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INHIBITORY EFFECT OF THE MEDICINAL PLANT, *ECLIPTA ALBA* LINN. ON SKIN CARCINOGENESIS IN SWISS ALBINO MICE

Sabera Sultana Rehman and *R Bharali
Department of Biotechnology, Gauhati University, Guwahati-781014, Assam, India

ABSTRACT

Chemoprevention using readily accessible natural substances from vegetables, fruits, herbs and spices is presently considered as one of the most noteworthy strategies for cancer prevention. The herb, *Eclipta alba* Linn (Family: Compositae) found commonly in moist places all over India is traditionally used as a tonic and deobstruent in hepatic and spleen enlargements .It has also got anti- inflammatory effect and may be applied to insect bites, stings, swellings and other skin diseases. The present investigation was undertaken to explore the anti tumor promoting activity of *Eclipta alba* on two stage skin carcinogenesis, induced by a single topical application of 7, 12 Dimethyl benz (a) anthracene (50µg/ 50µl of acetone) and two weeks later, promoted by repeated application of croton oil (1% in acetone for 3 times a week) till the end of the experiment (15 weeks). Topical application of the hydro alcoholic extract of the herb, *Eclipta alba* for 15 weeks at the pre, peri and post initiational stages on the shaven backs of Swiss albino mice was found to be effective in decreasing the tumor incidence (90, 77.77 and 66.6% respectively) in comparison to the control (100%). The cumulative number of papillomas, tumor yield and tumor burden were also found to be reduced significantly in *E. alba* treated mice. The histo-pathology of the affected skin tissue also indicated a significant reduction in the tumor size and slow growth of the tumors in the treated groups in comparison to the control. The results thus suggest a possible chemopreventive property of *E. alba* against DMBA induced skin papillomagenesis.

Keywords: DMBA, papillomagenesis, *Eclipta alba*, histopathology.

INTRODUCTION

Cancer chemoprevention is a concept defined as the prevention of cancer by the administration of natural or synthesized pure chemicals, or by daily foods enriched with cancer preventive components. Particularly, food phytochemical could be important for cancer prevention. In fact, a great number of epidemiological studies of the relationship between food and cancer, together with the research in the experimental animal models, have demonstrated that daily ingestion of some vegetables and fruits could undoubtedly contribute to cancer prevention (Murakami *et al*., 1994).

The medicinal herb *Eclipta alba* belonging to the family Asteraceae is found throughout India up to 2000 meters on the hills. The plant is used as tonic, deobstruent in hepatic and spleen enlargements (Kirtikar and Basu, 1981; Kanjilal, 1997). It is also used as a detoxifying deobstruent and antiseptic herb in vitiated blood, anaemia, splenic and liver enlargements, catarrhal jaundice, hyperacidity, gastritis and dysentery (Kumar, 2002; Khare, 2004). In Ayurvedic and Unani medicine, the juice of the plant is used for washing wounds and soft chancre and applied locally on skin infections, allergic urticaria, inflatuence and over swellings (Khare, 2004). The plant does not show any signs of toxicity and the minimum lethal dose was greater than 2.0 g/ Kg when given orally and intraperitonially in mice. The drug is traditionally considered safe (Indian Herbal Pharmacopoeia, 2002). The plant has a reputation as an anti ageing agent in Ayurveda. It is used externally for inflammation, minor cuts and burns and the fresh leaf juice is considered very effective in stopping bleeding (Sharma, 2003).

*Corresponding author email: rbharali@rediffmail.com

MATERIALS AND METHODS

Animals

Random bred 7-8 weeks old Female Swiss Albino mice were used to carry out the experiments. Permission was obtained from the Institutional Animal Committee of Gauhati University to pursue the experiment. The animals were obtained from the animal house of Zoology Department, Gauhati University and housed under normal conditions having natural photoperiod. They were provided with standard pellet diet and tap water ad libitum, under hygienic conditions. Three days before the onset of the experiment, the hair on the interscapular region of the mice were clipped and the resting phase of the hair growth cycle was observed. Only the mice showing no hair growth were taken for the experiment. Body weights of the mice were recorded on a weekly basis to keep a constant vigil on the health of the animals.

