Protective Effect of Glabridine on 7, 12-Dimethylbenz [A] Anthracene - Induced Skin Carcinogenesis in Swiss Albino Mice

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1. INTRODUCTION
Skin, the largest organ of the human body, is directly exposed to number of chemical mutagens and carcinogens in a day-to-day life. Skin cancer is one of the most common cancers worldwide and accounts for 30% of all newly diagnosed cancers. Glabridin is a bio-available isoflavone isolated from Glycyrrhiza glabra L. root. Glabridin is potent antioxidant that prevents oxidative DNA fragmentation and the activation of apoptosis-associated proteins in human keratinocytes. Glabridin has been reported to possess various biological activities including strong antioxidant activity, anti-Helicobacter pylori properties, radical scavenging activities, estrogenic activity, antioxidant against LDL oxidation, and neuroprotective activity. It appears to inhibit serotonin re-uptake, cytochrome P450 3A4, 2B6, and 2C9, inflammation and melanosynthesis. By decreasing the interaction of FAK and Src, Glabridin decreases the active forms of FAK and Src, and enhanced levels of inactivated phosphorylated Src (Tyr 527). Inhibition of the FAK/Src complex by glabridin also blocked Akt activation, resulting in reduced activation of RhoA and myosin light chain phosphorylation. Therefore, we hypothesized Glabridin may be potent against skin cancer as well. The present study was therefore designed to compare the chemopreventive potential of topically applied glabridin on DMBA-induced mouse skin carcinogenesis.

2. MATERIAL AND METHODS
2.1 Chemicals
DMBA and glabridine were purchased from Sigma Aldrich Chemical Pvt. Ltd., USA. All other Chemicals used were of analytical grade.

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Abstract
Cancer is a group of complex genetic diseases of aged cells. Chemoprevention of cancer is the attempt to use natural and synthetic compounds to get involved in the early stages of cancer, before persistent disease begins. The protective effect of glabridine on 7,12-dimethylbenz[a]anthracene-induced skin carcinogenesis in Swiss albino mice was assessed in this paper. The 24 mice were divided into 4 groups each group contain 6 mice. The depliated back of groups I to IV mice was painted with DMBA (25 mg in 0.1 ml acetone/mouse) two times weekly for 8 weeks. Group I mice served as vehicle-treated control. Group II mice received no other treatment. Group III and IV mice received topical application of glabridine (50 and 100 mgkg$^{-1}$ bw) starting 1 week before the exposure to the carcinogen and continued for 25 weeks (three times/week on alternate days). In DMBA-painted mice, a 100% tumor formation with mean tumor volume and tumor burden was observed. Topical application of glabridine and dehydroleucodine completely prevented tumor incidence in DMBA-painted mice.

2.2 Animals
Male Swiss albino mice, 4–6 weeks old, weighing 15–20 g were housed in polypropylene cages and provided standard pellet diet and water ad libitum. The mice were maintained under controlled conditions of temperature and humidity with a 12 h light/dark cycle.

2.3 Experimental Design
Institutional animal ethics committee approved the experimental design. The animals were maintained as per the principles and guidelines of the ethical committee for animal care. A total number of 24 male Swiss albino mice were divided into 4 groups of 6 each. Skin carcinogenesis was developed in Swiss albino mice according to the method of Azuine and Bhide. Anne French cream was applied to remove hair (2-8cm$^{2}$) from the back of each mouse and the mice were left untreated for 2 days. Mice having no hair growth after 2 days were selected for the experimental study. The depliated back of group I mice was painted with acetone (0.1 ml/mouse) two times weekly for 8 weeks (vehicle-treated control). The depliated back of groups I to IV mice was painted with DMBA (25 mg in 0.1 ml acetone/mouse) two times weekly for 8 weeks. Group II mice received no other treatment. Group III and IV mice received topical application of glabridine (50 and 100 mgkg$^{-1}$ bw) starting 1 week before the exposure to the carcinogen and continued for 25 weeks (three times/week on alternate days) thereafter.

At the end of experimental period all the animals were sacrificed by cervical dislocation. Tumors were counted and measured with a caliper. The tumor volume was measured by the formula $V = (4/3)\pi(D1/2/D2/2/D3/2)$, where D1, D2 and D3 are the three diameters (mm) of the tumors. Body weights were recorded at weekly intervals. At sacrifice, blood was collected, processed for plasma and erythrocytes and further for biochemical assays.

