PART-II:
CHEMOPREVENTIVE ACTION OF
BRASSICA COMPESTRIS VAR SARASON
ON DMBA INDUCED SKIN CARCINOGENESIS
MATERIALS
AND
METHODS
SKIN TUMOR MODEL SYSTEM

Animals:

Random-bred. male Swiss albino mice (7-8 weeks, old) were used for the experiments. These animals were maintained in the animal house. Temperature 24±3°C and a light : dark exposure period of 12 hours : 12 hours). Animals housed in polypropylene cages were fed standard mice feed from Hindustan Lever Ltd., India. Tap water was provided ad libitum. Multivitamin drops were given occasionally with the feed. Tetracyclin (mixed with water) was given to the animals to prevent infections.

Chemicals:

7. 12-dimethyl benz [a] anthracene (DMBA) and croton oil were procured from Sigma Chemicals Co. (St. Louis, MO, USA).

Plant material and preparation of crude extract of Brassica compestris var sarason

The mustard seed of Brassica compestris var sarason was procured from local market. Seed were identified for their authenticity. The seeds powdered mechanically were extracted exhaustively by soxhalation with 95% of ethanol at 60°C for 12 hours thrice (12x3 hrs.). The extract was filtered and concentrated under reduced pressure, where upon viscous brown mass was obtained.
Material and Methods

Mustard seed extract, dissolved in the vehicle, (double distilled water), was given at a dose of 800 mg/kg body weight to each mouse by oral gavage.

Induction of Tumors

For the induction of tumors, the two stage protocol consisting of initiation with a single, topical application of the carcinogen, DMBA, followed by thrice weekly treatment with a promoter (croton oil) was standardised.

Animals were assorted into control and experimental groups. The animals were marked and body weight was taken. On day 3rd, the hair on the dorsal region of the body (back) was removed. On day 6th, only those animals in the resting phase of the hair cycle, were topically applied with freshly prepared DMBA (100 μg / 50 μl acetone/animal) on the shaven area of the skin by a micro pipette. After an interval of 2 weeks 0.1 ml (100 μl) croton oil (1% in the 100 μl acetone) solution was applied on the initiated area and the treatment was continued thrice a week for 16 weeks. Preparation of DMBA solution and its application on the skin was done in subdued light to avoid its photo reaction.

Experimental design:

All the animals were divided into two groups and each group was given separate treatment. The day on which DMBA was applied was taken as day 0. 16 weeks later, the experiment was terminated. The animals were then sacrificed and tumors harvested, the tumor dimensions and tumor weight were determined. The animals were fed with standard mice feed throughout the experiment and water ad libitum.
C - EXPERIMENTAL DESIGN FOR THE OBSERVATION OF MODULATORY INFLUENCE OF AN ETHANOLIC SEED EXTRACT OF *BRASSICA COMPESTRIS* ON MOUSE SKIN PAPILLOMAGENESIS

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Animals</th>
<th>DMBA</th>
<th>Croton Oil</th>
<th>Brassica Compestris Extract</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>initial effective</td>
<td>100 μg/50 μl acetone</td>
<td>100 μl of 1%</td>
<td>800 mg/kg body weight in 50 μl double distilled water</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>10 8</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Control - Double distilled water 50 μl/animal was given orally for 16 weeks</td>
</tr>
<tr>
<td>II</td>
<td>10 8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Experimental - Oral treatment of Brassica compestris extract in double distilled water (15 days before DMBA treatment and continued throughout experimental protocol).</td>
</tr>
</tbody>
</table>

Statistical analysis was performed using the Student's t-test and χ²-test to test any significant difference in the experimental and the control group.
To observe the influence of mustard seed extract of *Brassica compestris* var. sarason on mouse skin papillomagenesis, the following parameters were studied.

1. **Tumor Rate / Tumor Incidence:**

   Number of mice carrying at least one tumor is expressed as percent incidence.