Chemicals

Chemicals for papillomagensis

The carcinogen, 7,12-dimethylbenz (a) anthracene (DMBA), and croton oil were procured from Sigma Chemicals Co., St. Louis; USA. DMBA was dissolved at

Table 1. Chemopreventive action of *Eclipta alba* extract on DMBA induced skin papillomagensis in mice.

Groups	Body weight (g) (Mean±S.E)		Cumulative number of papillomas	Tumor incidence (%)	Tumor yield	Tumor burden
	Initial	Final				
I (N = 10)	12.2±1.23	24.4±1.26	125	100	12.5*	12.5*
II (N = 10)	14.7±0.95	26.2±3.19	50	90	5*	5*
III (N = 10)	15±0.66	17.6±2.96	44	77.77	4.4*	4.8*
IV (N = 10)	13.5±1.4	27.8±1.9	26	66.66	2.6*	2.88*

N in parenthesis indicates the number of mice used in respective groups. * Indicates the significance level among different groups at $p < 0.05$.

a concentration of 50 μg in 50 μl acetone. Croton oil was mixed in acetone to give a solution of 1%.

Chemicals for histopathology

Alcohol (absolute, 90%, 70%, 50%, &30%), xylene, haematoxylin, eosin, paraffin, 10% formalin, Mayer's albumen and DPX.

Preparation of the Eclipta Alba Extract

The herb, *Eclipta alba* was collected locally from various parts of Guwahati, Assam, India after proper identification by a competent botanist from the Department of Botany, Gauhati University, Guwahati, Assam. The plant was washed and dried in shade without direct exposure to sunrays. It was then grounded and 50 gram of the material thus obtained was subjected to soxhlet extraction using 300 ml of hydro-alcoholic solvent (80% solvent: 20 % distilled water). The process was repeated 3 times with fresh material of the same amount. The alcohol was allowed to evaporate and then the residue obtained was stored at 4°c.The required dose for treatment was prepared by diluting the residue in acetone at a dose level of 5-mg/Kg body weights at par with the doses of the initiator and promoter concentrations. The aliquot obtained was a fine homogeneous suspension in acetone.

Preparation of Skin for Histology

Affected skin and skin with papillomas were fixed in 10% formalin and dehydrated with graded alcohols starting from 30% alcohol to absolute alcohol. Then the tissues were embedded in paraffin after clearing in xylene. Serial microtome sections (4μ) were stained with H and E. (Kehar and Wahi, 1967).

Experimental Design

A total of 40 animals were taken for the experiment and divided equally into 4 groups. The experiment was conducted for 15 weeks.

Group-I

A single dose of 50 μg of DMBA in 50 μl acetone was applied topically over the shaven area of the skin of mice. Two weeks later, croton oil (100 μl of 1 % croton oil in acetone) was applied three times per week until the end of the experiment.

Group-II

Animals received a topical treatment of an ethanolic extract of the herb, *Eclipta alba* (5mg / Kg body weight / day) in 100 μl acetone for 14 days (7 days before and 7 days after the application of a single dose of DMBA). Croton oil was applied as in Group-I and the experiment was continued for 15 weeks.

Group-III

Animals received a topical treatment of *Eclipta alba* extract, starting from the time of croton oil application and continued till the end of the experiment i.e., 15 weeks. A single dose of DMBA was given as in Group -I.

Group-IV

Animals were treated topically with *E. alba* extract (5mg/ Kg body weight/day) throughout the experimental period, i.e., before and after DMBA application and also at the promotional stage. Croton oil was given as in Group-I and the experiment was carried out for 15 weeks.

Morphological Observations of Papilloma Development

Body weight and papillomas appearing on the shaven area of the interscapular region of the skin of mice were recorded at weekly intervals. Only those papillomas that persisted for two weeks or more and were greater than or equal to 1 mm were considered for final evaluation of the data. Based on the following observations, the values of percent inhibition of tumor multiplicity, tumor incidence, tumor yield, tumor burden and cumulative number of papillomas were obtained and compared in all the four groups.

