CHAPTER 5

* ANTICARCINOGENICITY OF SPICES AND LEAFY VEGETABLES
INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are products of incomplete combustion of organic matter (1). These are initially released into the atmosphere but are subsequently deposited in soil and water, virtually contaminating all food sources. PAHs in food may originate from three major sources:

a) biosynthesis in plants,
b) pyrolytic formation due to cooking or processing and
c) environmental contamination.

In vegetables PAHs are end products of one or more biosynthetic pathways (2). PAH content of vegetable matter increases drastically during the process of decaying due to some catabolic process in the decaying plant (3). The high content of PAH in decaying vegetable matter also accounts for the PAH in soil and water to a large extent (4).

PAHs are also formed in substantial amounts when fatty meat or fish is cooked directly above a source of heat as in grilling or broiling and through smoking of fish and meat (5).

PAHs are formed in considerable amounts as a result of industrial activity and vegetables grown close to factories which emit a considerable quantity of smoke, have high PAH content (6).

After the early observation of high incidence of scrotal cancer in chimney sweeps by Percival Pott (7),
Yamagiwa and Ichikawa (8) induced skin cancer in the ears of rabbits by repeated applications of coal tar, in which carcinogenic compounds have been identified (9). Since then, polycyclic aromatic hydrocarbons have been proved to be carcinogenic in experimental animals and in culture. Out of the 13 PAHs identified in foods, only 6 are carcinogenic (10). Benzo(a)pyrene (BP), a potent carcinogen, is often identified in substantial amounts in foods (1).

Most of the studies on the carcinogenicity of PAHs have been directed towards the assessment of the effects of PAHs in inducing skin and lung cancers, because of the high risk involved due to the exposure to industries and smoke. BP and synthetic PAHs, such as 3-methyl cholanthrene (3MC) and 7,12-dimethylbenz(a)anthracene (DMBA) are the most frequently studied PAHs (1). Oral dosing of animals with these carcinogens leads to cancers, the most commonly encountered being leukaemias, forestomach tumours, hepatomas, pulmonary adenomas and mammary tumours (11).

The forestomach of mice is sensitive to a number of carcinogens (12). This experimental model has been found to be useful for the study of inhibitors of carcinogenesis, since this facilitates both the carcinogen and the inhibitor to come in to direct contact with the target tissue, rather than acting at a remote site (13).

Many coal tar dyes which are azo compounds are carcinogenic to animals. 4-Dimethylaminoazobenzene (butter
yellow) which was used as a food colourant is a potent hepatocarcinogen (14). 3'-Methyl, 4-dimethylaminoazobenzene (3MeDAB) is another potent carcinogen which causes hepatomas when administered orally to rats (14).

The metabolism and mechanism of action of PAHs have been widely studied. Figure 5.1 shows the general scheme of metabolism of BP and other carcinogenic PAHs. It is believed that PAHs exert their carcinogenic action by conversion to highly reactive metabolites, which covalently bind to nuclear DNA, thereby causing cellular mutations. One of the PAHs, BP is metabolised by a cytochrome P-450 dependent mixed function oxidation system and epoxide hydratase to BP 7,8-diol,9,10-epoxide (BPDE) (15). BPDE is the ultimate carcinogen of BP (15). The activation of aminoazo dyes such as 3MeDAB involves N-hydroxylation by a mixed function oxidase system of the endoplasmic reticulum followed by esterification by cytoplasmic sulphotransferases (16). These ultimate carcinogens bind to proteins and nucleic acids thereby initiating carcinogenesis (17).

The anticarcinogenic effects of plant products were evaluated in 2 models (i) squamous cell carcinoma of the stomach in Swiss mice induced by BP and (ii) hepatoma in Wistar rats induced by 3MeDAB.

MATERIALS AND METHODS

Chemicals:

3,4-Benzo(a)pyrene and 3'-methyl 4-dimethyl amino azo benzene were purchased from Sigma Chemical Company, U.S.A.
Figure 5.1: General Scheme of Metabolism of BaP and other Carcinogenic PAHs

Heavy arrows indicate pathways which may result in tumour formation. EH, epoxide hydrolase; TR, transferase.

