Cancer preventive potential of Momordica charantia L. against benzo(a)pyrene induced fore-stomach tumourigenesis in murine model system

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Bitter melon (Momordica charantia Linnæus) fruit extract was tested against 3,4 benzo(a)pyrene [B(a)P] induced fore-stomach papillomagenesis in Swiss albino mice. Extract of M. charantia in two concentrations, 2.5 and 5% of standard mice feed was used for the short-term and long-term studies. A significant decrease in tumour burden was observed in short and long-term treatment. Also in long-term treatment tumor incidence decreased to 76.92% with 2.5% dose and 69.23% with 5% dose of M. charantia. The possible mechanism involved in the cancer chemoprevention has also been discussed.

Keywords: Benzo(a)pyrene, Cancer prevention, Fore-stomach, Momordica charantia, Tumourigenesis

Bitter melon (Momordica charantia L, family: Cucurbitaceae) is a popular vegetable, and a constituent of the Indian traditional medicinal system. The mature fruits of M. charantia are also used for wound healing and for treatment of peptic ulcers in Turkish folk medicine. It exhibits strong anti-hyperglycaemic, anti-diabetic, anti-viral, anti-microbial and anti-mutagenic effects. M. charantia has proved its efficacy against mammary and skin papillomagenesis. Although, M. charantia is consumed by a large population worldwide and known for its medicinal applications, very little information is available about its chemopreventive ability against carcinogenicity. There is a need to study the preventive action of M. charantia against cancer using different model systems. In the present study, we have evaluated the short-term and long-term effect of M. charantia diet against 3,4 benzo(a)pyrene [B(a)P] induced fore-stomach tumourigenesis in Swiss albino mice. Our primary findings reveals the potent anti-carcinogenic properties of M. charantia against carcinogen-induced tumourigenesis at peri-initiation level.

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Chemicals — [B(a)P] was obtained from Sigma Chemical Co. (St. Louis, MO, USA). The peanut oil, which was used as a vehicle for carcinogen, was purchased locally and was of highest purity grade.

Preparation of extract — Fresh M. charantia fruits were bought from the local market. Seeds were removed and juice was extracted from the pulp (1 kg each time). The juice was added (w/v) in the standard feed powder according to the desired concentration (2.5% and 5%) and pellets were formed. Pellets with different concentration of M. charantia were stored in neat and clean bags and kept at 22 ± 2°C in the feed store room, animal house of the Jawaharlal Nehru University under strict sanitary conditions for further use.

Animals — Random-bred male Swiss albino mice (7-8 weeks old) were used for the present study. They were maintained in the air-conditioned animal facility (Jawaharlal Nehru University, New Delhi) with a 12-hr light/dark cycle, and provided (unless otherwise stated) with standard food pellets and tap water ad libitum. All animals were cared for according to the “Principles of Laboratory Animal Care” of the National Institute of Health (NIH, USA) and under strict adherence to Indian Animal Ethic Committee (IAEC).

Preparations of chemicals — [B(a)P] was dissolved in peanut oil and the concentration adjusted to 1 mg [B(a)P]/0.1 ml of peanut oil.

Experimental design — The experiment was done as described by Azuine and Bhide. This is a modified method originally described by Wattenberg et al. Fifteen animals in each group were assigned into the following groups (Scheme 1):

Group I — Animals were kept on a normal diet for two weeks. After that each mouse received eight doses of 1 mg [B(a)P]/0.1 ml of peanut oil (twice weekly for four weeks) by oral gavage route. This group of mice served as positive control group.

Group II — Animals were kept under 2.5% diet of M. charantia starting two weeks before, during and two weeks after the carcinogen treatment (eight doses of 1 mg [B(a)P]/0.1 ml of peanut oil) as given to Group I animals.

Group III — Animals were kept under 5% diet of M. charantia starting two weeks before, during and two weeks after the carcinogen treatment (eight doses of 1 mg [B(a)P]/0.1 ml of peanut oil) as given to Group I animals.
Group IV — Animals were kept under a 2.5% diet of *M. charantia* starting two weeks before and continued during and after the carcinogen treatment (eight doses of 1 mg [B(a)P]/0.1 ml of peanut oil) till the end of the experiment.

Group V — Animals were kept under a 5% diet of *M. charantia* starting two weeks before and continued during and after the carcinogen treatment (eight doses of 1 mg [B(a)P]/0.1 ml of peanut oil) till the end of the experiment.

Group VI — Animals were kept under a 5% diet of *M. charantia* throughout the experimental duration and served as negative control group.

Group II and Group III represent short-term treatment, while Group IV and Group V represent long term treatment. Animals were kept under observation, their body weights were noted at regular intervals and they were sacrificed at the end of 180 days. The fore-stomach was cut open longitudinally and the papillomas were counted under a dissecting microscope.

Mouse fore-stomach tumourigenesis — Table 1 depicts the result obtained by *M. charantia* supplementation on [B(a)P] induced fore-stomach tumorigenesis. No difference was noticeable in weight gain profile of animals treated with either doses of *M. charantia* diet as well as in the positive control group of mice. The control animals developed fore-stomach papillomas (100%) by [B(a)P] treatment. The mean number of papilloma/mouse (tumour burden) in this group of animals was 7.50±1.62. In contrast, in animals treated with 2.5 and 5% diet of *M. charantia*, tumor burden reduced to 1.33±0.98 and 2.00±1.68 respectively. In long-term studies the tumour burden was reduced to 1.73±0.90 and 1.77±1.59 by 2.5 and 5% diet of *M. charantia* respectively.