   The formula for calculating Percentage of animals with papillomas is:
   \[
   \text{Percentage} = \left( \frac{\text{No. of animals with tumors}}{\text{Effective Number of Animals}} \right) \times 100
   \]

2. **Tumor Yield:**

   Total number of tumors per group and mean number of tumors per effective mouse and is calculated by using the following formula:
   \[
   \text{Mean Tumor Yield} = \frac{\text{Total Number of Tumors}}{\text{Effective Number of Animals}}
   \]

3. **Diameter of each tumor**

4. **Weight of Tumors** of each animal at the termination of the experiment.

5. **Tumor Burden:** Tumor burden represent the ratio between average number of tumors and tumor bearing animals. The formula for calculating tumor burden is:
   \[
   \text{Tumor Burden} = \frac{\text{Average Number of Tumors}}{\text{Total Number of tumor bearing animals}}
   \]

6. **Average Latent Period** i.e. time lag between the application of the promoting agent and appearance of 50% of tumors. The average latent period will be computed by multiplying the number of tumors appearing each week by the time in weeks after the application of
the promoting agent and dividing the sum by the total number of tumors.

\[
\text{Average latent period } \frac{\sum Fx}{n}
\]

where:
F is the number of tumor appearing in each week.
X is the number of weeks.
n is the total number of tumors.

**Histopathology:**

**Fixation, dehydration, infiltration and block preparation**

The skin tumors were excised out and fixed in Bovin's fluid for 24-48 hours depending on the size of the tissue. The tissue were then dehydrated by passing through graded series of ethylalcohol (50%, 70%, 90% and 100% for one hour in each giving two changes).

These cleared tissues were placed for 5 minutes in xylene containing molten paraffin wax at 50-60º C for infiltration after giving two changes of molten paraffin (1 hour each). The tissues were embedded in fresh paraffin wax embedded tissue were sectioned (5-6μ thickness) on a microtome and spread on albumin coated glass slides. These were then dried at 35-40ºC.

**Staining Procedure:**

Sections were deparaffinized by dipping in xylene (2 changes). These slides were then passed through graded concentrations of ethyl alcohol (100%, 90%, 70%, 50% and 30%) with two changes of 2 minutes each and then kept in running water for 3 minutes. They were then stained with
Hariss Haematoxylin for 1 min and again washed in running water thoroughly. Slides were then passed through 50% and 70% ethyl alcohol and subsequently put into cosin stain (prepared in 70% ethyl alcohol). These were then passed through graded series of ethyl alcohol. [10%, 90% 100%, 100% alcohol + xylene (1:1) and finally give two changes of xylene. The slides were mounted with DPX, covered with glass cover slip and kept for drying at room temperature.

**STATISTICAL ANALYSIS:**

Statistical evaluation of data related to tumor mean per mouse, average tumor weight, average latent period of tumor and body weights of animals were carried out by using students - t-test using the following formula (Ipsen and Feigl. 1970):

\[
t = \frac{X_A - X_B}{\sqrt{\left(SE_A\right)^2 + \left(SE_B\right)^2}}
\]

and degree of freedom = n1 + n2 - 2

where

- \(X_A\) = Mean control value
- \(X_B\) = Mean treated value
- \(SE_A\) = Standard error of control group
- \(SE_B\) = Standard error of treated group
- \(n1\) = Number of variables in control group, and
- \(n2\) = Number of variables in treated group

The significant values are found at the following levels:

- \(p < 0.001\)
- \(p < 0.005\)
Material and Methods

Statistical evaluation of data relating percentage tumor incidence was carried out by using the chi-square special case of 2x2 contingency tables (Bailey, 1959):

This was given by the formula: \[ X^2 = \frac{n(ad - bc)^2}{(a + b)(c + d)(a + c)(b + d)} \]

2x2 Contingency table:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage Presence of tumor</th>
<th>Percentage Absence of tumor</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>a</td>
<td>b</td>
<td>a+b</td>
</tr>
<tr>
<td>Experimental</td>
<td>c</td>
<td>d</td>
<td>c+d</td>
</tr>
<tr>
<td>Total</td>
<td>a+c</td>
<td>b+c</td>
<td>n</td>
</tr>
</tbody>
</table>

degree of freedom = c-1

where C is the number of columns in the contingency table.