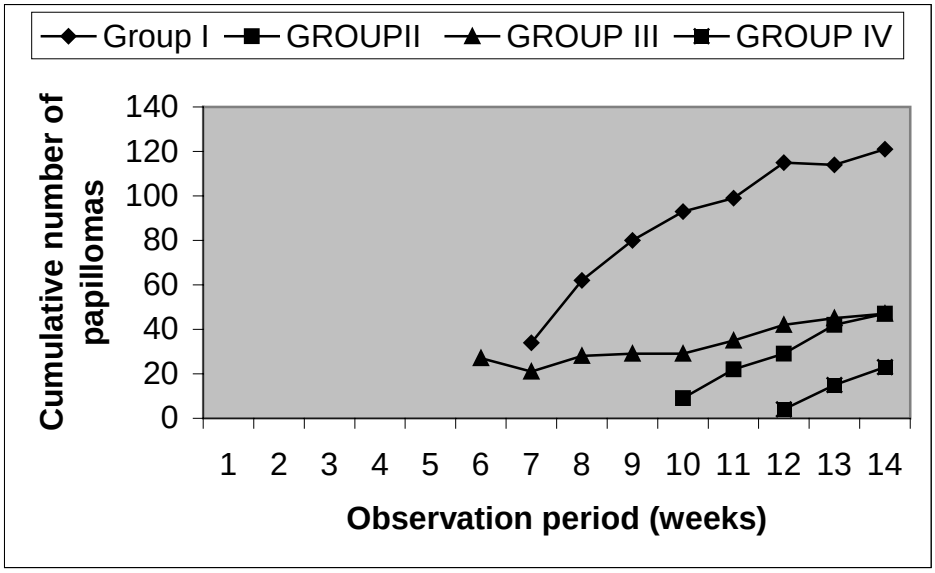


Fig. 1. Effect of *Eclipta alba* on cumulative number of papillomas in the treated groups of mice (group-II, III & IV) in contrast to the control (group I).

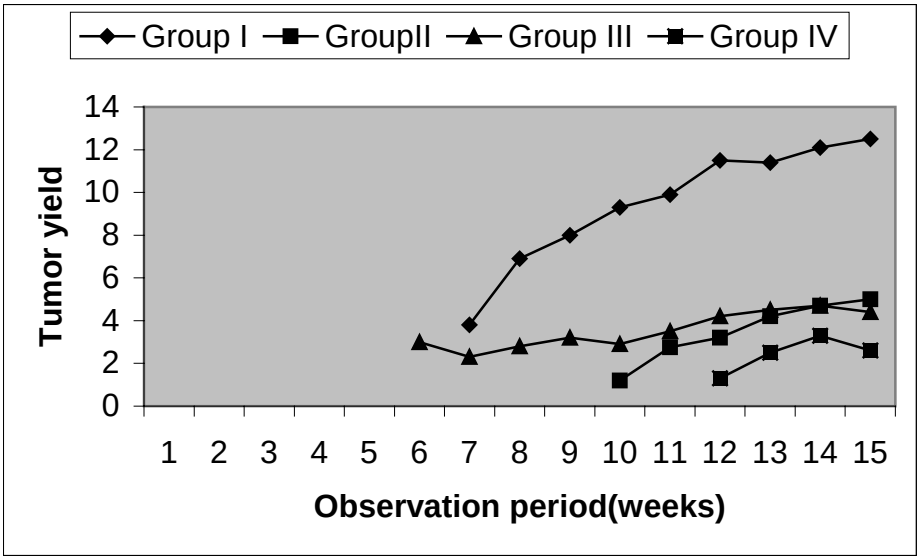


Fig. 2. Effect of *Eclipta alba* on the average number of papillomas per mouse (tumor yield) in the treated mice (group II, III & IV) in contrast to control (group I).

STATISTICAL ANALYSIS

The difference in the incidence of tumors among different groups were evaluated by student's t test and considered significant at 5 % significance level ($p<0.05$).

RESULTS

Effect of *E alba* on DMBA Induced Croton Oil Promoted Tumor Incidence, Cumulative Number, Tumor Yield and Tumor Burden of Papillomas

The findings of the present study have been depicted in table 1 and figures 1-4. The administration of the hydro alcoholic extract of *Eclipta alba* did not affect the body weight of the animals during the experimental period. Papillomas started appearing on the shaven interscapular region of the mice from 6-12 weeks during exposure to the initiator and promoter. The percent inhibition of tumor multiplicity reduced significantly in all the experimental groups in comparison to the control.

