INHIBITORY EFFECT OF ASHOKA ON CHEMICAL CARCINOGENESIS
5.1. INTRODUCTION

Chemical carcinogens are capable of causing detrimental mutations in structural genes leading to activation or altered expression of the proto oncogenes to oncogenes and tumour suppressor genes, that underpin the carcinogenic process (290). Chemical carcinogenesis in murine skin is a stepwise process involving at least three distinct stages viz., initiation, promotion and progression (291). Chemoprevention of cancer, therefore refers to the administration of chemical agents to prevent initiation and/or promotion and/or progression that occur during the multistage process of neoplastic development (292). The inhibitory effects of various naturally occurring flavones, retinoids, antioxidants, etc., on chemical carcinogenesis has been well established (293,294).

The active principle isolated from Saffron, Ixora javanica and Nigella sativa seeds have been shown to inhibit tumour promotion in mice (84,85). In this chapter, attempt has been made to study the inhibitory effect of Saraca asoca bark and flower extracts on DMBA and 20-methylcholanthrene - induced carcinogenesis.

5.2. Materials and Methods:

Chemicals: 7,12 Dimethyl benz (a) anthracene (DMBA) was obtained from Sigma Chemical Company, St. Louis, (MO) and 20 - methylcholanthrene from ICN Pharmaceuticals, New York. Croton oil was
obtained from the seeds of Croton tiglium by light petroleum extraction. Saraca asoca bark and flowers were obtained as stated in 2.2. These were extracted and purified as described in 2.2.2.

Animals: Inbred strain of male Swiss albino mice (9-10) weeks, 18 - 20 g) obtained from our animal facility were used for the experiment. Groups of ten mice were used for each set of experiment.

5.2.1. Determination of inhibition of DMBA induced carcinogenesis by Saraca asoca bark and flowers:

The mice were shaved with electric shears on dorsal skin areas between the cervical and caudal portions (2.5 x 4 cm) two days before the experiment. Animals showing no regrowth of hair were used for the experiment.

40 mice were divided into 4 groups of 10 animals each and were treated topically with 450 nmol DMBA in acetone(200 µl). After two weeks croton oil (50 µl) was applied topically on the same site twice weekly for six weeks.

The treated groups received Saraca asoca bark extract (100 mg/kg body weight) 30-40 minutes prior to Croton Oil (50 µl) application. The control groups were topically applied with 0.1 ml DMSO for the same period. A small section of papilloma was fixed in 10 % formalin and histopathological analysis was carried out after staining with haematoxylin eosin.
5.2.2. Determination of the inhibitory effect of *Saraca asoca* bark and flower on 20-methylcholangrene induced tumours:

All the mice were received subcutaneous injection of a single dose of 750 nmol of 20-methylcholangrene (20 MCA) in 0.1 ml DMSO. After thirty days the treated groups received *Saraca asoca* bark or flower extract orally using a gavage at a dose of 100 mg/kg body weight (2 mg in 0.1 sterile physiological saline) for 5 days successively. The control animals were treated with sterile saline (0.1 ml) orally. Soft tissue Sarcomas appeared in a span of 12 - 15 weeks. Tumour diameter was calculated using the formula:

\[
\text{Tumour diameter (cms)} = \frac{\text{Length of tumour} + \text{Width of tumour}}{2}
\]

and the inhibitory effects evaluated.

5.3. RESULTS:

5.3.1. Effect of *Saraca asoca* bark and flower extracts on DMBA induced carcinogenesis:

5.3.1.1 Inhibition of tumour promotion.

The anti-tumour-promoting activity of *Saraca asoca* flower and bark was tested on Swiss albino mice and the results were expressed as the percentage of inhibition of the number of papillomas per mouse.
and of papilloma bearing mice. The findings of the investigation are depicted in Table-32. When a single application of DMBA was followed 12 weeks later by repeated applications of Croton oil skin papillomas appeared Plate[A] in 90% of the animals(Fig.13.). Mice which received, in addition to DMBA and Croton oil, Saraca asoca bark and flower extracts showed only 37.5 % and 62.5 % tumour incidence respectively. A significant reduction (P < 0.01 bark) (P < 0.1 flower) in the mean number of papillomas was observed in the case of the treated groups i.e. 0.36 papillomas/mouse in the Saraca asoca bark extract and 1.5 papillomas/mouse in flower extract treated groups (Table-32). Plate C indicates the inhibitory effect of bark treated and Plate D flower treated Swiss albino mice.

