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ANTICANCER POTENTIAL OF *AEGLE MARMELOS* BARK EXTRACT AGAINST DMBA INDUCED SKIN PAPILLOMAGENESIS WITH REFERENCE TO OXIDATIVE STRESS

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ABSTRACT

Background: Cancer is among the second fatal diseases, next to cardiovascular diseases. Surgery, chemotherapy and radiotherapy or a combination of these are used worldwide for the treatment of Cancer. But, all these therapeutic options results in various side effects, to overcome these side effects, medicinal plants and herbs have now been used for their pharmacological active contents to treat cancer. **Aim:** The present study was aimed at evaluating the Chemopreventive and Antioxidant effects of hydromathanolic *Aegle marmelos* bark extract against 7, 12-dimethylbenzeneanthracene (DMBA) induced Papilloma in Swiss albino mice. **Material and Methods:** Crude extraction protocol was employed for the preparation of Hydromethanolic *Aegle marmelos* bark extract and Tumor was induced by the application of single dose of DMBA on the dorsal, shaved surface of mice and promotion by croton oil started after 2 weeks and in the treatment group, *A. marmelos* bark extract (900 mg/kg b.wt.) was given one hour before each application of croton oil 2 times/week up to 16 weeks. After the completion of the assay, Reduced Glutathione (GSH) level was estimated in control, Carcinogen control and treatment group. **Results:** In this Skin Papilloma model, Significant reduction in tumor burden, tumor incidence and cumulative number of papillomas and a marked increase in average latent period of tumor appearance and Reduced Gluatathione concentration was observed in *Aegle marmelos* bark extract treated group as compared to animals treated with DMBA and croton oil (carcinogen control) group. Thus, the present study suggests that the *Aegle marmelos* bark extract has antitumor and antioxidant potential against Chemical induced skin papillomagenesis.

KEYWORDS: Anticancer, Chemopreventive, Antioxidant, Hydromethanolic, *Aegle marmelos*, Papillomagenesis.

INTRODUCTION

Carcinogenesis is a multistage process involving cellular and molecular alterations, consisting of three linked but separate stages- Initiation, Promotion and progression. Initiation involves a chain of cellular changes arising spontaneously or induced by exposure to carcinogenic agent. In contrast to initiation, tumor promotion is recognized by further proliferation, leading to accumulation of actively proliferating preneoplastic cells. Progression is the final stage, in which successive changes in neoplastic transformation give rise to malignant sub populations.[1,2]

Cancer Chemoprevention is a mean of cancer control by the use of relatively non toxic chemical substance, either of natural or synthetic origin, to inhibit initiation and to act as suppressing agents. The use of herbal extracts and phytochemical contents derived from them have been extensively employed in cancer treatment.[3,4,5] It was also reported that phytochemicals with antioxidative and anti inflammatory properties can inhibit tumor initiation, promotion and progression.[6] Therefore, scientific validations of antitumor promoting agents present in medicinal plants should be done for its possible cure in the prevention and treatment of cancer.

Aegle marmelos, commonly known as Bael, is an indigenous medicine due to its various medicinal properties[7] and found all over the Sub-Himalayan forests, in Central and South India. It is a rich source of Coumarins, Vitamin C and Riboflavin.[8] It possesses antioxidant[9], antidiabetic[10], hepatoprotective[11], as anticarcinogenic agent during promotion/progression stages of different cancer[12] and antigenotoxic effects against doxorubicin induced micronucleus.[13] The fruit as well as the bark of the plant was reported to be a valuable Ayurvedic medicine for dysentery and various intestinal complaints. Thus, the present study was designed to investigate the chemopreventive potential of *Aegle marmelos* bark extract with reference to its antioxidative efficacy in Papilloma bearing mice.

MATERIAL AND METHODS

Identification of plant material

Identification of the plant ***Aegle marmelos*** (Family: Rutaceae) was done by competent botanist Dr. Shaukat S. Khan, Department of Botany, Saifia Science College, Bhopal, M.P.