Significance between control and experimental values was judged at the 0.001 level.
OBSERVATIONS
Observations

Skin Tumor Model System:

The data is summarised in tables I to VII and figures 1 to 8.

In the control group, in which a single topical application of DMBA was followed 2 weeks later by repeated applications of croton oil, skin papillomas appeared in all (100%) animals (table I, fig. 4), and the cumulative number of papillomas induced during the observation period 16 weeks was 71 (table IV, fig. 1).

The mean number of tumors per effective mouse was observed to be 8.9±1.7 (table III, fig. 6).

The average weight of tumor was observed to be 123.8 mg (table VII, fig. 8).

The number of the papillomas in the category 1-2 mm was 30 i.e. 42.25%, in 2.1-4 mm was 23 i.e. 32.39% and in the 4.1-10 mm category, there was 18 i.e. 25.35% papillomas (table IV, fig.5).

The average latent period was 7.8±0.17 weeks (table V, fig. 7) i.e. 50% of the animals develop at least one tumor by 7.8 weeks after the first application of the promoter. The tumor started appearing around the 5th week and the number of tumors continued increasing till almost the 13th week. After that no increase (table VI) in the number of tumors was noticed.

The average body weight of the animals at the termination of the experiment was (36.9±1.50 gm) (table VIII).

Morphologically the tumors could be classified into 2 broad categories (i) Peduncleated papillomas (ii) Flat papillomas.
The peduncleated papillomas were either branched or unbranched with relatively thin stalk. Fat papillomas were very broad at the base and their height was mostly less than their diameter.

All the tumors developed as a result of the treatment were benign (keratoacanthomas) containing large kertatinized masses of cells which are more or less flat in appearance.

2. Mice of the treatment group, given a continuous treatment of mustard seed extract of Brassica campestris var sarason orally at pre, peri initialional and promotional stages, showed significant reduction in the incidence of tumors i.e. 50% (p < 0.001) (table I, fig. 4) as well as the cumulative number of papillomas i.e. 9 (table VI, fig. 1) and the mean number of tumor per effective mouse 1.13±0.48 [p < 0.001] (table III fig. 6) as compared to the control group.

The average tumor weight was also significantly reduced to 5.27 mg (P < 0.001) (table VII, fig. 8).

The number of papillomas were subdivided into two different categories according to their size. The category of 1-2 mm had 6 i.e. 66.66% papillomas and the category of 2.1-4 mm recorded 3 i.e. 33.33% papillomas (table IV fig. 5).

The average latent period was increased significantly to 11.3±0.40 (table V, fig 7) as compared to the control group 7.8±0.17 (p < 0.005). The first appearance of tumor was considerably delayed (9th weeks) in the treatment group than in the control group (5th weeks) and the number of tumors continued increasingly till almost the 13th weeks. After that no increase in the number of tumor was noticed (table VI). There was no significant change in the body weight (37.5±1.29 gm) of the animals as compared to the control (table VIII).
TABLE I : THE TUMOR INCIDENCE RECORDED AFTER INITIATION BY DMBA, FOLLOWED 2 WEEKS LATER BY CROTON OIL TREATMENT, THREE TIMES WEEKLY FOR 14 WEEKS, WITH OR WITHOUT TREATMENT OF AN ETHANOLIC SEED EXTRACT OF \textit{BRASSICA COMPESTRIS}

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment &amp; Dose</th>
<th>Tumor Incidence</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>DMBA (100 μg/ 50 μl acetone) + Croton oil (100 μl of 1% conc.)</td>
<td>100%</td>
<td>CONTROL.</td>
</tr>
<tr>
<td>II</td>
<td>DMBA (100 μg/50 μl acetone) + Croton oil (100 μl of 1% conc.) + Brassica compestris extract (800 mg/kg b.w.)</td>
<td>50%*</td>
<td>TREATMENT - oral treatment of \textit{Brassica compestris} extract dissolved in double distilled water at pre-, peri- and post- initial stages continuously</td>
</tr>
</tbody>
</table>