PROCARCINOGEN → EPOXIDE → EH → DIOL → EPOXIDE (ultimate carcinogen) → binding to other macromolecules

P450

PHENOLS → TR

Sulphate + glucuronic acid conjugation

TR

EXCRETION

TUMOUR

PROMOTION

INITIATION

DNA BINDING → repair

GSH conjugates

mercapturic acids

EXCRETION
Plant products:

The following plant products were tested for their anticarcinogenic effects against BP and 3MeDAB - Cumin seeds, poppy seeds, asafoetida, turmeric, kandathipili, basil leaves, drumstick leaves, manathakkali leaves and ponnakanni leaves. The spices were powdered and leafy vegetables made into pastes as described in Chapter 1, Materials and Methods. They were administered according to the following schedule, mixed with the powdered chow: Cumin seeds, poppy seeds and turmeric - 160 mg/g diet, kandathipili 80 mg/g diet, asafoetida -40 mg/g diet and leafy vegetable paste at 600 mg/g diet.

Mouse tumour experiments, with benzo(a)pyrene:

Groups of 15 male Swiss mice, 8 weeks old and weighing 23±2.1 g, were used for all the experiments. Group 1 received standard pellet diet (negative control), while group 2 received BP alone (positive control). The other groups were fed the 9 plant products for 2 weeks after which they also received BP. BP at a dose of 0.3 mg/g diet (in groundnut oil) mixed with the diet was administered to mice thrice a week for 8 weeks (18).

The spices/leafy vegetables were administered to the mice during the period of BP administration (i.e. for 8 weeks), after which the mice were fed standard diet for six months.

The mice were then sacrificed by cervical dislocation. The stomach was fixed in an expanded state by intragastric
injection of 10% formalin. Subsequently they were split longitudinally and fixed. Tissues were embedded in paraffin wax, cut into thin sections, stained with haemotoxylin and eosin and examined for tumours.

Rat tumour experiments with 3MeDAB:

Groups of 15 male Wistar rats, 8 weeks old and weighing 85-100 g, were used for the experiment. Group I received standard diet (negative control), while group 2 received 3MeDAB alone (positive control). The other groups were fed the 9 plant products for 2 weeks after which they also received 3MeDAB. 3MeDAB (in groundnut oil), mixed in the diet at a concentration of 0.05% was given for 3 months (19). The spices/leafy vegetables were given to the rats during this period of carcinogen administration also (i.e., 3 months). After this, the animals were fed the standard diet alone for 6 months.

The rats were sacrificed by cervical dislocation. Tissues were fixed in 10% formalin, embedded in paraffin wax, cut into thin sections, stained with haematoxylin and eosin and examined for tumours (19).

The animals were weighed once a week from the commencement of the experiment for 2 months and then every two weeks for the rest of the experiment.

Statistical analysis:

Students 't' test (20) was used to analyse the significance of the results of food intakes and body weights of animals. $X^2$ test with correction for continuity (Yates) (20), was applied to evaluate the effects of plant products on BP and 3MeDAB induced carcinogenesis.
Taking into consideration the number of survivors also at the end of the experimental period, the suppression of tumourigenicity by cumin seeds, poppy seeds and basil leaves alone was statistically significant.
RESULTS

The effects of feeding plant products on food intakes and weights of mice fed BP are shown in Table 5.1. The weights of mice fed BP alone or along with the different plant products were not significantly different from those of mice fed only standard diet. The food intakes/day of different groups of mice fed different plant products + BP, or BP alone were not significantly different from that of normal mice.

BP causes squamous cell carcinoma of the stomach when administered in the diet to mice. (figure 5.2). The effects of plant products on BP-induced squamous cell carcinoma of the stomach are shown in Table 5.2.

