RESEARCH COMMUNICATION

Chemopreventive Action of Emblica Officinalis on Skin Carcinogenesis in Mice

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Abstract

Chemoprevention with food phytochemicals is currently regarded as one of the most important strategies for cancer control. *Emblica officinalis* (Family: Euphorbiaceae) indigenous to India, is valued for its unique tannins and flavanoids, which contain very powerful antioxidant properties. The inhibition of tumor incidences by fruit extract of this plant has been evaluated on two-stage process of skin carcinogenesis in Swiss albino mice, induced by a single application of 7, 12-dimethyabenz(a)anthrecene (100 μg/ 100 μl acetone), and two weeks later, promoted by repeated application of croton oil (1% in acetone/thrice a week) till the end of the experiment (16 weeks). The tumor incidence, tumor yield, tumor burdon and cumulative number of papillomas were found to be higher in the control (without EO treatment) as compared to experimental animals (EO treated). The differences in the values of the results of experimental groups were statistically analysed and found to be significant in comparison to the control group ($p< 0.05$). The present study demonstrates the chemopreventive potential of Emblica officinalis fruit extract on DMBA induced skin tumorigenesis in Swiss albino mice.

Key Words: Emblica officinalis - skin-carcinogenesis - chemoprevention - antioxidant enzymes - lipid peroxidation

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Introduction

A number of antioxidant nutrients and phytochemicals exhibit antimutagenic properties when administered after chemical exposure but it is unclear if this results in an anticarcinogenic effect. It is generally accepted that diets rich in fruits and vegetables reduce cancer risk, but definitive randomized controlled trials on prevention of specific cancers by antioxidants and phytochemicals have not been completed (Byers, 1999). Chemoprevention refers to the administration of chemical agents to prevent the initiational and promotional events that occur during the process of neoplastic development. This prescription approach to cancer prevention supplements the conventional prescription approach of eliminating or avoiding carcinogens in the environment, when they can be identified, and of screening for the early detection of precancerous and cancerous lesions.

Detrimental effects caused by free radicals occur as a consequence of an imbalance between formation and inactivation of these species. Oxidative damage may be involved in the pathogenesis of major diseases such as cancer, atherosclerosis (Knight, 1995), and certain neurological disorders (Jenner, 1994). The relevance of free radicals/reactive oxygen species (ROS) in tissue damage and carcinogenesis has been reported (Oberly and Beuttner, 1979). Inactivation and removal of ROS depend on reactions involving the antioxidative defense system. Chemoprevention aims to directly modulate specific steps in the carcinogenic process, i.e., block mutagenic carcinogens, prevent DNA damage by free radicals, suppress epithelial cell hyperproliferation and/ or modulate epithelial cell differentiation and apoptosis (programmed cell death). Chemoprevention with food phytochemicals is currently regarded as one of the most important strategies for cancer control.

Emblica officinalis (Amla), belonging to family euphorbiaceae, is one of the most celebrated herbs in the Indian traditional medicine system. It is extensively found all over India, as well as Srilanka, Malaya, China, Pakistan and Bangladesh. The fruits of the plant have been used in Ayurveda as a potent Rasayana (Satyavati et al, 1976) and also for treatment of diseases of diverse etiology. Although Emblica is reputed to have the highest content of vitamin C of any natural plant and is revered for its rejuvenating powers, most of the current scientific interest has centered on its unique tannins and flavanoids, which contain very

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powerful antioxidant properties. Even more exciting studies (Bhattacharya et al 2000, Rajak et al 2004) indicate that Emblica has the ability to stimulate our natural antioxidant enzyme systems including catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX).

Scientists at Nagasaki University in Japan recently found that Emblica contains 18 compounds that inhibit the growth of gastric and uterine cancer cells (Bhattacharya et al, 2000). Additionally, a recent study at the University of Ferrara in Italy showed that Emblica extract inhibited the growth of *in vitro* human breast cancer cells (Zhang et al, 2004).