In the control group (group I), skin papillomas appeared in all the animals (100% tumor incidence). The cumulative number of papillomas, tumor yield and tumor burden were recorded as 125, 12.5 and 12.5 respectively (Fig. 1).

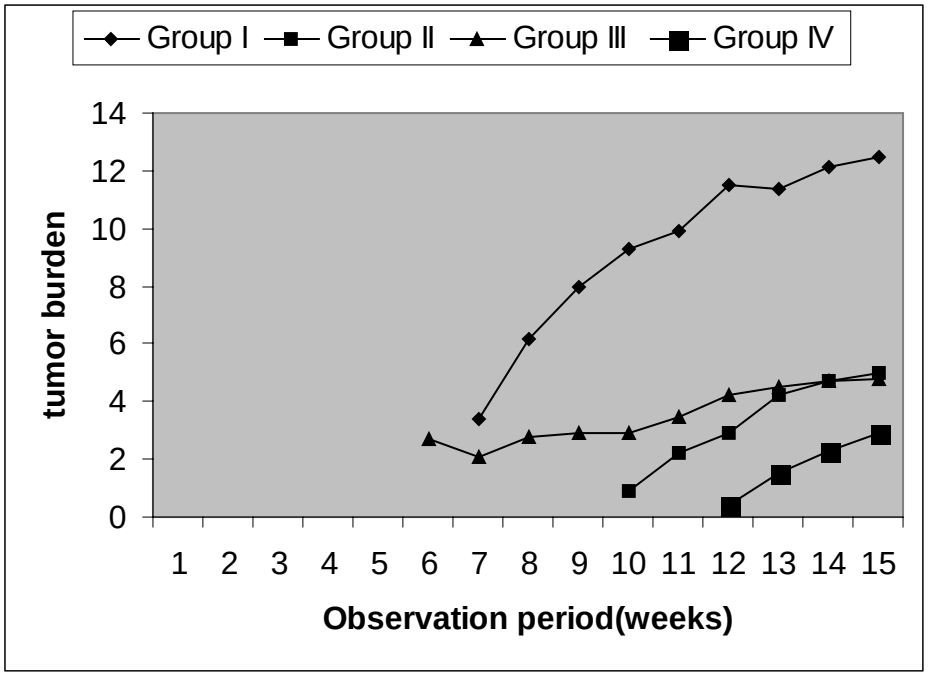


Fig. 3. Effect of *Eclipta alba* on papillomas per papilloma bearing mice (tumor burden) in the treated mice (group II, III & IV) in contrast to control (group I).