The average number of papillomas per untreated control group varied between 2.6 per mouse (mean 2.6/mice). The papillomas growth at the 20th week in the DMBA/Croton oil treated control group of animals is shown in (Table-32).[Plate B].

The histopathological examination of the papillomatous growth revealed diffusely infiltrating papillary projections giving a highly convoluted and involuted pattern with severe degree of epithelial hyperplasia in the epidermal layers [Plate E], depicts the convoluted and involuted pattern. ( ) indicates the hyper plastic changes in epidermal layers.

While the group treated with Saraca asoca bark extract did not reveal any papillary projection and the epidermal layers had more or
Table 32. Effect of *Saraca asoca* bark and flower extracts on DMBA induced Papillomas in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Drug</th>
<th>Mean number of Papillomas/mouse</th>
<th>P. Value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6th week</td>
<td>9th week</td>
</tr>
<tr>
<td>DMBA Croton oil</td>
<td>-</td>
<td>0.85±0.9</td>
<td>2.2±0.5</td>
</tr>
<tr>
<td>(450 nmol in 0.1 ml DMSO)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMBA Croton oil Saraca asoca bark extract</td>
<td>0.21±0.8</td>
<td>0.26±0.13</td>
<td>0.36±0.13</td>
</tr>
<tr>
<td>(450 nmol in 0.1 ml DMSO)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMBA Croton oil Saraca asoca flower extract</td>
<td>0.56±0.25</td>
<td>0.86±0.3</td>
<td>1.5±0.3</td>
</tr>
<tr>
<td>(450 nmol in 0.1 ml DMSO)</td>
<td></td>
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<td></td>
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</table>

Values are mean ± SD from 3 separate experiments using 10 male albino mice/group/expt.
Fig. 13: Inhibitory effects of *Saraca asoca* bark or flower extract on 7,12 Dimethyl benz(a) anthracene (DMBA) and Croton Oil induced papillomas in mice.

(■) Papilloma bearing control mice.

(■) Treated mice topically applied with 100mg/kg of *Saraca asoca* bark.

(■) Treated mice topically applied with 100mg/kg of *Saraca asoca* flower.
less normal appearance. The dermis showed loose ariolar connective tissue with a few intact gross sections of the hair follicles (Plate F).

In the group treated with the *Saraca asoca* flower extract lesser degree of papillary projections were noticed, but the hyperplastic changes were only mild to moderate. Keratin was present in moderate amounts (Plate G).

5.3.2. Effect of *Saraca asoca* bark and flower extract of 20 Methylcholanthrene induced soft tissue Sarcoma formation:

*Saraca asoca* bark and flower extracts also had a significant inhibitory effect on the carcinogenesis induced by 20 methylcholanthrene. It was found that all the animals which did not receive extracts, developed tumour within 12 weeks and only 10% and 17% in the bark and flower extracts of *Saraca asoca* treated animals produced tumour respectively under the same condition. Methylcholanthrene injection were invasive and the control animals died of tumour burden within 20 weeks, while the tumour incidence reached only 37.3% and 46.4% in the *Saraca asoca* bark and flower extracts treated animals under the same span of time (Fig.14).

The oral administration of active principle of *Saraca asoca* resulted in a significant (*P < .005*) reduction in the tumour diameters (Table-33).
Fig. 14: Effect of *Saraca asoca* bark or flower extract on soft tissue fibrosarcomas induced by 20 - methylcholangrene (MCA) in mice.

( ) Fibrosarcoma bearing control mice.

( ) Treated mice orally administered 100mg/kg of *Saraca asoca* bark extract.