* Significant (P < 0.001) using χ² test.
TABLE II: TUMOR BURDEN* OBSERVED AFTER INITIATION BY DMBA FOLLOWED 2 WEEKS LATER BY CROTON OIL TREATMENT, THREE TIMES WEEKLY, FOR 14 WEEKS, WITH OR WITHOUT TREATMENT OF AN ETHANOLIC SEED EXTRACT OF *BRASSICA COMPESTRIS*

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment &amp; Dose</th>
<th>Tumor Burden</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>DMBA (100 μg/50 μl acetone) + Croton oil (100 μl of 1% conc.)</td>
<td>8.9</td>
<td>CONTROL.</td>
</tr>
<tr>
<td>II</td>
<td>DMBA (100 μg/50 μl acetone) + Croton oil (100 μl of 1% conc.) + Brassica compestris extract (800 mg/kg b.w.)</td>
<td>2.3</td>
<td>TREATMENT - oral treatment of Brassica compestris extract dissolved in double distilled water at pre- peri- and post-initiaional stages continuously</td>
</tr>
</tbody>
</table>

*Tumor burden is the average number of tumors per tumor bearing mouse.*
TABLE III: TUMOR MEAN OBSERVED AFTER INITIATION BY DMBA, FOLLOWED 2 WEEKS LATER, BY CROTON OIL TREATMENT, THREE TIMES WEEKLY, FOR 14 WEEKS, WITH OR WITHOUT TREATMENT OF AN ETHANOLIC SEED EXTRACT OF BRASSICA COMPESTRIS

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment &amp; Dose</th>
<th>Tumor Mean ± S.E./mouse</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>DMBA (100 µg 50 µl acetone) + Croton oil (100 µl of 1% conc.)</td>
<td>8.9±1.7</td>
<td>CONTROL.</td>
</tr>
<tr>
<td>II</td>
<td>DMBA (100 µg/50 µl acetone) + Croton oil (100 µl of 1% conc.) + Brassica compestris extract (800 mg/kg b.w.)</td>
<td>1.13±0.48*</td>
<td>TREATMENT - oral treatment of Brassica compestris extract dissolved in double distilled water at pre-, peri- and post-initiational stages continuously</td>
</tr>
</tbody>
</table>

*Values differ significantly from control at the level of P < 0.001.
<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment &amp; Dose</th>
<th>Total number of papillomas</th>
<th>Size</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1-2 mm</td>
<td>2.1-4 mm</td>
</tr>
<tr>
<td>I</td>
<td>DMBA (100 μg/50 μl acetone) + croton oil (100 μl of 1% conc.)</td>
<td>71</td>
<td>30</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(42.25%)</td>
<td>(32.39%)</td>
</tr>
<tr>
<td>II</td>
<td>DMBA (100 μg/50 μl acetone) + Croton oil (100 μl of 1% conc.) + Brassica compestris extract (800 mg/kg b.w.)</td>
<td>9</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>
TABLE V: THE AVERAGE LATENT PERIOD OBSERVED ON INITIATION BY DMBA, FOLLOWED 2 WEEKS LATER, BY CROTON OIL TREATMENT, THREE TIMES WEEKLY, FOR 14 WEEKS, WITH OR WITHOUT TREATMENT OF AN ETHANOLIC SEED EXTRACT OF *BRASSICA COMPESTRIS*

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment &amp; Dose</th>
<th>Average Latent Period (week)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>DMBA (100 µg/50 µl acetone) + Croton oil (100 µl of 1% conc.)</td>
<td>7.8±0.17</td>
<td>CONTROL</td>
</tr>
<tr>
<td>II</td>
<td>DMBA (100 µg/50 µl acetone) + Croton oil (100 µl of 1% conc.) + Brassica compestris extract (800 mg/kg b.w.)</td>
<td>11.3±0.40*</td>
<td>TREATMENT - oral treatment of Brassica compestris extract dissolved in double distilled water at pre-, peri- and post-initiation stages continuously</td>
</tr>
</tbody>
</table>