Animals

The study was conducted on male Swiss albino mice (6-7 weeks old: Body weight 25±2). These animals were kept under controlled conditions of temperature (25±1^{o}c) and light (12 light: 12 dark). All animal studies were conducted according to the guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The animals were fed on standard mice feed procured from Golden feeds, New Delhi, and water *ad libitum*. Three days before the treatment, the dorsal hair of all the animals was shaved (2x2cm).

- Investigation was approved by **Institutional Animal Ethical Committee (IAEC)**, project no. **500/01/a/2001/19th/proj-1/27-7-09.**

Chemicals

The known skin initiator DMBA and Croton oil (used as promoter) were obtained from Sigma Chemicals Co. (St. Louis, USA). DMBA was prepared in acetone at a concentration of 104µg/100µl. Croton oil was diluted in acetone to give a solution of 1% dilution.

Experimental protocol

Experiment was performed as per the method reported by **Berenblum (1975)**[14] and standardized by **Agrawal *et. al* (2009)**.[15] A total of 30 animals were randomly divided in to 5 groups of 6 mice in control and experimental group. Initiation of tumor was done by application of single dose of DMBA on the dorsal, shaved surface and promotion by croton oil started after 2 weeks.

Group-I: Vehicle Control- received topical application of acetone (100µl/mouse) on the shaven dorsal skin, 2 times/week up to 16 weeks.
Group-II: DMBA alone- a single dose of 104µg DMBA in 100µl of acetone was applied topically over the shaven area of the skin of the mice.
Group-III: Croton oil alone- 100µl of 1% croton oil in acetone was applied two times per week until the end of the experiment.
Group-IV: Drug treated Control- 100µl of AMB at the dose of 900 mg/kg was applied two times per week until the end of the experiment.
Group-V: Carcinogen treated (Positive control) - applied topically with a single dose of DMBA (104µg/100µl of acetone) over the shaven area of the skin of the mice. Two weeks later, croton oil (1% in acetone) was applied two times per week up to 16 weeks.
Group-VI: AMB Experimental- applied topically with a single dose of DMBA over the shaven area of the skin of mice. Two weeks later, they were treated with *A. marmelos* stem bark extract (900 mg/kg b.wt.) which was given one hour before each application of 1% croton oil 2 times/week up to 16 weeks.

The body weights of the animals from each group were recorded at the beginning and at the termination of the experiment. The mice from all the groups were sacrificed at 16 weeks after the last dose of AME for the following studies.

a) Morphological observations of papilloma development

During the study, each animal in all the groups of all stages were weighed and shaved weekly for an easy application of the carcinogens/tested extracts and skin lesion observation.

The following morphological parameters were studied in the following groups:

- Cumulative no. of Papillomas
- Tumor incidence
- Tumor yield
- Tumor burden
- Average latent period

(b) Biochemical

The whole liver and blood from eye was taken out for each mouse for the study of Glutathione activity (GSH). The level of reduced GSH was determined by the method of **Moron *et. al.*, 1979**.[16] The GSH content in both the samples was measured Spectrophotometrically using Ellman's reagent with 5, 5'-dithiobis 2-nitrobenzoic acid (DTNB) as a coloring agent, according to the method of **Beutlar *et al*, (1963)**.[17]

Data Analysis

Values are given as Mean ± SE of six mice per group. The results were statistically evaluated using Student's t-test. The differences between the groups were considered as significant at *p<0.05.

RESULT

Morphological Analysis

As shown in **Table 1**. Control group-V, with a single topical application of DMBA followed by repeated application of croton oil (twice a week) 2 weeks later, skin papilloma appeared in all animals which started appearing from 5th week onwards and the cumulative no. of Papillomas induced during the observation period was 40. In the treated group it was only 7. Tumor incidence was to be 100% in Carcinogen treated control (group V) and drug treted group (group VI) it was 50%. The average no. of Papilloma per mouse (tumor yield) as well as the Papilloma per Papilloma bearing mice (tumor burden) was found to be 6.6±1.4 in Carcinogen treted control group and in drug treted group it was 2.3±0.6 and 1.16±0.5, respectively. The average latent period in carcinogen treated control and drug treated group was 5.1±1.5 and 7.8±2.1.

