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# Chemopreventive Action of *Bacopa monnieri* (Brahmi) Hydromethanolic Extract on DMBA- Induced Skin Carcinogenesis in Swiss Albino Mice

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*Bacopa monnieri* (L.) Wettst. (Brahmi) (Family: Scrophulariaceae), has been used in the Ayurvedic system of medicine for centuries. In the present study, Cancer Chemopreventive property of *B. monnieri* was evaluated on 7,12-dimethyl benz(a)anthracene (DMBA) induced skin papillomagenesis in male Swiss albino mice (6-7 weeks old). A single topical application of 7,12-dimethyl benz(a)anthracene (104 µg/100 µl of acetone), followed 2 weeks later by repeated application of croton oil (1% in 100 µl acetone two times in a week) and continued till the end of the experiment (After 16 weeks) exhibited 100% tumor incidence. In contrast, mice topically treated on the shaven dorsal side with the *Bacopa monnieri* Hydromethanolic extract (BMH) (dose 120 mg/kg body wt.) & (dose 240mg/kg body wt.) at one hour before each application of 1% Croton oil two times in a week., a significant reduction in the values of tumor incidence, average number of tumors per tumor bearing mouse and papillomas per papilloma bearing mouse were observed. Thus results showed that BMH possesses a Chemopreventive activity and provide evidences for its traditional usage in clinical studies.

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**Keyword:** *Bacopa monnieri*, Chemoprevention, Croton oil, DMBA, Papillomas.

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### 1. Introduction

*Bacopa monnieri*, a member of the Scrophulariaceae family, is a small, creeping herb with numerous branches, small oblong leaves, and light purple flowers. In India and the tropics it grows naturally in wet soil, shallow water, and marshes. The herb can be found at elevations from sea level to altitudes of 4,400 feet, and is easily cultivated if adequate water is available.

Flowers and fruit appear in summer and the entire plant is used medicinally (Mukherjee & Dey 1966, Chopra 1958). Compounds responsible for the pharmacological effects of *Bacopa* include alkaloids, saponins, and sterols. Many active constituents - the alkaloids Brahmine and herpestine, saponins d-mannitol and hersaponin,

acid A, and monnierin – were isolated in India over 40 years ago. Other active constituents have since been identified, including betulinic acid, stigmastanol, beta-sitosterol, as well as numerous bacosides and bacopasaponins. The constituents responsible for *Bacopa*'s cognitive effects are bacosides ( Anbarasi *et al.* 2006). Free radical scavenging activity and protective effect on DNA damage of plant were observed (Ghosh *et al.* 2008). Antimicrobial fractions of various aerial parts of the plant were studied (Ghosh *et al.* 2007).

### 2. Materials and Methods

**2.1. Animals:** The study was conducted on random bred, 6-7 weeks old and 24 - 28 gm body weight bearing, male *Swiss albino* mice. Animals

were maintained under controlled conditions of temperature and light (Light: dark, 10 hrs: 14 hrs.). They were provided in the standard mice feed (procured from Hindustan Levers Ltd. India.) and water *ad libitum*. The study protocol is approved by Institutional Animal Ethical Committee (IAEC) and confirms to the guidelines set by World Health Organization, Geneva, Switzerland and Indian National Science Academy (INSA), New Delhi (India) (Project No. 500/01/a/200/19<sup>th</sup> /proj 2/27-7-09).

**2.2. Chemicals:** The chemicals 7, 12-dimethylbenz (a) anthracene (DMBA) and croton oil were procured from Sigma Chemicals Co., St. Louis, USA. DMBA was dissolved at a concentration of 104 µg/100 µl in acetone. Croton oil was mixed in acetone to give a solution of 1% dilution.

### 2.3. Preparation of the *Bacopa monnieri* Hydromethanolic Extract (BMH)

The plant of *Bacopa monnieri* collected from Herbal Garden of NBRI, Lucknow (U.P.). After authentication (Voucher specimen No: BM/15/2009), the aerial parts of plant material were shade dried & powdered by a mechanical grinder. Powdered material weighed & soaked in petroleum ether for ½ an hr. & then the extract was taken out and dried and then again it was soaked in 50% methanol in a pear shaped separating funnel. Mixture was agitated at regular intervals for 24 hours. The extract was filtered using Whatman filter paper (No.1) and then concentrated in a vacuum at 45°C in Water bath. The extracts were stored at about 2-8°C. Pellets of the drug were obtained and the required dose for treatment was prepared by dissolving the pellets in DMSO at a dose level of 120 and 240 mg/ kg body weight.