Values given in mean ± S.E.M.
*Values differ significantly from control at the level of P < 0.005.
<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment &amp; Dose</th>
<th>Weeks</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>DMBA (100 μg/50 μl acetone) + Croton oil (100 μl of 1% conc.)</td>
<td>5 5 8 12 27 44 50 59 71 71</td>
<td>CONTROL.</td>
</tr>
<tr>
<td>II</td>
<td>DMBA (100 μg/50 μl acetone) + Croton oil (100 μl of 1% conc.) + Brassica compestris extract (800 mg/kg b.w.)</td>
<td>2 6 6 7 7 9 9 9</td>
<td>TREATMENT - oral treatment of Brassica compestris dissolved in double distilled water at pre-, peri- and post-initiation stages continuously</td>
</tr>
</tbody>
</table>
**TABLE VII: THE AVERAGE WEIGHT OF TUMOR RECORDED AFTER INITIATION BY DMBA, FOLLOWED 2 WEEKS LATER, BY CROTONE OIL TREATMENT, THREE TIMES WEEKLY, FOR 14 WEEKS, WITH OR WITHOUT TREATMENT OF AN ETHANOLIC SEED EXTRACT OF **_BRASSICA COMPESTRIS_**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment &amp; Dose</th>
<th>Average weight of a tumor</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>DMBA (100 μg/50 μl acetone) + croton oil (100 μl of 1% conc.)</td>
<td>123.8 ± 16.62 mg.</td>
<td>CONTROL</td>
</tr>
<tr>
<td>II</td>
<td>DMBA (100 μg/50 μl acetone) + Croton oil (100 μl of 1% conc.) + <em>Brassica compestris</em> extract (800 mg/kg b.w.)</td>
<td>5.27 ± 2.36 mg. *</td>
<td>TREATMENT - oral treatment of Brassica compestris dissolved in double distilled water at at pre-, peri- and post-initiation stages continuously</td>
</tr>
</tbody>
</table>

Value given in mean ± S.E.M.

*Values differ significantly from control at the level of $p < 0.001$. 
TABLE VIII: THE AVERAGE WEIGHT OF THE ANIMALS AFTER INITIATION BY DMBA, FOLLOWED 2 WEEKS LATER, BY CROTON OIL TREATMENT, THREE TIMES WEEKLY, FOR 14 WEEKS, WITH OR WITHOUT TREATMENT OF AN ETHANOLIC SEED EXTRACT OF *BRASSICA COMPESTRIS*

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment &amp; Dose</th>
<th>Average body weight</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>DMBA (100 µg/50 µl acetone) + croton oil (100 µl of 1% conc.)</td>
<td>36.9±1.50 g</td>
<td>CONTROL</td>
</tr>
<tr>
<td>II</td>
<td>DMBA (100 µg/50 µl acetone) + Croton oil (100 µl of 1% conc.) + <em>Brassica compestris</em> extract (800 mg/kg b.w.)</td>
<td>37.5±1.29 g</td>
<td>TREATMENT - oral treatment of <em>Brassica compestris</em> dissolved in double distilled water at pre-, peri- and post-initiational stages continuously</td>
</tr>
</tbody>
</table>