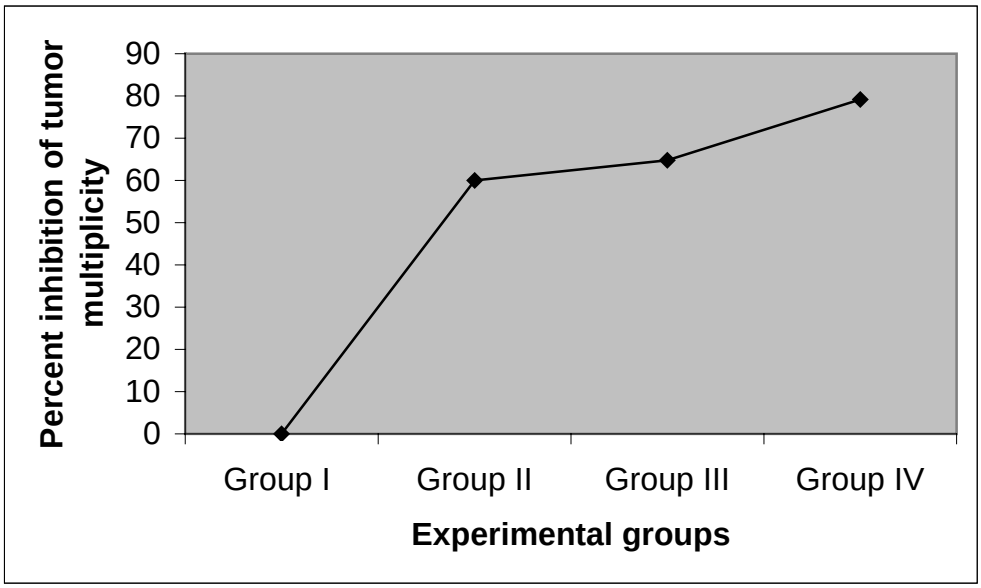


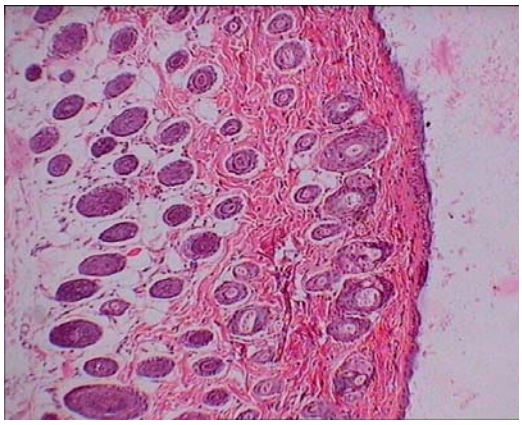
Fig. 4. Effect of *Eclipta alba* on percent inhibition of tumor multiplicity in treated mice (group II, III & IV) in contrast to control (group I).

In group II, the tumor incidence, the cumulative number of papillomas, tumor yield and the tumor burden was found to be 90%, 50, 5 and 5 respectively (Fig. 2).

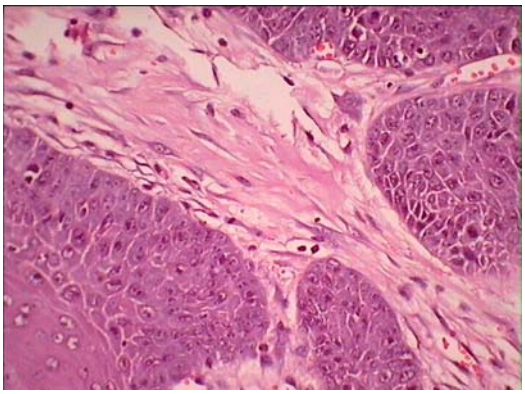
In group III, the tumor incidence, the cumulative number of papillomas, tumor yield and the tumor burden was found to be 77.77%, 44, 4.4 and 4.8 respectively (Fig. 3).

In group IV, the tumor incidence, the cumulative number of papillomas, tumor yield and the tumor burden was found to be 66.66 %, 26, 2.6 and2.88 respectively (Fig. 4).

The difference in the values of results of group-II, III and IV were statistically analyzed (Table 1) and found to be



A



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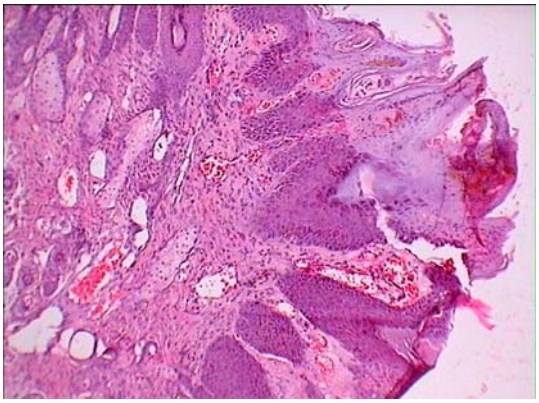

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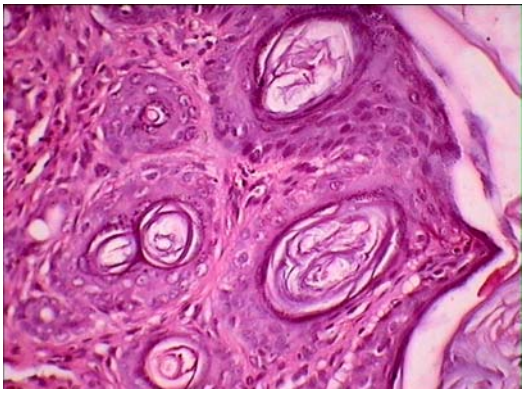

D

significant in comparison to the control (Group I) at 5% probability level.

Effect of *E alba* on the Histopathology of the Skin after Treatment with DMBA (Inducer), Croton Oil (Promoter) and *E alba Extract* (Modulator)

The animals were sacrificed at the end of the experiment (after 15 weeks of treatment) and a section of skin from the interscapular region of the mice was taken for histo-pathological studies. The stains used were Haematoxylin and eosin.

