

CHEMOPREVENTIVE ACTION OF *BOERHAAVIA DIFFUSA* ON DMBA-INDUCED SKIN CARCINOGENESIS IN MICE

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Abstract : *Boerhaavia diffusa*, Linn (Fam: Nyctagenaceae), is widely used for the treatment of Jaundice in various parts of India. In the present study, cancer chemopreventive property of *B. diffusa* 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 (50 µg/50 µl of acetone), followed 2 weeks later by repeated application of croton oil (1% in acetone three times a week) and continued till the end of the experiment exhibited 100% tumor incidence. In contrast, mice treated topically on the shaven backs with the *Boerhaavia diffusa* extract at either the peri-initiational phase (i.e. 7 days before and 7 days after the application of DMBA; Group II), post initiational phase (i.e. from the day of start of croton oil treatment and continued till the end of the experiment; Group III) or continuously at the peri- and post-initiational stages (i.e. 7 days prior to DMBA application and continued till the end of the experiment; Group IV), a significant reduction in the values of tumor incidence (Group II – 65%; Group III – 30%; Group IV – 25%), average number of tumors per tumor bearing mouse (Group II – 2.8; Group III – 0.75; Group IV – 0.35) and papillomas per papilloma bearing mouse (Group II – 3.1; Group III – 2.5; Group IV – 1.2) were observed.

Key words : chemoprevention
DMBA

Boerhaavia diffusa Linn
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INTRODUCTION

Boerhaavia diffusa, Linn (Fam: Nyctagenaceae) commonly known as “Punarnava” in the Indian system of medicine is a perennial creeping herb found throughout India (1). The plant serves as a

non-conventional vegetable, specially in the North-Eastern region of India (2). A large number of tribes in India use the plant for the treatment of Jaundice and various other liver disorders (1). The plant is also reported to be diuretic and laxative and are given for the treatment of anasarca, ascites and

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jaundice (3, 4). The roots of *Boerhaavia diffusa* have been found to have anti-inflammatory, diuretic, fibrinolytic, nephrotic syndrome and anti-convulsant activities (5–10). Investigations on the chemical constituents of the plant has indicated the occurrence of two novel alkaloids, Punarnavine-1 and Punarnavine-2, belonging to the group quinolizidine (11). From the roots, seeds and leaves of *Boerhaavia diffusa*, isolation of β -sitosterol, β -sitosterol- β -D-glucoside, tetracosanoic, hexacosanoic, stearic, palmitic, arachidic acids, hextriactane, urosolic acid has been reported (12–14).

METHODS

Random bred, male, Swiss albino mice (6–7 weeks old : body weight 12–15 gms.) were obtained from animal house of Biotechnology Department, Gauhati University and housed under normal condition having natural photoperiod (12 hours light/dark cycle) at temperature $25\pm 1^\circ\text{C}$ and 50–60% humidity. Animal experimentation protocols conform to the Institutional Animal Ethics Committee's guidelines. They were provided with standard feed and tap water *ad libitum*. The hairs on the dorsal skin of the animals in the interscapular area was shaved 3 days before the commencement of the experiment and only those animals in the resting phase of the hair cycle were taken for the study, because resting hair retains carcinogens for a longer period. Body weight of the animals were recorded at weekly intervals and also at the time of autopsy.

7, 12-dimethylbenz(a)anthracene (DMBA) and croton oil were obtained from Sigma

Chemicals Co. (St. Louis, USA). DMBA was prepared in acetone at a concentration of $50\ \mu\text{g}/50\ \mu\text{l}$. Croton oil was diluted in acetone to give a solution of 1% dilution.

The fresh leaves and stems of the plant were collected from the foothills of Nilachal ranges (Guwahati) after proper identification. The leaves were washed, shade-dried and ground. A known quantity of leaves (50 gm) were then subjected to soxhlet extraction using 80% hydro-alcoholic solvent (80% ethanol : 20% distilled water). The final extract was filtered and the remaining alcohol was allowed to evaporate. The yield was 7.78%. The thick paste obtained was stored at 4°C till further use. After appropriate dilution in acetone the extract was topically applied over the shaven area of the skin of mice at a dose level of 5 mg/kg body weight/day.

The animals were separated into the following groups:–

Group I (n = 20): A single dose of $50\ \mu\text{g}$ of DMBA in $50\ \mu\text{l}$ of acetone was applied topically over the shaven area of the skin of the mice. Two weeks later croton oil ($100\ \mu\text{l}$ of 1% croton oil in acetone) was applied three times per week until the end of the experiment.

Group II (n = 20): Animals received a topical application (on the shaven area of the skin) of the extract of *Boerhaavia diffusa* (5 mg/kg body weight in $100\ \mu\text{l}$ of acetone) 7 days before and 7 days after the application of DMBA. Croton oil was given as in Group I.