Materials and Methods

The animal care and handling was done accordingly with the guidelines set by the World Health Organization (WHO), Geneva, Switzerland and the INSA (Indian National Science Academy), New Delhi, India. The inhibition of tumor incidence by fruit extract of Emblica officinalis (EO) has been evaluated on two-stage process of skin carcinogenesis, induced by a single application of 7, 12-dimethyabenz(a)anthrecene (initiator) and two weeks later, promoted by repeated application of croton oil (promoter) thrice weekly, using the following protocol for 16 weeks.

Animals

The study was conducted on random bred, 6-7 weeks old and 25 $\pm$ 2 gm body weight bearing, male Swiss albino mice. These were maintained under controlled conditions of temperature (25$\pm$2˚C) and light (14 light: 10 dark). The animals were fed on standard mice feed procured from Brook Bond Lipton India Limited, Calcutta and water ad libitum. Four animals were housed in a polypropylene plastic cage containing saw dust (procured locally) as bedding material. As a precaution against infections, tetracycline hydrochloride water was given to these animals once in a fortnight. Three days before the commencement of the experiment, hair on the interscapular region of the mice were clipped. Only the mice showing no hair growth were considered for the study.

Chemicals

The carcinogen, 7, 12-dimethylbenz(a)anthrecene (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.

Emblica officinalis (EO) Extract Preparation

Fresh fruits of Emblica officinalis were collected locally after proper identification (I. N0.: RUBL- 19885) by a competent botanist from the Herbarium, Department of Botany, University of Rajasthan, Jaipur. The fruits were washed, air dried, powdered and extracted with double distilled water by refluxing for 36 hrs. at 80˚C. The required dose for treatment was prepared by dissolving the extract in double distilled water at a dose level of 100 mg/ kg body weight.

Experimental Design

A total of 48 animals were assorted into the following groups:

Group-I

Animals of this group (control) were applied topically a single dose of DMBA (100 µg/ 50 µl of acetone) over the shaven area of the skin of the mice. Two weeks later, croton oil (1% in 100 µl of acetone) was applied three times per week until the end of experiment (16 weeks). They were given orally double distilled water equivalent to the dose of EO extract (100 mg/ kg. b.wt.) throughout the experiment.

Group-II

A single dose of DMBA (100 µg/ 100 µl of acetone) was applied topically over the shaven area of the skin of the mice and two weeks later, promoted by repeated application of croton oil (1% in 100 µl of acetone/thrice a week) till the end of experiment. These animals received aqueous fruit extract of EO (100 mg/ kg. b.wt.) by oral gavage for 7 days, before and after DMBA application.

Group-III

Animals of this group received EO extract (100 mg/ kg b. wt.) orally, starting from the time of croton oil treatment and continued till the end of experiment (i.e. 16 weeks).

Group-IV

These animals received EO extract (100 mg/ kg b. wt. / day) throughout the experimental period, i.e., before and after DMBA application as well as at the promotional stage. Croton oil was given as in Group-I.

Morphological Observations of Papilloma Development

Body weights and papillomas appearing on the shaven area of the skin of mice were recorded at weekly intervals. Only those papillomas which persisted for two weeks or more have been taken into consideration for final evaluation of the data. Based on the following observations, the values of per cent inhibition of tumor multiplicity, tumor incidence (percentage of papilloma bearing mice), tumor yield (average number of papillomas per mouse), tumor burdon (papillomas per papilloma bearing mice) and cumulative number of papillomas were derived and compared in all the groups.

Statistical Analysis

The differences in the incidence of tumors among different groups were evaluated by Studentûs t test and considered significant at 5% significance level ($p<0.05$).