In the Control animals, normal cellular structure of the skin was observed i.e. the skin consists of two major layers -the epidermis and the dermis. The epidermis consists of stratified squamous epithelium .The outermost region consists of many layers of dead usually flattened squamous cells which forms a protective covering or stratum corneum on the skin surface. The deepest layer of cells in the epidermis is called the stratum germinativum or malpighian layer that consists of a single row of living columnar cells and is separated from the underlying dermis by a basement membrane (Reith and Ross, 1977; Kotpal, 1995).

The dermis or corneum is the inner layer of skin and is composed of fibrous connective tissue and contains nerve endings, blood vessels and lymphatic vessels. Pigment cells or melanocytes are mostly located in the dermis (Kotpal, 1995) (Fig. A).

In group I animals, presence of keratinized tissue along with the papillomas was observed. The skin epithelium showed multiple papillomas characterized by the development of finger like projections protruding over the surface. Each papilloma was consisted of hyperplastic stratified squamous epithelial cells with central connective tissue core along with a large number of newly formed blood vessels. The outer lining appears to be covered by keratinized tissue (Fig. B).

In group II animals, the tumor mass appears to project out from the surface. The stratum cornium of the skin was almost sloughing out. The stratum germinativum layer showed proliferative changes with large number of mitotic bodies along with large nucleoli. The cells were hyperchomic in character and extended into the dermal layer. The proliferated cells developed a mass of solid sheets, occasionally with bizarre character. Few cells showed vaculation (Fig. C).

In group III animals, the papillary outgrowths were found to be much smaller then the group I. The skin epithelium showed hyperplastic papilleric outgrowths of stratified squamous epithelium along with development of cell

nests structure of keratinized tissue. The epithelial lining showed excessive presence of melanin pigments, which was outwardly covered by degenerated keratinized tissue. Core tissue is not that distinct (Fig. D).

In group IV animals, the stratified squamous epithelium focally showed papillary projection with or without presence of core tissue. The granular layer showed large haematoxylin positive granules in the cytoplasm. The germinativum layer showed bizarre arrangement with mitotic bodies. At places the hyperplastic cells showed focal penetration into the dermis. Only remnants of cornified layer could be seen (Fig. E).