**2.4. Experimental Protocol:** Three days before the commencement of the experiment, hair on the interscapular region of the mice were shaved. Only the mice showing no hair growth were selected for the study. The animals were randomly allocated into 7 groups comprising six mice each. The treatment was provided topically

on shaved area using the following protocol Berenblum, 1975.

### 2.5. Treatment Groups:

**I Group: (Vehicle Alone):** 100 µl acetone 2 times /week up to 16 weeks

**II Group: (DMBA Alone):** 104 µg DMBA was dissolved in 100 µl acetone and single application was given.

**III Group: (Croton Oil Alone):** 1% Croton oil was applied on skin 2 times a week up to 16 weeks.

**IV Group: (BMH extract alone):** (dose 240 mg/kg body wt.) (100 µl/mouse) was applied on skin 2 times a week up to 16 weeks.

**V Group: (DMBA+Croton oil):** 104 µg DMBA was dissolved in 100 µl acetone and single application was given afterwards 1 % Croton oil was applied on skin 2 times a week up to 16 weeks.

**VI Group: (DMBA+ BMH extract + Croton oil):** 104 µg DMBA was dissolved in 100 µl acetone and single application was given afterwards the 100 µl dose of BMH at the dose of 120 mg/kg b. wt. dose was given one hour before the each application of 1% croton oil 2 times a week up to 16 weeks.

**VII Group: (DMBA+ BMH extract + Croton oil):** 104 µg DMBA was dissolved in 100 µl acetone and single application was given afterwards the 100 µl dose of BMH at the dose of 240 mg/kg b. wt. dose was given one hour before the each application of 1% croton oil 2 times a week up to 16 weeks.

The animals of all groups were kept under observation for gross and microscopic changes in skin. During the 16 weeks of experimentation, papillomas appearing on the shaven area of the skin were examined & recorded at weekly intervals in all above groups. Only those papillomas which persisted for two weeks or more, with a diameter greater than 2mm, have been taken into consideration for final evaluation of the data. Skin papillomas, which regressed after one observation, were not considered for counting.

### 2.6. Biochemical Study:

Biochemical alterations were studied in all the groups at the time of termination of the experiment (i.e., at 16th week). The hepatic level of glutathione (GSH) was determined by the method of Moron *et al.* 1979. The GSH content in blood was measured spectrophotometrically using Ellman's reagent with 5-5, dithiobis-2-nitrobenzoic acid (DTNB) as a coloring reagent, according to the method of Beutler *et al.* 1963.

### 2.7. Data Analysis:

The differences in the incidence of tumors among different groups are considered to be significant at 5% significance level ( $p < 0.05$ ) when evaluated by Student 't' test.

### 3. Result

The results of the present investigation are furnished in Table 1 & 2. The gain in the body weight in mice was not affected either by the carcinogen or by *B. monnieri* extract administration. A gradual increase in body weight was noted in all animal groups that were near to normal values of the vehicle treated control animals. The vehicle treated control animals (Group I), DMBA alone group (Group II), Croton oil alone group (Group III) as well as the BMH extract alone administered (Group IV) did not show any tumor incidences.

In the BMH extract treated experimental group VI & VII, in which extract was given topically at a dose of 120 mg/kg. body wt. & 240 mg/kg. body wt./animal, mice showed a significant

decrease in tumor number and weight as compared with that of the carcinogen control group (Group V). In the carcinogen treated control group (Group V) skin papillomas appeared in all the animals (100% tumor incidence) from week 7 onwards. The cumulative number of papillomas as induced during the observation period of 16 weeks was 28. The tumor yield as well as the tumor burden was found to be 4.66. These were significantly reduced in the group which received the treatment of BMH additionally at the dose at 120 and 240 mg/kg body weight (Group VI and VII). The tumor incidence in these groups was found to be 66.6% and 50% by the end of the experiment (16 weeks). The values of cumulative number of papillomas and tumor yield were recorded 11 and 8 and 1.8 and 1.5 respectively. The results also indicate that BMH extract has prolonged the average latency period (i.e. time lag between the application of the promoter and the appearance of 50% of tumors) of tumor occurrence. The latency period was found to be 10.51 week in the carcinogen treated control group, whereas it was significantly higher i.e. 13.43 & 12.96 weeks respectively in BMH extract experimental group. A significant fall in glutathione (GSH) activity was noticed in blood and liver in the carcinogen control animals as compared to BMH experimental (Groups VI- VII), at the time of termination of the experiment (i.e., 16 weeks). Treatment of BMH resulted in an enhanced level of GSH ( $p < 0.05$ ) in such groups.