Biochemical Analysis

A considerable reduction in the level of GSH in blood and liver was observed in carcinogen treated control group. Treatment with *Aegle marmelos* bark extract resulted in an enhanced level of antioxidant protein GSH in blood and liver in group III (Table 2).

TABLE. 1. Effect of *Aegle marmelos* Bark extract on Mouse Skin Papillomagenesis.

Group	Treatment	Cumulative no. of Papilloma	Tumor Incidence (%)	Tumor Burden	Tumor Yield	Average Latent Period	Number of Papilloma with Tumor size, mm	
							<2	2-4
I	Vehicle alone (100µl acetone)	0	0	0	0	0	0	0
II	DMBA alone	0	0	0	0	0	0	0
III	Croton oil	0	0	0	0	0	0	0
IV	**AMB** extract alone	0	0	0	0	0	0	0
V	DMBA+CO (carcinogen control)	40	100	6.6±1.4	6.6±1.4	5.1±1.5	31	9
VI	DMBA + Croton oil + **AMB**(Drug treated)	07	50	1.16±0.5*	2.3±0.6*	7.8±2.1	7	0

(*) denotes Level of significance as compared to carcinogen control at p<0.05, n= no. of animals in each group.

Table. 2. Showing the level of Glutathione (GSH) in blood and liver sample of Papilloma bearing Swiss albino mice receiving treatment of *Aegle marmelos* extracts.

S.No.	Treatment Group	Glutathione level	
		Blood (µg/ml)	Liver (µmoles/gm)
I.	Normal mice	5.1±.06	54.9±0.67
II.	Carcinogen control (DMBA+CO)	2.27±0.09	23.2±1.3
III.	DMBA+*Aegle marmelos* bark extract (900 mg/kg)+CO	4.8±0.06*	33.2±1.8*

Data are reported as Mean ±SE, n=6.
* Significance level among different groups at p< 0.05.

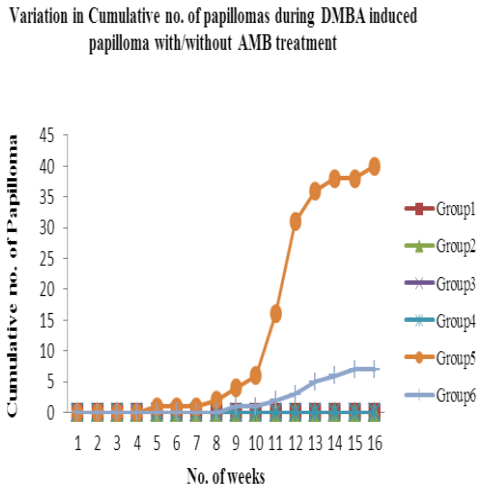


Figure (1).

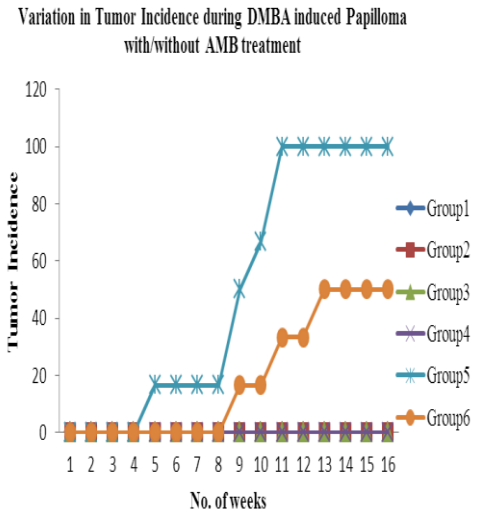
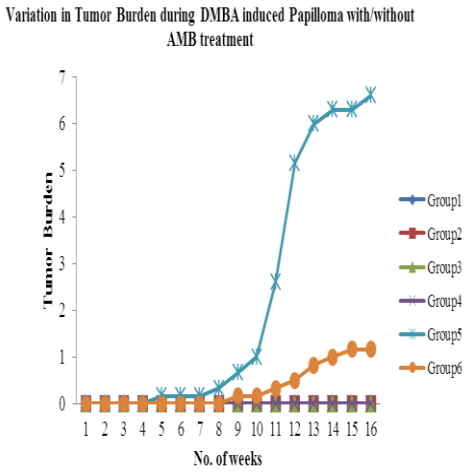


Figure (2).



