weekly for 6 weeks. Treated group received Withania extract (20mg/dose animal i. p.) for 5 consecutive days before initiation and continued twice weekly for 10 weeks. Number of animals developing papillomas was recorded.
Table VIII - 2

Effect of *Withania somnifera* on the mean number of Papilloma

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days : -</th>
<th>40</th>
<th>80</th>
<th>120</th>
<th>160</th>
<th>180</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>2</td>
<td>4</td>
<td>7</td>
<td>9</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Treated</td>
<td></td>
<td>Nil</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Swiss albino mice were treated with 7-12 -Dimethyl Benzantracene (DBMA) (470 nm) and 10% croton oil twice weekly for 6 weeks. Treated group received *Withania* extract (20mg/dose animal i. p.) for 5 consecutive days before initiation and continued twice weekly for 10 weeks. Number of animals developing papillomas/animal was recorded.
Table VIII - 3

Effect of *Withania somnifera* on enzyme levels (Liver)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GSH Nano moles/mg protein</th>
<th>GST Nano moles of CDNB/min/mg protein</th>
<th>Glutathione Peroxidase Nano moles of NADPH/min/mg protein</th>
<th>Catalases Nanomoles of H$_2$O$_2$ decomposed/min/mg protein</th>
<th>Lipid peroxide Nano moles of MDA/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>6.33 ± 0.15</td>
<td>362 ± 0.09</td>
<td>99.21 ± 3.53</td>
<td>67.59 ± 1.24</td>
<td>144 ± .87</td>
</tr>
<tr>
<td>Control</td>
<td>3.13 ± 0.23</td>
<td>186 ± 1.85</td>
<td>77.89 ± 2.15</td>
<td>28.32 ± 2.51</td>
<td>282 ± .92</td>
</tr>
<tr>
<td>Treated</td>
<td>6.05 ± 0.14*</td>
<td>323 ± 1.93*</td>
<td>88.09 ± 2.92**</td>
<td>58.21 ± 2.1*</td>
<td>168 ± .86*</td>
</tr>
</tbody>
</table>

Swiss albino mice were treated with Dimethyl Benzantracene (DBMA) (470 nm) and 10% croton oil twice weekly for 6 weeks. Treated group received *Withania* extract (20mg/dose animal i. p.) for 5 consecutive days before initiation and continued twice weekly for 10 weeks.

*P < 0.001

**P < 0.01
Table VIII - 4

Effect of *Withania somnifera* on enzyme levels (SKIN)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GSH Nano moles/mg protein</th>
<th>GST Nano moles of CDNB/min/mg protein</th>
<th>Glutathione Peroxidase Nano moles of NADPH/min/mg protein</th>
<th>Catalases Nanomoles of H$_2$O$_2$ decomposed/min/mg protein</th>
<th>Lipid peroxide Nano moles of MDA/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>12.73 ± 1.59</td>
<td>749 ± 2</td>
<td>91.21 ± 88</td>
<td>93.6 ± 1.5</td>
<td>166 ± 1.2</td>
</tr>
<tr>
<td>Control</td>
<td>6.05 ± 1.01</td>
<td>301 ± 3</td>
<td>72.2 ± 93</td>
<td>38.3 ± 2.4</td>
<td>398 ± 1.4</td>
</tr>
<tr>
<td>Treated</td>
<td>8.66 ± 0.98*</td>
<td>625 ± 4*</td>
<td>82.9 ± 97**</td>
<td>79.6 ± 2.1*</td>
<td>226 ± 1.5*</td>
</tr>
</tbody>
</table>

Swiss albino mice were treated with Dimethyl Benzanthracene (DBMA) (470 nm) and 10% croton oil twice weekly for 6 weeks. Treated group received *Withania* extract (20 mg/dose animal i. p.) for 5 consecutive days before initiation and continued twice weekly for 10 weeks.

*P < 0.001

**P < 0.01
Table VIII - 5

Effect of *Withania somnifera* on Sarcoma development

<table>
<thead>
<tr>
<th>Treatment</th>
<th>40th day</th>
<th>60th day</th>
<th>80th day</th>
<th>105th day</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-methyl cholanthrene</td>
<td>8/15</td>
<td>8/15</td>
<td>15/15</td>
<td>15/15</td>
<td>15/15</td>
</tr>
<tr>
<td>20-methyl cholanthrene + Withania</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3/15</td>
<td>3/15</td>
</tr>
</tbody>
</table>

A single dose of 20-methyl cholanthrene (200μg/0.1ml/mouse) was injected subcutaneously on the dorsal skin. *Withania* was given i. p. (20mg/dose/animal) 5 doses prior to carcinogen administration and continued twice weekly for 10 weeks.
### Table VIII - 6

**Effect of *Withania somnifera* on enzyme analysis in Methyl cholangthrene treated animals (Liver)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GSH (Nano moles/mg protein)</th>
<th>GST (Nano moles of CDNB/min/mg protein)</th>
<th>Lipid peroxide (Nano moles of MDA/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>6.33 ± 0.48</td>
<td>365 ± 3.9</td>
<td>144 ± 6.7</td>
</tr>
<tr>
<td>20-methyl cholangthrene</td>
<td>3.96 ± 0.23</td>
<td>205 ± 4.2</td>
<td>196 ± 6.2</td>
</tr>
<tr>
<td>20-methyl cholangthrene + Withania</td>
<td>7.7 ± 0.51**</td>
<td>451 ± 5.6*</td>
<td>152 ± 4.1**</td>
</tr>
</tbody>
</table>

*P < 0.001

**P < 0.01

A single dose of 20-methyl cholangthrene (200μg/0.1ml/mouse) was injected subcutaneously on the dorsal side. *Withania* was given i. p. (20mg/dose/animal) 5 doses prior to carcinogen administration and continued twice weekly for 10 weeks.
Table VIII -7

Effect of *Withania somnifera* on the survival of Methyl Cholanthrene induced sarcoma

<table>
<thead>
<tr>
<th>Treatment</th>
<th>60th day</th>
<th>80th day</th>
<th>105th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-methyl cholangrene</td>
<td>15/15</td>
<td>8/15</td>
<td>6/15</td>
</tr>
<tr>
<td>20-methyl cholangrene + Withania</td>
<td>15/15</td>
<td>15/15</td>
<td>15/15</td>
</tr>
</tbody>
</table>

A single dose of 20-methyl cholangrene (200µg/0.1ml/mouse) was injected subcutaneously on the dorsal skin. Withania was given i. p. (20mg/dose/animal) 5 doses prior to carcinogen administration and continued twice weekly for 10 weeks.