Inhibition of BP-induced neoplasia by more than 50% was demonstrated by the following plant products - cumin seeds (83% inhibition), poppy seeds and basil leaves (62% inhibition). Kandathipili and manathakkali leaves inhibited BP-induced neoplasia by 20%, turmeric by 40%, ponnakanni leaves by 35% and drumstick leaves by 26%. The effect of asafoetida on the incidence of tumours was negligible.

The food intake/day and body weights of the rats fed the different plant products + 3MeDAB, and only standard diet are given in Table 5.3. The body weights of rats fed 3MeDAB alone were significantly different (p<0.001) from those of rats fed standard diet at 20 weeks and 32 weeks studied (from commencement of 3MeDAB administration). The body weights of rats fed different plant products and 3MeDAB were not significantly different from those of rats fed
Table 5.1: Effect of plant products on food intakes and body weights in mice fed BP

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Plant product Dose (mg spices/leafy vegetable paste/g diet)</th>
<th>Food intake (g/mouse/day)</th>
<th>Body weight ('g) at the end of 16 weeks</th>
<th>Body weight ('g) at the end of 32 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None (Negative control)</td>
<td>-</td>
<td>3.5 ± 0.3</td>
<td>29.0 ± 2.6 (15)</td>
<td>36.5 ± 4.1 (15)</td>
</tr>
<tr>
<td>2</td>
<td>BP alone (Positive control)</td>
<td>-</td>
<td>3.2 ± 0.3</td>
<td>26.5 ± 3.1 (14)</td>
<td>34.5 ± 3.8 (13)</td>
</tr>
<tr>
<td>3</td>
<td>Cumin seeds + BP</td>
<td>160</td>
<td>3.4 ± 0.2</td>
<td>28.0 ± 3.0 (15)</td>
<td>38.1 ± 3.5 (15)</td>
</tr>
<tr>
<td>4</td>
<td>Poppy seeds + BP</td>
<td>160</td>
<td>3.2 ± 0.2</td>
<td>26.8 ± 2.9 (15)</td>
<td>35.5 ± 2.9 (14)</td>
</tr>
<tr>
<td>5</td>
<td>Asafoetida + BP</td>
<td>40</td>
<td>3.2 ± 0.1</td>
<td>26.7 ± 2.4 (14)</td>
<td>34.1 ± 4.6 (13)</td>
</tr>
<tr>
<td>6</td>
<td>Kandathipili + BP</td>
<td>80</td>
<td>3.2 ± 0.1</td>
<td>27.8 ± 2.8 (14)</td>
<td>35.1 ± 4.0 (13)</td>
</tr>
<tr>
<td>7</td>
<td>Turmeric + BP</td>
<td>160</td>
<td>3.3 ± 0.2</td>
<td>27.1 ± 2.5 (15)</td>
<td>38.5 ± 4.0 (13)</td>
</tr>
<tr>
<td>8</td>
<td>Basil leaves + BP</td>
<td>600</td>
<td>3.3 ± 0.1</td>
<td>28.3 ± 3.2 (15)</td>
<td>37.6 ± 4.5 (14)</td>
</tr>
<tr>
<td>9</td>
<td>Drumstick leaves + BP</td>
<td>600</td>
<td>3.4 ± 0.2</td>
<td>28.9 ± 3.9 (15)</td>
<td>38.2 ± 5.1 (14)</td>
</tr>
<tr>
<td>10</td>
<td>Manathakkali leaves + BP</td>
<td>600</td>
<td>3.4 ± 0.1</td>
<td>29.2 ± 3.0 (14)</td>
<td>37.5 ± 4.9 (14)</td>
</tr>
<tr>
<td>11</td>
<td>Ponnakanni leaves + BP</td>
<td>600</td>
<td>3.5 ± 0.2</td>
<td>28.4 ± 3.4 (15)</td>
<td>37.0 ± 5.3 (15)</td>
</tr>
</tbody>
</table>

Values are Mean ± SD
Numbers in parentheses indicate the number of mice alive at the time of measurement.
There were no significant differences in food intake and body weight (p > 0.05) between normal mice (negative control) and the different groups fed the plant products and BP, and those fed BP alone.
Figure 5.2: Tumour tissue from stomach (of Swiss mice) showing well differentiated squamous cell carcinoma with areas of keratinisation.

