



International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR)

[Impact Factor – 0.852]

Journal Homepage: www.eijppr.com

Research Article

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

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Article info

Article History:
Received 16 February 2014
Accepted 08 July 2014

Keywords:

DMBA, Glabridine, Tumor

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 depilated 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.

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 cancers¹.

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 keratinocytes².

Glabridin has been reported to possess various biological activities including strong antioxidant activity³, anti-Helicobacter pylori properties, radical scavenging activities, estrogenic activity⁴, antioxidant against LDL oxidation⁵, and neuroprotective activity⁶. It appears to inhibit serotonin re-uptake, cytochrome P450 3A4, 2B6, and 2C9, inflammation and melanogenesis⁷. 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 phosphorylation⁸. 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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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^{9,10}. Anne French cream was applied to remove hair (2-8cm²) 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 depilated back of group I mice was painted with acetone (0.1 ml/mouse) two times weekly for 8 weeks (vehicle-treated control). The depilated 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 = (4/3) [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).[11] 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.

The results of the examinations showed that co-administration of glabridine 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 dehydroleucodine 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

Groups/parameters	Tumor incidence	Total number of tumors
Group I	0	0
Group II	100%	38/6
Group III	28%	3/1
Group IV	16%	1/1

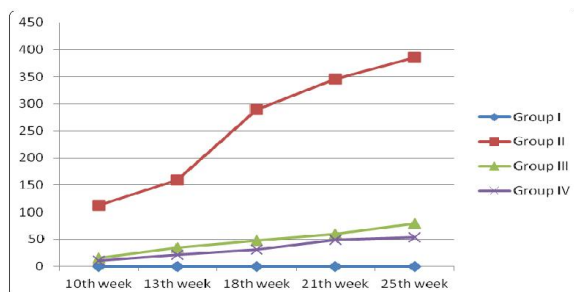


Fig.1: Tumor Volume (mm³)

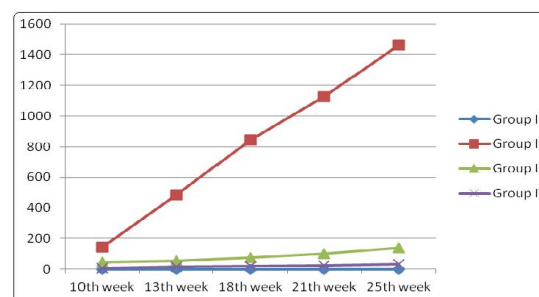


Fig. 2: Tumor Burden (mm³)

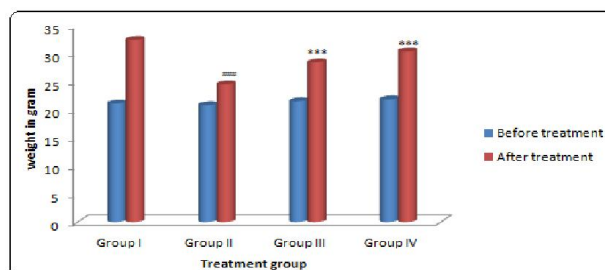


Fig. 3: Change in body weight before and after the treatment with Glabridine

$P < 0.001$ when compared to normal control vs disease control;

*** $p < 0.001$ when compared to disease control vs. Glabradin treated group.

Table 2: Change in biochemical Parameters in different groups

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

$P < 0.001$ when compared to normal control vs disease control;

*** $p < 0.001$ when compared to disease control vs. Glabradin treated group.

REFERENCES

- Vellaichamy L, Balakrishnan S, Panjamurthy K, Manoharan S, Alias LM. Chemopreventive potential of piperine in 7,12-dimethylbenz[a]anthracene-induced skin carcinogenesis in Swiss albino mice. *Environmental Toxicol Pharmacol*. 2009; 28(1): 11–18.
- Veratti E, Tiziana R, Stefania G, Luisa B, Giorgia B, Daria M. 18β-Glycyrrhetic Acid and Glabridin Prevent Oxidative DNA Fragmentation in UVB-irradiated Human Keratinocyte Cultures. *Anticancer Res*. 2011; 31(6): 2209-15.
- Belinky PA, Aviram M, Fuhrman B, Rosenblat M, Vaya J. The antioxidative effects of the isoflavan glabridin on endogenous constituents of LDL during its oxidation. *Atherosclerosis*. 1998; 137(1): 49-61.
- Somjen D, Knoll E, Vaya J, Stern N, Tamir S. Estrogen-like activity of licorice root constituents: glabridin and glabrene, in vascular tissues in vitro and in vivo. *J Steroid Biochem Mol Biol*. 2004; 91(3): 147-55.
- Michael Aviram. Interaction of oxidized low density lipoprotein with macrophages in atherosclerosis, and the antiatherogenicity of antioxidants. *Eur J Clin Chem Clin Biochem*. 1996; 34: 599-608.
- Yu XQ, Xue CC, Zhou ZW, Li CG, Du YM, Liang J, Zhou SF. In vitro and in vivo neuroprotective effect and

- mechanisms of glabridin, a major active isoflavan from *Glycyrrhiza glabra* (licorice). *Life Sci.* 2008; 82(1-2): 68-78.
7. Yokota T, Nishio H, Kubota Y, Mizoguchi M. The inhibitory effect of glabridin from licorice extracts on melanogenesis and inflammation. *Pigment Cell Res.* 1998; 11: 355-61.
 8. Hsu YL, Wu LY, Hou MF, Tsai EM, Lee JN, Liang HL, Jong YJ, Hung CH, Kuo PL. Glabridin, an isoflavan from licorice root, inhibits migration, invasion and angiogenesis of MDA-MB-231 human breast adenocarcinoma cells by inhibiting focal adhesion kinase/Rho signaling pathway. *Mol Nutr Food Res.* 2011; 55(2): 318-27.
 9. Azuine MA, Bhide SV. Chemopreventive effect of turmeric against stomach and skin tumors induced by chemical carcinogens in Swiss mice. *Nutr Cancer.* 1992;17(1):77-83.
 10. Hossein B, Ali Reza M, Hassan R, Mohamed A. Inhibitory effect of tannic acid on iron–dextran-augmented and 7,12-dimethyl benz(a)anthracene-initiated skin carcinogenesis. *The J Trace Elements in Exp Med.* 2004; 17: 21–29.
 11. Sarwat S, Aftab A, Khan N, Sharma S. Inhibition of cutaneous oxidative stress and two-stage skin carcinogenesis by *Hemidesmus indicus* (L.) in Swiss albino mice. *Indian J Exp Bio.* 2013; 41(12): 1416-23.
 12. Kaur J, Sharma M, Sharma PD, Bansal MP. Antitumor activity of Lantadenes in DMBA/TPA induced skin tumors in mice: Expression of transcription factors. *Am J Biomed Sci.* 2010; 2(1): 79-90.
 13. Gali-Muhtasib HU, Yamout SZ, Sidani MM. Tannins protect against skin tumor promotion induced by Ultraviolet-B radiation in hairless mice. *Nutr Cancer* 2000;37:73-77.
 14. Vaqar MA, Khan N, Mukhtar H. Cancer Chemoprevention by Pomegranate: Laboratory and Clinical Evidence. *Nutr Cancer.* 2009; 61(6): 811–15.