Anticarcinogenic Effects of Solanum lycopersicum Fruit Extract on Swiss Albino and C57 Bl Mice

RC Agrawal1*, Rachana Jain2, Wasim Raja1, M Ovais3

Abstract

In the present studies, the effect of Solanum lycopersicum extract on DMBA induced skin papillomas and B6 F10 melanomas was studied. Topical single application of DMBA at the dose of 4 mg/kg b.wt. followed by 1% croton oil for 16 weeks produced a 100% incidence of skin papillomas which started appearing from the 6th week onwards. The mice which additionally received S. lycopersicum extract at 0.6 g/kg 2 day/week for 16 weeks showed a significant decrease in the number and incidence of tumors (p< 0.05), with a delay in their appearance to week 10. Histopathological examination showed well and poorly differentiated squamous cell carcinomas in the group which received DMBA + Croton oil treatment whereas hyperkeratosis and hyperplasia were more prevalent in DMBA + Croton oil + Lycopersicum extract treated animals. In a second experiment the effect of cyclophosphamide alone and in combination with S. lycopersicum extract was studied in B16F10 melanoma tumour bearing mice. The inhibition rate was 25.9% in the cyclophosphamide treated group but this increased to 37.7% with S. lycopersicum. The life span of tumour bearing animals was also increased. Thus in two models, S. lycopersicum extract exerted protective potential against skin tumors.

Key Words: Chemoprevention - Solanum lycopersicum - skin papillomas - melanomas

Introduction

In recent years, the role of the diet in preventing the occurrence of cancer has been a popular and important area of research. The botanical name of tomato is Solanum lycopersicum Linn which belongs to Solanaceae family. Epidemiological studies suggest that diets rich in tomato may account for a reduction in the risk of several different types of cancer (Franceschi et al., 1994; Giovannucci and Clinton, 1998; Okajima et al., 1998; Michaud et al., 2000). Initial studies have suggested that cooked tomatoes (i.e., tomato sauce or paste) are a better source of available lycopene than raw tomato juice because the heating action allows the body to quickly absorb the lycopene and it is reported as a powerful antioxidant (Rao and Agarwal, 1998). A population-based case-control study found that Solanum lycopersicum-based foods was associated with a small reduction in risk for prostate cancer (Giovannucci and Clinton, 1998). High concentration of lycopene in prostate tissues resulted in a nearly three-fold increase in programmed cell damage among cancer cells. In animal studies the antitumour effect of lycopene was reported in S180 tumors (Pan et al., 2004).

The antitumor effect may be related to its immune function and antioxidative effect. Pre-treatment with lycopene had significantly reduced the frequency of MNNG-induced bone marrow micronuclei and chromosomal aberrations (Velmurugan et al., 2004). Lycopene did not caused direct maternal or developmental toxicity in rats or rabbits at dosages as high as 2000 or 3000 mg/kg/day (Christian et al., 2003).

We have therefore undertaken to study the anticarcinogenic effects in Swiss albino mice in skin papillomas and B16F10 melanoma cell line in C57 BL hybrid mice.

Materials and Methods

Animals

The study was conducted on random bred, 6-7 weeks old and 24-28 gm body weight male Swiss albino mice. and C57 BL hybrid mice. Animals were maintained under controlled conditions of temperature and light (light: dark, 12 hrs: 12 hrs.). They were provided standard mice feed and water ad libitum. The study protocol was approved by the Institutional Animal Ethical Committee. (IAEC, Ref. No.-2157/225/2006)

Chemicals

The chemicals, 7, 12-dimethylbenz (a) anthracine (DMBA) and croton oil were procured from Sigma Chemicals Co., St. Louis, USA. DMBA was dissolved at a concentration of 100 µg/100 µl in acetone. Croton oil was mixed in acetone to give a solution of 1% dilution.
Skin Bioassay Protocol

The animals were randomly divided into 7 groups. Each group comprised of 6 animals. The skin of the mice was shaved in 2cm^2 area with the help of hair removing cream in interscapular region initially and after every 2 weeks hair were removed with the help of scissors. The treatments were provided topically on shaved area up to 16 weeks using the following protocol.

Group 1 (Untreated control) No treatment
Group 2 (Vehicle control) 100 µl acetone 2 times/week up to 16 weeks
Group 3 (DMBA Alone): 104 µg DMBA was dissolved in 100 µl acetone and a single application was given
Group 4 DMBA + Croton Oil: DMBA followed by 1% Croton oil applied on skin 2 times a week up to 16 weeks
Group 5 DMBA + Croton Oil + Solanum lycopersicum (tomato) fruit extract, 100µl dose one hour before the each application of 1% croton oil
Group 6 Croton oil alone
Group 7 Tomato Extract alone

Melanoma Skin Bioassay

Melanoma cell line were obtained from National Cell science centre, Pune and maintained in our laboratory. The C57 BL hybrid mice of both sexes of the mean weight of 25 gm and 6-7 weeks old were obtained from the animal colony of our institute. They were housed in good light and temperature condition. Cell diet and water ad libitum. All the mice were kept at laboratory condition and were given standard mouse pellet diet and water ad Libitum. Those papillomas which persisted for two weeks or more weekly intervals. For final evaluation of the data, only those papillomas, which started appearing on the dorsal skin of mice were recorded at 16 weeks on the skin of animals, the tumor incidence was followed by 1% croton oil for 16 weeks produced skin papillomas, and tumor yield were significantly (p< 0.05) lower than group-5. The first tumor appeared in week 10.