Figure 5.3: Adenocarcinoma with adjacent normal liver cells.
Table 5.2: Effect of plant products on BP induced neoplasia of the stomach

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of mice at risk</th>
<th>No. of mice with tumours</th>
<th>Per cent incidence of tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal diet (Negative control)</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>BP alone (Positive control)</td>
<td>13</td>
<td>10</td>
<td>77</td>
</tr>
<tr>
<td>3.</td>
<td>Cumin seeds + BP</td>
<td>15</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>4.</td>
<td>Poppy seeds + BP</td>
<td>14</td>
<td>4</td>
<td>29</td>
</tr>
<tr>
<td>5.</td>
<td>Asafoetida + BP</td>
<td>12</td>
<td>9</td>
<td>75</td>
</tr>
<tr>
<td>6.</td>
<td>Kandathipili + BP</td>
<td>13</td>
<td>8</td>
<td>62</td>
</tr>
<tr>
<td>7.</td>
<td>Turmeric + BP</td>
<td>13</td>
<td>6</td>
<td>46</td>
</tr>
<tr>
<td>8.</td>
<td>Basil leaves + BP</td>
<td>14</td>
<td>4</td>
<td>29</td>
</tr>
<tr>
<td>9.</td>
<td>Drumstick leaves + BP</td>
<td>14</td>
<td>8</td>
<td>57</td>
</tr>
<tr>
<td>10.</td>
<td>Manathakkali leaves + BP</td>
<td>13</td>
<td>8</td>
<td>62</td>
</tr>
<tr>
<td>11.</td>
<td>Ponnakanni leaves + BP</td>
<td>14</td>
<td>7</td>
<td>50</td>
</tr>
</tbody>
</table>
Table 5.3: Effect of plant products on food intakes and body weights on rats fed 3MeDAB

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Plant product Dose (mg spices/leafy vegetable paste/g diet)</th>
<th>Food intake (g/rat/day) 20 weeks</th>
<th>Body weight (g) at the end of 20 weeks</th>
<th>Body weight (g) at the end of 32 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>None ('Negative control')</td>
<td>-</td>
<td>18.0 ± 2.0</td>
<td>203 ± 26 (15)</td>
<td>282 ± 35 (15)</td>
</tr>
<tr>
<td>2.</td>
<td>3MeDAB alone ('Positive control')</td>
<td>-</td>
<td>16.5 ± 1.8</td>
<td>155 ± 21a(13)</td>
<td>212 ± 29a(12)</td>
</tr>
<tr>
<td>3.</td>
<td>Cumin seeds + 3MeDAB</td>
<td>160</td>
<td>18.3 ± 2.0</td>
<td>210 ± 31 (14)</td>
<td>291 ± 39 (13)</td>
</tr>
<tr>
<td>4.</td>
<td>Poppy seeds + 3MeDAB</td>
<td>160</td>
<td>17.8 ± 1.9</td>
<td>198 ± 18 (13)</td>
<td>276 ± 26 (11)</td>
</tr>
<tr>
<td>5.</td>
<td>Asafoetida + 3MeDAB</td>
<td>40</td>
<td>16.9 ± 2.1</td>
<td>186 ± 25 (13)</td>
<td>270 ± 30 (10)</td>
</tr>
<tr>
<td>6.</td>
<td>Kandathipili + 3MeDAB</td>
<td>80</td>
<td>17.3 ± 2.4</td>
<td>198 ± 22 (13)</td>
<td>281 ± 33 (13)</td>
</tr>
<tr>
<td>7.</td>
<td>Turmeric + 3MeDAB</td>
<td>160</td>
<td>19.6 ± 3.0</td>
<td>215 ± 33 (15)</td>
<td>298 ± 31 (14)</td>
</tr>
<tr>
<td>8.</td>
<td>Basil leaves + 3MeDAB</td>
<td>600</td>
<td>19.8 ± 2.9</td>
<td>211 ± 32 (15)</td>
<td>301 ± 36 (12)</td>
</tr>
<tr>
<td>9.</td>
<td>Drumstick leaves + 3MeDAB</td>
<td>600</td>
<td>19.4 ± 2.6</td>
<td>208 ± 25 (14)</td>
<td>290 ± 29 (14)</td>
</tr>
<tr>
<td>10.</td>
<td>Manathakkali leaves + 3MeDAB</td>
<td>600</td>
<td>20.5 ± 2.0</td>
<td>216 ± 30 (14)</td>
<td>305 ± 25 (14)</td>
</tr>
<tr>
<td>11.</td>
<td>Ponnakanni leaves + 3MeDAB</td>
<td>600</td>
<td>18.3 ± 1.6</td>
<td>206 ± 27 (15)</td>
<td>298 ± 31 (15)</td>
</tr>
</tbody>
</table>