**Table 1:** Effect of BMH on DMBA-induced papillomas in *Swiss albino* mice

S.N.	Groups	Body Weight (gm)		Cumulative No. of Papillomas	Tumor Incidence	Tumor Yield	Tumor Burden	Average Latent Period
		Initial	Final					
I	Vehicle alone	25.31±0.51	31.56±0.49	0	0	0	0	0
II	DMBA alone(one application)	25.7±0.46	31.60±0.36	0	0	0	0	0
III	C.O. alone	26.75 ±0.44	35.6±0.63	0	0	0	0	0
IV	BMH extract alone	26.88±0.29	32.78±0.60	0	0	0	0	0

V	(240mg/kg) DMBA + C. O.	26.63±0.37	32.48±0.67	28	6/6 (100%)	4.66±0.33	4.66±0.33	10.51
VI	(DMBA+ BMH (120mg/kg) + C.O.)	26.6±0.56	32.96±0.48	11	4/6 (66.6%)	1.83±0.65*	2.75±0.25*	13.43
VII	(DMBA+ BMH (240mg/kg) + C.O.)	25.83±0.33	34.12±0.42	9	3/6 (50%)	1.5±0.71*	3.0±0.57*	12.96

\* Significance level among different groups at p< 0.05.

**Table 2:** Effect of BMH on GSH level in blood (µg/ml) & liver (µ mole/gm) in DMBA induced papilloma model.

S.N.	Treatment group	Glutathione level	
		Blood (µg/ml)	Liver (µ mole/gm)
I	Normal mice	4.65 ± 0.28	49.02±0.57
II	Carcinogen (DMBA + C.O.)	2.53 ± 0.05	23.46±0.48
III	DMBA+ BMH (120mg/kg body wt.)+ C.O.	3.02±0.06*	27.0±0.58*
IV	DMBA+ BMH (240 mg/kg body wt.) + C.O.	3.23±0.03*	28.1±0.45*

\* Significance level among different groups at p< 0.05.



**Fig 1:** Showing the Skin tumor papilloma induced by DMBA + Croton oil for 16 weeks



**Fig 2:** Showing reduced Skin tumor papilloma which received the treatment.

#### 4. Discussion

There has been considerable scientific evidence, epidemiologic and experimental, accumulated in the past two decades indicating that modifications in lifestyle, including diet, can have a major effect on the risk for numerous cancers (Martinez 1997). To reduce the occurrence of cancer, one promising approach is its prevention, especially by chemical intervention through minor nutritional dietary constituents (Boone *et al.* 1990, Wattenberg 1985). The present study demonstrates the chemopreventive property of BMH, on the two-stage mechanism of DMBA induced skin papillomagenesis in male Swiss albino mice.

Literature suggests that one subminimal dose of carcinogen “initiates” tumorigenesis and the treatment with croton oil “promotes” development to the visible tumor stage (Berenblum & Shubik 1947). The application of promoter to the mice skin results in the rapid accumulation of inflammatory cells such as neutrophils and macrophages (Lewis & Adams 1987) and an increase in the release of active oxygen species (Copeland 1983, Cerrutti 1985). Topical application of TPA (active constituent of croton oil) has been reported to increase production of free radicals (Huachen & Krystyna 1991). This is perhaps due to the free radical oxidative stress that has been implicated in the pathogenesis of a wide variety of clinical disorders (Das 2002). Glutathione is one of the antioxidant enzymes that act as the first line of defense against prooxidant stress.

Several studies have shown that compounds that possess anti-inflammatory property inhibit 12-O-tetradecanoyl phorbol-13-acetate induced tumor promotion in mouse skin while **Aurore** and co-workers reported that anti-inflammatory steroids drastically inhibits epidermal DNA synthesis and cellular proliferation induced by phorbol ester tumor promoters, a pre-requisite for tumorigenesis (Aurore *et al.* 1977). The cancer chemopreventive efficacy is assessed by the ability to modulate the activities of enzymes associated with drug metabolism and bifunctional modulators reduced the availability of ultimate

carcinogen metabolites in the epithelial stage. It is known that application of promoter containing phorbol ester generates free radicals (Copeland 1983, Cerrutti 1985) which are scavenged by plant products possessing anti-oxidant property (Ziboh 1985, Huang *et al.* 1988). It was supposed that the inhibition of tumorigenesis by the plant extract might have been executed either by preventing the formation of active carcinogens from their precursors or by augmenting detoxification process, preventing promotional events in the mouse skin through free radical scavenging mechanism. However, further studies are required to elucidate the exact mechanism underlying the chemopreventive property of *Bacopa monnieri*.

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