Results

The findings of the present study have been depicted in Table-1 and Figs. 1-5. The administration of Emblica officinalis fruit extract orally for 7 consecutive days did not affect the body weight of the animals during the experimental period. Papillomas started appearing on the skin area from

Table 1. Chemopreventive action of *Emblica officinalis* fruit extract on DMBA induced carcinogenesis in mice

Groups	Body weight (g) (mean ± S.E.)		Cumulative Number of papillomas	Tumor Incidence (%)	Tumor Yield	Tumor Burdon
	Initial	Final				
I (n=12)	26 ± 1.2	31.2 ± 1.4	59.0	100	4.9	4.9
II (n=12)	25.8 ± 2.1	29.8 ± 2.6	32.0	83.3	2.6*	3.2*
III (n=12)	25.6 ± 1.7	32.0 ± 2.1	20.0	66.6	1.6*	2.5*
IV (n=12)	26.0 ± 1.9	30.0 ± 2.3	14.0	41.6	1.2*	2.3*

n in parenthesis indicates the number of mice used in respective group.
* Significance level among different groups at $p < 0.05$

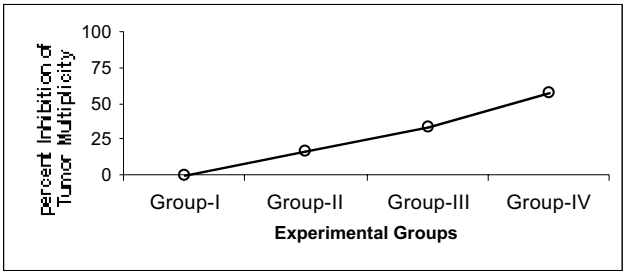

Figure 1. Effect of *Emblica officinalis* on per cent inhibition of tumor multiplicity in the treated mice (Group-II, III and IV) in contrast to Control (Group-I)

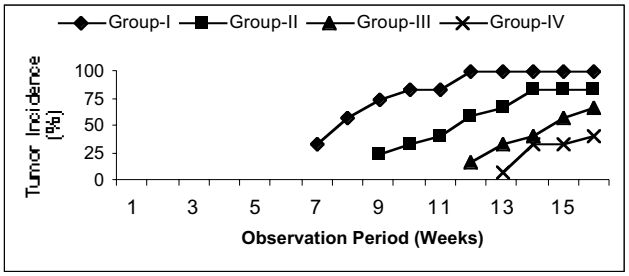

Figure 2. Effect of Emblica officinalis on percentage of papillomas bearing mice (tumor incidence) in the treated mice (Group-II, III and IV) in contrast to Control (Group-I)

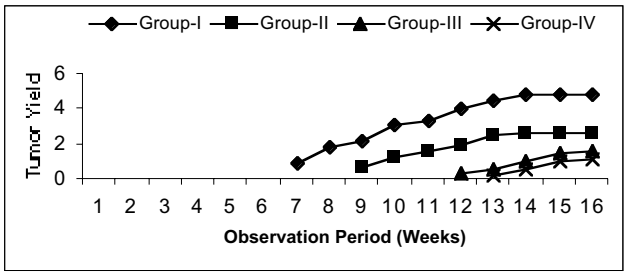

Figure 3. Effect of Emblica officinalis on average number of papillomas per mouse (tumor yield) in the treated mice (Group-II, III and IV) in contrast to Control (Group-I)

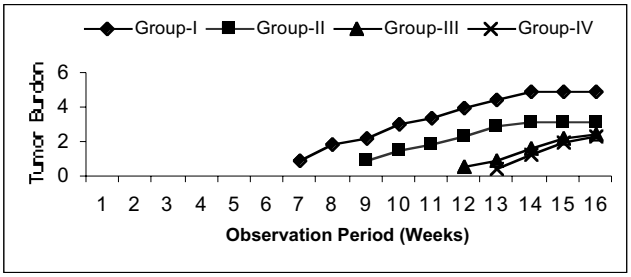

Figure 4. Effect of Emblica officinalis on papillomas per papilloma bearing mice (tumor burdon) in the treated mice (Group- II, III and IV) in contrast to Control (Group-I)