With DMBA + Croton oil infiltrating nests of neoplastic squamous epithelium were observed. Tumour cells exhibited a high nuclear/cytoplasmic ratio. Moderate cytoplasm and dense clumped chromatin were also seen. Adjacent epithelium showed marked hyperkeratosis. This is suggestive of kerating squamous cell carcinoma grade II

With theDMBA + Croton oil + Lycopersicum extract most of the sections showed hyperkeratosis and hyperplasia. One section showed mild dysplasia but no malignancy was observed.

Table 2 shows the effects of cyclophosphamide alone, S. lycopersicum extract + Cyclophosphamide and S. lycopersicum alone on B16F10 melanoma tumour bearing mice. Tumour growth inhibition was evident with increase in life span in the S. lycopersicum extract + cyclophosphamide group as compared to cyclophosphamide alone and S. lycopersicum extract alone groups.

Discussion

The present study demonstrates that when S. lycopersicum extract was given one hour before the each application of DMBA at the dose of 4 mg/kg b.wt. followed by 1% croton oil for 16 weeks produced skin papillomas which started appearing from 6th week onwards. The mice which received S. lycopersicum extract showed a significant decrease in the number and incidence of tumor appearance as compared with that of the DMBA + croton oil group. When S. lycopersicum extract was topically applied at the dose of 0.6 g/kg 2 day/week for 16 weeks on the skin of animals, the tumor incidence was found to be 50% and the values for cumulative number of papillomas, and tumor yield were significantly (p< 0.05) lower than group-5. The first tumor appeared in week 10.

With theDMBA + Croton oil + Lycopersicum extract most of the sections showed hyperkeratosis and hyperplasia. One section showed mild dysplasia but no malignancy was observed.

Table 2. Effects of Solanum lycopersicum Fruit Extract on DMBA-induced Papillomas in Swiss albino Mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight</th>
<th>No. of Papillomas</th>
<th>Tumour Yield</th>
<th>Inc*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle alone</td>
<td>26.8±1.2</td>
<td>30.9±1.1</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>DMBA alone</td>
<td>26.7±1.6</td>
<td>30.3±1.9</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Croton oil alone</td>
<td>25.0±1.3</td>
<td>30.0±1.3</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>DMBA + Croton</td>
<td>24.7±2.2</td>
<td>28.8±2.8</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>DMBA+S. lycopersicum + Croton</td>
<td>26.0±1.4</td>
<td>30.9±1.4</td>
<td>17</td>
<td>2.8</td>
</tr>
<tr>
<td>S. lycopersicum ext. alone</td>
<td>26.1±1.8</td>
<td>30.1±2.4</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*Inc, Incidence

The results are summarized in Table 1. Topical single application of DMBA at the dose of 4 mg/kg b.wt. followed by 1% croton oil for 16 weeks produced skin papillomas which started appearing from 6th week onwards. The mice which received S. lycopersicum extract showed a significant decrease in the number and incidence of tumor appearance as compared with that of the DMBA + croton oil group. When S. lycopersicum extract was topically applied at the dose of 0.6 g/kg 2 day/week for 16 weeks on the skin of animals, the tumor incidence was found to be 50% and the values for cumulative number of papillomas, and tumor yield were significantly (p< 0.05) lower than group-5. The first tumor appeared in week 10.

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Table 2. Effects of Solanum lycopersicum Fruit Extract on B16F10 Melanoma Tumors

<table>
<thead>
<tr>
<th>Group</th>
<th>Volume</th>
<th>VDT (days)</th>
<th>IR%</th>
<th>ILS%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.55±0.49</td>
<td>3.5±1.2</td>
<td>-</td>
<td>23.5</td>
</tr>
<tr>
<td>CP alone</td>
<td>0.71±0.05</td>
<td>5.5±2.1</td>
<td>25.9</td>
<td>28.2</td>
</tr>
<tr>
<td>Ext. + CP</td>
<td>0.60±0.13</td>
<td>10.0±0.8</td>
<td>37.7</td>
<td>54.7</td>
</tr>
<tr>
<td>Ext. alone</td>
<td>0.85±0.13</td>
<td>3.8±1.8</td>
<td>11.6</td>
<td>32.1</td>
</tr>
</tbody>
</table>

VDT, volume doubling time; IR, inhibition rate; ILS, increase in life span
Anticarcinogenic Effects of Tomato Extract in Mouse Skin


References


Michaud DS, Feskanich D, Rimm EB, et al (2000). Intake of specific carotenoids and risk of lung cancer in 2 prospective treatment of croton oil, the incidence and the number of skin papillomas were significantly decreased. The appearance time of papillomas was also prolonged in the *S. lycopersicum* experimental groups in comparison to the carcinogen treated animals. The reduction in tumor counts may be due to effect in the promotional phase of tumorigenesis which prevent the reduction of free radicals (Huachen and Krystyn, 1991).

Antitumour effects of lycopene, an active component of *S. lycopersicum* (tomato) fruit extract was also reported is S180 transplanted tumors (Pan et al., 2004). Antioxidant and antimutagenic effect of lycopene have also been reported (Velumurugan et al., 2004). Topical application of TPA (active constituent of croton oil) has been reported to increase production of free radicals (Huachen and Krystyn, 1991). This is perhaps due to the free radical oxidative stress that has been implicated in the pathogenesis of a wide variety of clinical disorders (Hursting et al., 1999; Das, 2002; Kausar et al., 2003). The exact mechanism of anticarcinogenicity of *S. lycopersicum* extract is not fully understood but it may be possible the free radical scavenging activity of lycopene which is reported to powerful antioxidant This result is important because the tomato is an important vegetable in Indian diet and considerable important has been given for the role of tomato and lycopene in prevention of prostate and other type of cancers.