DISCUSSION

The skin carcinogenesis model in experimental animals has been found to be very useful system for investigating the influence of dietary chemopreventors both mechanistically and operationally (Morse and Stoner, 1993). A number of naturally occurring as well as synthetic substances have been shown to cause inhibition of chemical carcinogenesis either by preventing the formation of active carcinogens from their precursors, by preventing carcinogenic compounds by reaching reacting with critical target sites in cells or by inhibiting/ suppressing the expression of neoplasia in cells which have already reacted with carcinogens (Wattenberg, 1983, 1985). The present study demonstrates the chemopreventive potential of *Eclipta alba* on DMBA induced skin papillomagensis in Swiss Albino mice. Berenblum and Shubik (1947) has suggested that one sub minimal dose of carcinogen initiates the process of carcinogenesis and the treatment with croton oil promotes them to visible tumor stage. The current study also exhibited the same with 100% tumor incidence in the control group. But the administration of the hydro alcoholic extract of *Eclipta alba* at pre, peri and post initiational phases showed a significant reduction in tumor incidence, tumor yield, tumor burden and cumulative number of papillomas. The histo-pathology of the skin showed visible reduction in the size of the tumors in comparison to the control (Fig. A-E). The treated groups also have not exhibited any distinct core tissues, thereby ruling out the possibility of newly formed blood vessels. This is perhaps due to the presence of phytoestrogens like flavonoids and coumestans present in the herb, which are considered to have an inhibitory role during the initiational and promotional phases of cancer development (Messina and Barnes, 1991; Messina *et al*., 1994). Several natural and dietary compounds from vegetables, fruits, herbs and spices are being considered for the primary and secondary prevention of cancer (Mishra *et al*., 2003). One such compound is the phytoestrogen, which is a naturally derived compound found in plants. Two phytoestrogens (coumestans), wedelolactone and desmethyl-wedelolactone were isolated as the main active principles present in E.alba (Saxena *et al*., 1993). Both constituents showed anti hepato toxic activity in assays using liver enzyme induced cyto toxicity in cultured rat hepatocytes. These constituents also showed a significant stimulatory effect on liver regeneration (Wagner *et al*., 1986). Evidences suggest that *E. alba* exerts its protective action through a reduction in GSH depletion (Wagner *et al*., 1986; Saxena *et al*., 1993). Besides the herb also contains the flavonoids, apigenin and luteolin as minor constituents in addition to the active principles (Indian Medicinal Plants, 1994; Indian Herbal Pharmacopoeia, 2002). Studies have shown that apigenin acts as proteasome inhibitor and apoptosis inducer in human leukemia cells (Chen *et al*., 2005). Studies have also have revealed that apigenin induce cell cycle arrest in activated microglia (Elsisi *et al*., 2005). Researches have also shown that apigenin can inhibit pancreatic cell proliferation through G2/M cell cycle arrest (Ujiki *et al*., 2006) and the expression of vascular endothelial growth factor and angiogenesis in human lung cancer cells (Liu *et al*., 2005) which may be one of the many reasons that accounted for the lack of distinct core tissues and reduction in tumor size as seen in the histopathological slides in the plant extract treated groups (Fig. C, D, E) in comparison to the DMBA and croton oil treated group (Fig. B). Studies have also revealed that luteolin is effective in the protection of human single cell DNA from oxidative attack which indicates that *E. alba*, which has luteolin as one of its constituents may have a preventive or curative effect on the oxidative stress caused by free radicals which is responsible for a wide variety of clinical disorders including cancers (Horvathova *et al*., 2004). The use of the herb as a healing and restorative agent against skin diseases, inflammations, wounds and ulcers have led to the supposition that it might have either acted as an anti-inflammatory agent or inhibited the epidermal ornithine decarboxylase, a rate limiting enzyme in the biosynthesis of polyamines which appear to be a pre requisite for cell proliferation, differentiation and neoplastic transformation (Katiyar *et al*., 1996). Thus the reduction in tumorigenesis in groups II (where animals were treated with the modulator 7 days before and 7 days after the application of a single dose of DMBA), III (where the modulator treatment was started from the time of croton oil application and continued till the end of the experiment) and IV(where the modulator treatment was continued throughout the experiment) may be due to the inhibition of epidermal ornithine decarboxylase .Similar reduction of tumorigenesis through the inhibition of epidermal ornithine decarboxylase in mice by *Emblica officinalis (amla)*, a popular fruit in India have also been reported earlier (Mou *et al*., 1988; Sancheti *et al*., 2005). Similar inhibition of epidermal ornithine decarboxylase in male Wistar rats by *Butea monosperma*,a medicinal plant have been reported by Sehrawat and Sultana (2006) and by Sancheti and Goyal (2006) in mice using the extract of

Rosemarinus officinalis, which is an evergreen shrub having medicinal properties. Further it is suggested that aryl hydrocarbon hydroxylase, a cytochrome dependent carcinogen metabolizing enzyme present in the skin appears to play an important role in the activation of polycyclic aromatic hydrocarbons into reactive moieties that can bind to DNA and that may directly induce cancer (Bickers and Kappas, 1978). So there is a possibility that E.alba might have an inhibitory influence on the aryl hydrocarbon hydroxylase enzyme system, thereby reducing tumorigenesis in the treated animals. Similar inhibition of aryl hydrocarbon hydroxylase enzyme system in rats using garlic oil have been reported by Siddiqui and Pawar (1984) and Sadhana *et al*. (1988) using garlic oil in mice. The significant reduction in the tumor incidence, tumor burden, tumor yield and also in the cumulative number of papillomas in the treated animals (groups II, III and IV) may be attributed to the individual or shared effects of one of the constituents of the herb, Eclipta alba.

Eclipta alba is already popularly used as a home remedy for numerous purposes such as minor skin burns or cuts, wounds, insect bites, stings etc (Indian Medicinal Plants, 1994). The present study suggests that *E. alba* should be explored further for cancer chemopreventive prospective, in addition to its existing utility as a medicine for treating several diseases in the traditional medicine system of India, Ayurveda. Advance researches on hepatic detoxifying and anti oxidant enzymes by the extract of the herb, *E.alba* on Swiss albino mice are in progress.

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