Values given in mean ± S.E.M.
Figure 1: CUMULATIVE NUMBER OF PAPILLOMAS in the DMBA (100μg / 50 μl acetone) + croton oil treatment (100 μl of 1% conc.) three times weekly for 16 weeks and in the experimental group: *Brassica campestris* seed extract (800 mg/kg b.w.) orally in double distilled water given at pre-, peri- and post initiation stages + DMBA + croton oil treatment
Figure 2: TUMOR BURDEN (NUMBER OF TUMORS/TUMOR BEARING MOUSE) in the DMBA (100µg / 50 µl acetone) + croton oil treatment (100 µl of 1% conc.) three times weekly for 16 weeks and in the experimental group: *Brassica campestris* seed extract (800 mg/kg b.w.) orally in double distilled water given at pre-, peri- and post initiation stages + DMBA + croton oil treatment.
Figure 3: TUMOR INCIDENCE (PERCENTAGE OF TUMOR BEARING MICE) in the DMBA (100µg / 50 µl acetone) + croton oil treatment (100 µl of 1% conc.) three times weekly for 16 weeks and in the experimental group: *Brassica campestris* seed extract (800 mg/kg b.w.) orally in double distilled water given at pre-, peri- and post initiational stages + DMBA + croton oil treatment.
Figure 4: TUMOR INCIDENCE (PERCENTAGE OF TUMOR BEARING MICE) in the DMBA (100 μg/50 μl acetone) + croton oil treatment (100 μl of 1% conc.) three times weekly for 16 weeks (I) and in the experimental group: Brassica compestris seed extract (800 mg/kg b.w.) orally in double distilled water given at pre-, peri- and post-initiation stages + DMBA + croton oil treatment (II)
Figure 5 : PERCENTAGE OF PAPILLOMAS OF SIZE 1-2 mm, 2.1-4 mm and 4.1-10 mm in the DMBA (100 \( \mu g/50 \mu l \) acetone) + croton oil treatment (100 \( \mu l \) of 1\% conc.) three times weekly for 16 weeks(I) and in the experimental group : Brassica compestris seed extract (800 mg/kg b.w.) orally in double distilled water given at pre-, peri- and post- initiational stages + DMBA + croton oil treatment (II)
Figure 6: TUMOR MEAN (MEAN NUMBER TUMORS PER EFFECTIVE MOUSE) in the DMBA (100 µg / 50 µl acetone) + croton oil treatment (100 µl of 1% conc.) three times weekly for 16 weeks and in the experimental group: *Brassica campestris* seed extract (800 mg/kg b.w.) orally in double distilled water given at pre-, peri- and post initiation stages + DMBA + croton oil treatment
Figure 7: AVERAGE LATENT PERIOD (TIME TAKEN IN WEEKS FOR 50% OF TUMORS TO DEVELOP IN THE MICE) in the DMBA (100µg / 50 µl acetone) + croton oil treatment (100 µl of 1% conc.) three times weekly for 16 weeks and in the experimental group: *Brassica campestris* seed extract (800 mg/kg b.w.) orally in double distilled water given at pre-, peri- and post initiational stages + DMBA + croton oil treatment.
Figure 8: AVERAGE WEIGHT OF A TUMOR in the DMBA (100µg / 50 µl acetone) + croton oil treatment (100 µl of 1% conc.) three times weekly for 16 weeks and in the experimental group: *Brassica compestris* seed extract (800 mg/kg b.w.) orally in double distilled water given at pre-, peri- and post initiational stages + DMBA + croton oil treatment
Plate - 1

Normal male Swiss albino mice

Plate - 2

Normal male Swiss albino mice with hair clipped on back
Plate - 3

Mice showing large number of skin papillomas branched, flat type having broad base with height less than diameter in the control group treated with DMBA (initiator) + croton oil (promoter) for 16 weeks.

Plate - 4

Mice showing small number of skin papillomas of conspicuous size in the experimental group treated with DMBA (initiator) + croton oil (promoter) + an ethanolic extract of Brassica compestris var sarason at a dose of 800 mg/kg b.w. till the termination of the experiment (16 weeks)
Plate - 5

Male Swiss albino mice with small sized skin papilloma (one only) in the experimental group treated with DMBA (initiator) + Croton oil (promoter) + an ethanolic seed extract of *Brassica campestris* var sarason at a dose of 800 mg/kg b.w. till the termination of the experiment (16 weeks)

Plate - 6

Swiss albino mice without any skin papilloma in the experimental group treated with DMBA (initiator) + Croton oil (promoter) + an ethanolic seed extract of *Brassica campestris* var sarason at a dose of 800 mg/kg b.w. till the termination of the experiment (16 weeks)
Plate - 7

Cross section of normal skin showing epidermis (EP) keratin (K) and dermis with sebaceous glands and hair follicles

X100

Plate - 8

Section of skin papilloma showing hyperplasia (HEP), clear keratin (K) and connective tissue core (CT)

X100
Cross section of skin papilloma showing branching

X4