3. RESULTS AND DISCUSSION
3.1 Tumor incidence, volume and burden
Topical application of TPA has been reported to increase of free radicals. Many tumor promoters have been shown to exert their action by production of oxygen species (ROS).
DMBA/TPA mediated skin cancer. In detriment effect of oxidant or reactive metabolites of carcinogens. Like SOD, CAT, GPx and GSH level were significantly decreased in erythrocytes and skin tissues of tumor bearing animals (group II) as compared to control animals. Topical application of dehydroecdysone to DMBA-painted animals did not show significant effect. Control mice treated with ferulic acid exhibited a chemopreventive effect on the experimental carcinogenesis induced by DMBA in mice. The preventive effect was evident as a significant reduction in tumor incidence, multiplicity and accompanying delay in tumor latency period. Fig 1 shows the increase in tumor volume after every 5 week interval. Fig 2 shows the decrease in the tumor burden after treatment with Glabridine.

3.2 Body weight
Body weight of control and experimental animals in each group were given in fig. 3. The body weight was significantly decreased in DMBA-treated animals as compared to control animals. Topical application of glabridine three times per week for 25 weeks significantly (p<0.001) increased the body and liver weight in DMBA-treated animals.

In the present study, chemopreventive effect of glabridine on DMBA/TPA induced carcinogenesis on mouse skin was studied, a significant decrease of the average body weights as compared to the normal control mice was observed. Carcinogenesis induced by DMBA/TPA on mice skin showed reduction in body weights which can be attributed to the initiation carcinogenesis or result of metabolic changes. However supplementation of glabridine to DMBA/TPA treated mice showed significant increase in weight when compared with DMBA/TPA treated mice.

3.3 Enzymatic and non-enzymatic antioxidants status
The activities of enzymatic antioxidants (SOD, CAT, GPx) and non-enzymatic antioxidant (GSH) level in erythrocytes and skin tissues of control and experimental animals in each group respectively. The activities of SOD, CAT, GPx and GSH level were significantly decreased in erythrocytes and skin tissues of tumor-bearing animals (group II) as compared to control animals. Topical application of glabridine three times per week for 25 weeks to DMBA-painted animals reverted the activities of enzymatic and non-enzymatic antioxidants level to near-normal range. However, topical application of dehydroecdysone to DMBA-painted animals did not show significant effect. Control mice treated with ferulic acid alone (group V) showed no significant difference in erythrocytes and skin tissue enzymatic antioxidants and non-enzymatic antioxidant status as compared to control mice (group I).

The collective action of both antioxidants and detoxification enzyme like SOD, CAT, GPx and GSH is afford protection against the detrimental effect of oxidant or reactive metabolites of carcinogens. In conclusion, our data suggest that Glabridine may be an effective chemopreventive agent and may offer protection against DMBA/TPA mediated skin cancer.

Table 1: The Tumor incidence and number

<table>
<thead>
<tr>
<th>Groups/parameters</th>
<th>Tumor incidence</th>
<th>Total number of tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group II</td>
<td>100%</td>
<td>38/6</td>
</tr>
<tr>
<td>Group III</td>
<td>28%</td>
<td>3/1</td>
</tr>
<tr>
<td>Group IV</td>
<td>16%</td>
<td>1/1</td>
</tr>
</tbody>
</table>

**Table 2: Change in biochemical Parameters in different groups**

<table>
<thead>
<tr>
<th>Groups/Parameters</th>
<th>SOD</th>
<th>CAT</th>
<th>GPx</th>
<th>GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>3.14 ± 0.02</td>
<td>0.58 ± 0.04</td>
<td>142.56 ± 4.25</td>
<td>25.45 ± 1.24</td>
</tr>
<tr>
<td>Group II</td>
<td>1.85 ± 0.04</td>
<td>0.22 ± 0.01</td>
<td>86.14 ± 5.12</td>
<td>16.45 ± 1.05</td>
</tr>
<tr>
<td>Group III</td>
<td>2.86 ± 0.02</td>
<td>0.42 ± 0.01</td>
<td>124.68 ± 6.15</td>
<td>22.85 ± 1.36</td>
</tr>
<tr>
<td>Group IV</td>
<td>3.02 ± 0.01</td>
<td>0.53 ± 0.02</td>
<td>135.27 ± 4.87</td>
<td>26.78 ± 1.08</td>
</tr>
</tbody>
</table>

**P<0.001 when compared to normal control vs disease control; ***p<0.001 when compared to disease control vs. Glabradin treated group.**

REFERENCES
6. Yu XQ, Xue CC, Zhou ZW, Li CG, Du YM, Liang J, Zhou SF. In vitro and in vivo neuroprotective effect and