( ) Treated mice orally administered 100kg/kg of *Saraca asoca* flower extract.
Table 33: Effect of *Saraca asoca* bark and flower extracts on 20 - Methylcholanthrene (MCA) induced Sarcomas in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Drugs</th>
<th>Quantal Survival</th>
<th>Mean Tumour Diameter (Cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>8th 12th 20th 8th 12th</td>
<td></td>
</tr>
<tr>
<td>MCA (745 nmol SC x 2 days)</td>
<td>Sterile Saline (0.1 ml (oral))</td>
<td>8/10 5/10 0/10 0.63+0.41 3.15+0.52</td>
<td></td>
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<tr>
<td>MCA (745 nmol SC x 2 days)</td>
<td><em>Saraca asoca</em> bark extract 100 mg/kg body weight oral x 5 days</td>
<td>10/10 9/10 7/10 - 0.67+0.27**</td>
<td></td>
</tr>
<tr>
<td>MCA (745 nmol SC x 2 days)</td>
<td><em>Saraca asoca</em> flower extract 100 mg/kg body weight oral x 5 days</td>
<td>10/10 7/10 6/10 - 0.80+0.5**</td>
<td></td>
</tr>
</tbody>
</table>

Sarcomas were initiated with 20 - methyl cholangrene (MCA).
*Saraca asoca* bark and flower extracts were administered 30 days after tumour initiation.
Mice treated with DMSO *Saraca asoca* bark or flower extract in absence of MCA did not develop tumours.
Values are mean ± SD from 3 experiments using 10 male albino mice/group/experiment. Significance was ** P < 0.005
5.4. DISCUSSION:

Epidemological studies in the past few years has generated great interest in determining whether specific compounds from natural products consumed by the general population are responsible for the observed reduction in cancer rates (75). The chemical analysis of the active compound from the bark and flower extract of Ashoka showed that (-)-epicatechin was responsible for the observed activity. There is growing body of evidence in literature that catechins and its derivatives can protect laboratory animals against tumorigenesis induced by chemical carcinogens (86,292,293).

It was interesting to note that topical application of bark and flower extract of Ashoka inhibited the growth of papillomas induced by the classical two stage initiation/promotion protocol. A significant delay in the onset of papilloma formation and a reduction in the mean number of papillomas was observed in the treated groups as compared to untreated controls. Reduced glutathione constitutes a major part of intracellular free thiols and is important in many biological reactions such as DNA synthesis, detoxification and the metabolic process towards the protection of cells from tumourigenesis (295). Recent reports on chemopreventive agents and antioxidants have also pointed out that catechins, (-)-epicatechins and other cinnamic acid derivatives can scavenge free radicals especially the OH radical (86) and inhibit lipid peroxidation, a critical process underlining carcinogenesis. The enhancement of reduced glutathione
(GSH) glutathione reductase (GSH - R) and glutathione-S-transferase (GSH-S) levels in cultured tumour cells (3.3.5) which act as non-critical nucleophiles may be attributed to the anticarcinogenic/antiproliferative potentials of *Saraca asoca* bark and flower, indicative of protective mechanism of these extracts against tumorigenesis probably scavenging OH radicals. Most of the anticarcinogenic compounds have been shown to inhibit chemical carcinogenesis by inhibiting epidermal ODC and/or protein kinase C inhibiting arachidonic acid metabolism and protein phosphorylation, all of which are believed to represent non-specific markers of tumour promotion.

Another exciting observation is the histopathological findings in which treated group reveal lesser degree of papillary projections and less hyperplastic changes of the epidermal layers. This suggests the reduction of the expression of the papillomas in the treated group compared with the control group.

Oral administration of 100 mg/kg of bark or flower extracts elicited considerable tumour growth inhibitory effects on fibrosarcomas induced by MCA, further supporting the antitumorigenic role of these extracts against chemically induced tumours in mice. The exact mechanism by which *Saraca asoca* bark or flower extract induces antitumorigenic/antipromoting activity in the above studies remains to be further investigated.
The above observation suggest that bark and flower extract of Ashoka may prove to be a useful antitumorigenic/antipromoting agent in cancer prevention.