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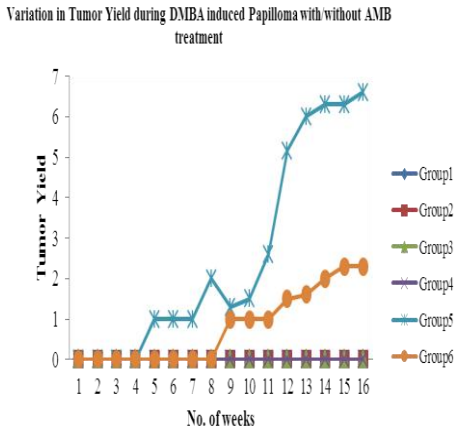

Figure (4).

Photograph. 1 CARCINOGEN CONTROL.

Photograph. 2 DRUG TREATED.

DISCUSSION

Today, human beings have been exposed to a variety of mutagenic and carcinogenic agents which may drive the formation of cancer. Carcinogenesis is a process exemplified by initiation, promotion and progression in which different events determine the neoplastic conversion of normal cells.[18, 19] Initiation phase is a fast, irreversible reaction and usually begin with spontaneous mutations, supported by normal occurrence such as DNA depurination, deamination and errors in DNA replication.[20] Initiation is done by the application of 7, 12-dimethyl benzanthracene (DMBA), is a well known carcinogen and is further promoted by croton oil application. The last stage of carcinogenesis is progression, in which transformation of preneoplastic lesions in to malign lesions occurs and is characterized by irreversibility, genetic instability, invasion and metastization. DMBA toxicity is elucidated by its oxidative stress created due to the formation of free radicals, being highly reactive binds to nucleophilic sites on cellular macromolecules eliciting cancerous responses.[4,5]

Meanwhile, chemoprevention has evolved as an effective strategy fight against this deadly disease. Medicinal plants or herbs with a great diversity in their phytochemical constitution have been shown to be a rich source of cancer chemoprevention.[3] The present study showed the chemopreventive activity of *Aegle marmelos* bark extract on DMBA and croton oil induced papillomagenesis in Swiss albino mice. Significant reduction in tumor incidence, tumor burden, and cumulative number of papilloma was observed, with a marked increase in the average latent period of tumor appearance and Reduced GSH concentration in *Aegle marmelos* bark extract treated group as compared to the animals treated with DMBA and croton oil.

Significant effects were observed indicating that the extract may have either inhibited the metabolism of DMBA to its active form, delayed the promotion of carcinogenesis phase or downregulated ROS (Reactive oxygen species) formation. The chemopreventive activity of the plant extract is may be due to the presence

of secondary metabolites such as tannins, terpenoids, glycosides, and flavonoids present in the hydromethanolic *Aegle marmelos* bark extract [21]. Evidences have also been present which showed that these secondary metabolites possess anticancer activity.[4,22]

Thus, the present piece of work demonstrates that hydromethanolic *Aegle marmelos* bark extract exhibited antitumor and antioxidant activity against DMBA induced skin papillomagenesis.

CONCLUSION

The biochemical fluctuations observed in papilloma bearing mice may be due to the presence of free radicals which resulted in low levels of GSH in carcinogen control group followed by DMBA application. The data obtained from the present study inferred the chemopreventive and antioxidant potentiality of *Aegle marmelos* bark extract on DMBA and croton oil induced papilloma in Swiss albino mice. Further investigation can be carried out to know the molecular mechanism of the drug action.

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