Values are Mean ± SD
Numbers in parentheses indicate the number of rats alive at the time of measurement
a: p < 0.001 (compared to negative control)

There were no significant differences in food intake and body weights (p > 0.05) between normal rats (negative control) and rats fed different plant products + 3MeDAB.
standard diet (negative control). The food intakes/day of the different test groups were not significantly different from that of control rats, fed only normal standard diet.

Wistar rats when fed 3MeDAB in the diet for 3 months, developed hepatomas (adenocarcinoma) after 6 months (Figure 5.3).

At the end of experiment, 11 out of 15 rats fed the azo dye were alive and 9 had developed tumours. The per cent incidence was 82%. The effects of plant products on 3MeDAB induced hepato carcinogenesis are presented in Table 5.4.

Among the spices, cumin seeds, poppy seeds, turmeric and kandathipili decreased the tumour incidence by 72%, 33%, 39% and 24% respectively. Asafoetida failed to prevent the development of hepatomas induced by 3MeDAB. All the 4 leafy vegetables decreased the incidence of tumours in rats, basil leaves by 70%, drumstick leaves by 39%, manathakkali leaves by 31% and ponnakanni leaves by 35%.

DISCUSSION

Among the plant products studied for their effects against BP-induced neoplasia, cumin seeds, poppy seeds, turmeric and basil leaves are extremely efficient, while turmeric kandathipili, ponnakanni leaves, drumstick leaves and not-significant manathakkali leaves are moderate anticarcinogens.

PAHs are present in foods and are important etiological agents in the causation of gastro-intestinal tract cancers (21). Many people are exposed to relatively high levels of
Table 5.4: Effect of plant products on 3MeDAB induced hepatomas