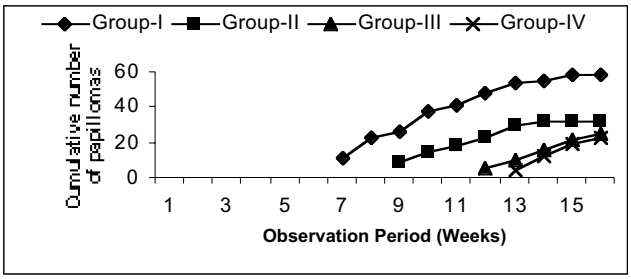

Figure 5. Effect of Emblica officinalis on cumulative number of papillomas in the treated mice (Group- II, III and IV) in contrast to Control (Group-I)

7-13 weeks during exposure to the initiator and the promoter. Per cent inhibition of tumor multiplicity reduced significantly in all the experimental groups as compared to control.

In Group-I (control), treated with a single dose of DMBA and two weeks later promoted by repeated application of croton oil, all animals developed skin papillomas (100% tumor incidence). The average number of papillomas per mouse (tumor yield) as well as the papillomas per papilloma bearing mice (tumor burdon) were found to be 4.9. Cumulative number of papillomas in these mice were recorded as 59.

In the animals of Group-II, receiving similar treatment of DMBA and croton oil when subjected to oral

administration of EO for 14 days (i.e., 7 days before & 7 days after DMBA application), tumor incidence, tumor yield, tumor burdon and cumulative number of papillomas were recorded as 83.3%, 2.6, 3.2 and 32 respectively, which was found to be significantly lower than Group-I.

In Group-III, tumor incidence, tumor yield, tumor burdon and cumulative number of papillomas were found to be 66.6%, 1.6, 2.5 and 20 respectively, when the mice were administered EO for 14 weeks from the time of croton oil application. When the animals of Group-IV were orally treated with EO continuously (i.e., 7 day prior to DMBA application and continued till the end of experiment) tumor incidence, tumor yield, tumor burdon and cumulative number of papillomas were recorded to be 41.6%, 1.2, 2.3 and 14 respectively.

The difference in the values of the results of Group-II, III and IV were statistically analysed (Table-1) and found to be significant in comparison to the control group ($p< 0.05$).

Discussion

There have been few studies on the effect of antioxidant like vitamin E (tocopherol) on tumor growth in animals. The studies of Kagerud et al (1978) and Kagerud and Peterson (1981) demonstrated that tocopherol at doses of 50-500 mg/kg b.wt. given intramuscularly or orally (acetate form) at 7 days before tumor irradiation significantly enhanced the radiation-induced retardation of growth of a rat sarcoma.

Clinical trials of interventions used to treat oral mucosities or its associated pain in cancer patients receiving chemotherapy and/or radiotherapy were recently reviewed (Worthington et al, 1973). Treatment with a combination of pentoxifylline (800 mg/day) and Vitamin E (1000 IU/day) for at least 6 months resulted in clinical regression and functional improvement Delanian et al, 1999).

Now-a-days, chemoprevention is an important strategy to control the process of cancer induction. Therefore, there is a need for exploring medicinal plants or other natural agents that can work as chemopreventive agents. The present study demonstrates the chemopreventive potential of Emblica officinalis extract on DMBA induced skin tumorigenesis in male Swiss albino mice. Literature suggests that one sub-minimal dose of carcinogen initiates the process of carcinogenesis and the treatment with croton oil promotes them to visible tumor stage (Berenblum and Shubik, 1947).