**Scheme 1** — Experimental design for investigating the modulatory influence of *Momordica charantia* on forestomach tumourigenesis in male Swiss albino mice. Numbers in the boxes represent age of the mice in weeks, animals on Low (2.5%) dose of *Momordica charantia*; animals on high (5%) dose of *Momordica charantia*. Twice a week treatment of benz(a)pyrene.
Table 1 — Effect of different doses of *Momordica charantia* on benzo(a)pyrene induced forestomach tumourigenesis in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Total tumour incidence (%)</th>
<th>Tumour burden (tumour/mouse)</th>
<th>% Inhibition of tumour burden</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td></td>
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<tr>
<td>Benzo(a)pyrene</td>
<td></td>
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<tr>
<td></td>
<td>22.0 ± 2.22</td>
<td>25.75 ± 1.76</td>
<td>100</td>
<td>7.50 ± 1.62</td>
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<tr>
<td><strong>Short-term</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B(a)P + <em>M. charantia</em> (2.5%)</td>
<td>21.58 ± 2.54</td>
<td>25.92 ± 1.83</td>
<td>83.33</td>
<td>1.33 ± 0.98&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>B(a)P + <em>M. charantia</em> (5%)</td>
<td>23.08 ± 2.91</td>
<td>26.75 ± 2.09</td>
<td>90.90</td>
<td>2.00 ± 1.68&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td><strong>Long-term</strong></td>
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</tr>
<tr>
<td>B(a)P + <em>M. charantia</em> (2.5%)</td>
<td>22.67 ± 2.31</td>
<td>27.50 ± 1.09</td>
<td>76.92</td>
<td>1.73 ± 0.90&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>B(a)P + <em>M. charantia</em> (5%)</td>
<td>21.17 ± 2.41</td>
<td>27.67 ± 1.61</td>
<td>69.23</td>
<td>1.77 ± 1.59&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>M. charantia</em> (5%)</td>
<td>22.50 ± 2.21</td>
<td>28.0 ± 1.12</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

<sup>d</sup>(P < 0.001), represent significant changes against control

In short term treatment groups, the tumour incidence by low and high doses of *M. charantia* was 83.33 and 90.90%, compared to 100% tumor incidence in the carcinogen treated control group. The long-term treatment of *M. charantia* reduced the tumor incidence further to 76.92 and 69.23% respectively by 2.5 and 5% diet. Long-term treatment of the high dose of *M. charantia* did not give rise to any tumor nor did the treatment have any apparent toxic effect on the survival and body weight gain profile of the animals. In the short-term study, the percentage inhibition of tumour burden was 82.23 and 76.97% by the low and high doses of *M. charantia* treatment respectively. Whereas in the long-term study, the percentage inhibition of tumour burden was 73.33 and 76.41% by low and high doses respectively.

Stomach cancer is one of the most common forms of cancer in the world. [B(a)P] employed in initiating stomach cancer is the prototypical and best characterized member of the polycyclic aromatic hydrocarbons (PAH) family of chemical carcinogens which are widespread in the environment and suspected human carcinogens<sup>10</sup>. We found that dietary administration of *M. charantia* exerted a strong chemopreventive effect against [B(a)P]-induced forestomach tumours in Swiss albino mice. [B(a)P] might have been metabolised to ultimate carcinogen 7,8-dihydroxy 9,10-epoxy,7,8,9,10-tetrahydrobenza-pyrene (anti-BPDE) which in turn induced forestomach tumourigenesis. The chemopreventive agents are found to exert their effect by modulating the carcinogen detoxifying enzymes. These enzymes have been categorized into two groups namely phase I enzymes (cytochrome P450 system) and phase II enzymes [glutathione S transferase (GST), DT diaphorase (DTD)]. Phase I enzymes metabolically activate xenobiotic/carcinogens to generate reactive electrophiles and phase II enzymes convert these electrophiles to more water soluble forms which are readily eliminated from the cells<sup>11-13</sup>. Therefore, the cancer chemopreventive potential of *M. charantia* could be ascribed to its ability to induce phase I and phase II enzymes. Also, it has been shown that mutations affecting regulation of cell cycle and apoptosis lead to carcinogenesis. As *M. charantia* is known to possess strong anti-mutagenic properties and the same might also be responsible for its cancer chemopreventive action.

It may be mentioned that *Helicobacter pylori* infection has recently been identified as an important cause of stomach cancer as a result of the consistent association between its infection and stomach cancer<sup>14</sup>. The fruit extract of *M. charantia* possesses anti-*helicobacter pylori* activity<sup>15</sup>. Therefore, cancer chemopreventive action of *M. charantia* is likely to be closely associated with its selective anti-*helicobacter pylori* activity.

In conclusion, present study strongly suggests the cancer preventive potential of *M. charantia* against fore-stomach tumourigenesis, with no toxic effect even with long-term dietary supplementation. Its chemopreventive effect could be attributed to the modulation of enzymes involved in the carcinogen metabolism. Antimutagenic, antioxidant and anti-*helicobacter pylori* properties of *M. charantia* might have also contributed to its cancer preventive action. However, further investigations are to be carried out to understand the chemopreventive effect of *M. charantia* in different tumour model systems.
References