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of rats at risk</th>
<th>No. of rats with tumours</th>
<th>Per cent incidence of tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal diet (Negative control)</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>3MeDAB (Positive control)</td>
<td>11</td>
<td>9</td>
<td>82</td>
</tr>
<tr>
<td>3.</td>
<td>3MeDAB + Cumin seeds</td>
<td>13</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td>4.</td>
<td>3MeDAB + Poppy seeds</td>
<td>11</td>
<td>6</td>
<td>55</td>
</tr>
<tr>
<td>5.</td>
<td>3MeDAB + Asafoetida</td>
<td>10</td>
<td>8</td>
<td>80</td>
</tr>
<tr>
<td>6.</td>
<td>3MeDAB + Kandathipili</td>
<td>13</td>
<td>8</td>
<td>62</td>
</tr>
<tr>
<td>7.</td>
<td>3MeDAB + Turmeric</td>
<td>14</td>
<td>7</td>
<td>50</td>
</tr>
<tr>
<td>8.</td>
<td>3MeDAB + Basil leaves</td>
<td>12</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>9.</td>
<td>3MeDAB + Drumstick leaves</td>
<td>14</td>
<td>7</td>
<td>50</td>
</tr>
<tr>
<td>10.</td>
<td>3MeDAB + Manathakkali leaves</td>
<td>14</td>
<td>8</td>
<td>57</td>
</tr>
<tr>
<td>11.</td>
<td>3MeDAB + Ponnakanni leaves</td>
<td>15</td>
<td>8</td>
<td>53</td>
</tr>
</tbody>
</table>
carcinogenic PAHs (1). It is generally accepted that in man, PAH can cause skin tumours following topical exposure and respiratory tract tumours on inhalation (21). Populations consuming large amounts of smoked fish, nitrate, salted foods and low amounts of vitamin C as in Japan and Iceland face a high risk of gastric cancer (22). Carcinogenicity studies in animals indicate that high levels of BP in the diet cause cancers of the gastro-intestinal tract and mammary glands (11). In vitro studies indicate that human and animal tissues have the capacity to metabolise PAHs to form reactive metabolites, which bind covalently to DNA and cause mutations (1). The increased levels of GSH and GST induced by these plant products may contribute to their anticarcinogenic effects. GST and GSH detoxify the ultimate carcinogen of BP - BPDE, and may thus inhibit the deleterious effects of BP (23). Garlic, onion and orange oil have been found to increase GST activity. These compounds also significantly decreased the carcinogenicity of PAHs (24,25).

In humans, genetic deficiency in the μ isozyme of GST has been associated with increased lung cancer risk (26). Recent results have demonstrated a causal relationship between cancer risk, impaired carcinogen detoxification and mutagen sensitivity and suggest that GST μ deficiency can be a predictor of chromosomal sensitivity to classes of mutagens (26). More over GST is capable of detoxifying a wide range of carcinogens, including nitrosamines,
aflatoxins and also lipid and DNA - hydroperoxides (23). Clearly, the frequent intake of these plant products might mitigate the toxic and deleterious effects of dietary carcinogens.

Cumin seeds and basil leaves are extremely efficient inhibitors of hepatocarcinogenesis by 3MeDAB, while turmeric, poppy seeds, kandathipili, drumstick leaves, manathakkali leaves and ponnakanni leaves are moderately effective. N-hydroxylation is an important step in the metabolic activation of azo dyes, followed by N-esterification (16). The ultimate carcinogens thus produced react with GSH to give non-toxic products (27). GSTs are known in some cases to bind to azo dyes and detoxify them (16). Evidently, the same mechanism viz., increased GST and GSH levels in the liver may be operative as one of the methods for the chemopreventive potential exhibited by plant products.

It has been found that there is good correlation between generation of free radicals and carcinogenicity of amino azo dyes (28). Leafy vegetables contain substantial levels of β-carotene and vitamin E, which may scavenge free radicals produced by the azo dyes. This may also account for the inhibition of 3MeDAB induced-neoplasia in liver by leafy vegetables. Basil leaves contain eugenol as their main constituent (29). Eugenol is a potent antioxidant and anticarcinogen (30). Hirayama et al (31) have reported that eugenol was able to significantly inhibit N-hydroxylation of
aromatic amino compounds. This may account for the potent anticarcinogenic effects of basil leaves against 3MeDAB induced hepatomas.

Kuttan and Unnikrishnan (32) have reported that *Piper longum* Linn possessed antitumour activity. Our results show that kandathipili is a moderate antimutagen and anticarcinogen, but not a significant anticarcinogen.
SUMMARY

1. The effects of feeding the following plant products viz., cumin seeds, poppy seeds, asafoetida, kandathipili, turmeric, basil leaves, drumstick leaves, manathakkali leaves and ponnakanni leaves on BP induced squamous cell carcinoma in stomach of Swiss mice and on the 3 Me DAB-induced hepatomas in Wistar rats were studied.

2. Cumin seeds and basil leaves significantly inhibited BP-induced neoplasia in mice and 3 MeDAB induced hepatomas in rats. Poppy seeds inhibited BP-induced neoplasia only. Others were ineffective.
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