The present investigation exhibited the same with 100% tumor incidence and number of tumor yield as well as tumor burdon as 4.9 in Group-I (control) animals. This is perhaps due to the free radical oxidative stress that has been implicated in the pathogenesis of a wide variety of clinical disorders, resulting usually from deficient natural antioxidant defences as well as lipid peroxidation. On the other hand, animals of Groups II, III and IV received similar treatment of DMBA and croton oil; when subjected to an oral administration of E. officinalis extract, a significant reduction ($p< 0.05$) in tumor incidence, tumor yield and tumor burdon were recorded. The cumulative number of papillomas were found to be reduced in the E. officinalis fruit extract treated groups (II, III & IV) when compared to the Group-I (control) mice. The present findings also show an increase in the percent inhibition of tumor multiplicity in the EO extract treated groups in comparison to control group.

Preventive, curative and health restorative properties of E. officinalis leads to the assumption that the plant extract may have either acted as an anti-inflammatory agent inhibiting DMBA-induced skin papillomagenesis or inhibited the epidermal ornithine decarboxylase, thereby decreasing the tumor incidence, tumor yield and tumor burdon. Similar depletion of tumorigenesis owing to the inhibition of epidermal ornithine decarboxylase, epidermal DNA synthesis and promotion of skin tumors by cucurmin has been earlier reported (Mou et al, 1988).

The property of E, officinalis as an antioxidant as well as an antioxidant enzyme system stimulant, lead to the supposition that it has played a role in augmenting the activities of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX), concomitant with reduction in lipid peroxidation. Similar reduction of tumorigenesis by the inhibition of oxidative tissue damage has been reported by Bhattacharya et al (1999). The E. officinalis extract rich in emblicanin A and B, was found to significantly increase the cortical and striated concentration of the antioxidant enzymes, SOD, CAT and GPX, and to reduce lipid peroxidation in rat brain areas (Bhattacharya et al, 2000).

Free radicals and lipid peroxidation are known to cause initiation and promotion of carcinogenesis (Bauer et al, 1980). The most abundant free radicals generated in living cells are superoxide anions and derivatives, particularly the highly reactive and damaging hydroxyl radical, which induces peroxidation of cell membrane lipids. The end products of lipid peroxidation are known to induce cellular damage and have been responsible for oxidative free radical induced human disease (Halliwell and Gutteridge, 1989).

Superoxide is inactivated by SOD, the only enzyme known to use a free radical as a substrate. However, the free radical scavenging activity of SOD is effective only when it is followed up by increase in the activity of CAT and/or GPX, since SOD generates hydrogen peroxide as a metabolite, which is more tissue toxic than oxygen radicals and has to be scavenged by CAT or GPX. GPX and CAT play important roles in cellular defence as well as maintenance of cellular membranes from oxidative damage of free radicals by eliminating H2O2 (Sunde et al 1980, Moser et al 1996). Thus, a concomitant increase in CAT and /or GPX activity is essential if a beneficial effect from increase in SOD activity is to be expected (Harman 1991). A significant reduction in skin papilloma formation due to the increased activity of antioxidant enzymes in the liver tissue of mice treated with saffron (a naturally occurring spice and food colorant) have also been reported by Das et al (2004).

Antioxidants may interfere with the initial mediation of apoptosis by reactive oxygen species (ROS) (Salganic, 2001), as well as later membrane lipid peroxidation.

Protection against carcinogenic apoptosis in damaged cells is also relevant to chemoprevention strategies in populations exposed to environmental carcinogens. Further in vivo studies are required that focus on antioxidant effects, e.g. on carcinogen induced apoptosis, in specific tumors and normal tissues.

If human disease is considered to result from an imbalance between oxidative stress and antioxidant defence, then it is conceivable that it may be possible to limit oxidative tissue damage and, hence, prevent or ameliorate disease progression, by supplementing antioxidant defence. By virtue of their properties and clinical use in Ayurveda, Emblica officinalis may provide potential to be used as chemopreventive agent. This present work demands additional study to evaluate the exact mechanism of chemoprevention of carcinogenesis by E. officinalis or by its active principles.

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