Group III (n = 20): Animals received a topical application of *Boerhaavia diffusa* extract (5 mg/kg body weight in 100 µl acetone) starting from the time of croton oil application till the end of the experiment (15 weeks). DMBA was given as in Group I.

Group IV (n = 20): Animals were treated topically with *Boerhaavia diffusa* extract (5 mg/kg body weight in 100 µl of acetone) throughout the experimental period i.e. before and after DMBA application and also at the promotional stage till the end of the experiment (15 weeks). Croton oil was given as in Group I.

Papillomas appearing on the shaven area of the skin were recorded at weekly intervals. Only those papillomas that persisted for two weeks or more have been considered for final analysis of the data. Chi-square test was employed to evaluate the significance level of difference between control and experimental values.

RESULTS AND DISCUSSION

Table I depicts the findings of the present investigation. The gain in body

weight is not affected by the application of *Boerhaavia diffusa* extract. In the control group (Group I) in which a single topical application of DMBA was followed 2 weeks later by repeated application of croton oil, showed 100% tumor incidence and the average number of papillomas per mouse as well as the number of papillomas per papilloma bearing mouse was found to be 5.4. Animals of Group II, which received the *Boerhaavia diffusa* extract treatment at the peri-initiational phase of tumorigenesis, showed 65% tumor incidence and the average number of tumors per mouse and papillomas per papilloma bearing mouse were 2 and 3.1 respectively. All animals in Group III which were treated with *Boerhaavia diffusa* extract at the promotional stage, showed only 30% tumor incidence. The average number of tumors per mouse and papillomas per papilloma bearing mouse were observed to be 0.75 and 2.5 respectively. Mice of Group IV, given a continuous application of the extract at the peri- as well as at the post-initiational phases, showed a significant reduction in the incidence of tumor (25%) as well as in the average number of tumors per mouse (0.35) and papillomas per papilloma bearing mouse (1, 2).

TABLE I: Chemopreventive effect of *Boerhaavia diffusa*, Linn, on DMBA-induced skin papillomagenesis in mice.

Groups	Total number of animals		Body weight(g) (Mean±SD)		Tumors per tumor bearing mice	Mice with papillomas(%)	Average number of papillomas per mouse
	Initial	Effective	Initian	Final			
I	20	20	13.25±1.45	22.25± 2.94	5.4	100	5.4
II	20	20	13.7±3.23	21.9± 3.46	3.1*	65*	2.0*
III	20	20	13.15±1.46	22.75± 5.58	2.5*	30*	0.75*
IV	20	20	14.1±1.33	25.0± 4.66	1.2*	25*	0.35*

*P<0.05

The difference in the values of control and treated groups were found to be significant at 5% probability level.

Cumulative number of papillomas and percentage inhibition of tumor multiplicity in control and experimental groups during the observation period has been shown in Fig. 1 and 2 respectively.

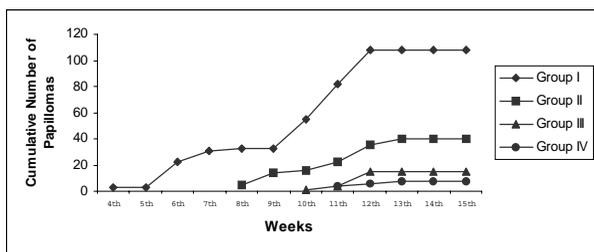


Fig. 1: Effect of *Boerhaavia diffusa*, Linn on cumulative number of papillomas in the treated groups (Group II, III and IV) in contrast to the control (Group I) mice.

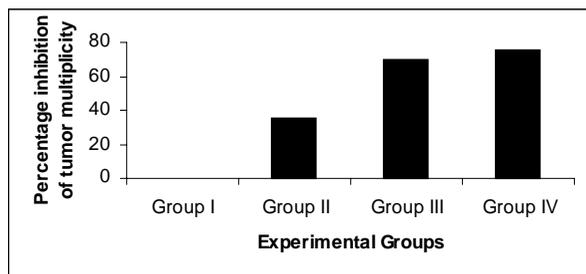


Fig. 2: Effect of *Boerhaavia diffusa*, Linn on percentage inhibition of tumor multiplicity in the treated groups (Group II, III and IV) in contrast to the control (Group I) mice.

To reduce the occurrence of cancer, one promising approach is its prevention, specially by chemical intervention through minor nutritional dietary constituents (15, 16). The present study demonstrates the chemopreventive property of *Boerhaavia diffusa* extract, 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 (17). The present findings revealed the same with 100% tumor incidence in the control group (Group I). The application of promoter to the mice skin results in the rapid accumulation of inflammatory cells such as neutrophils and macrophages (18) and an increase in the release of active oxygen species (19, 20). 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 (21). Though the exact mechanism underlying the anti-inflammatory action of *Boerhaavia diffusa* has not been ascertained, it may be inferred that presence of steroidal compounds including sitosterol (12) and the anti-inflammatory property of *Boerhaavia diffusa* which has been reported by many workers (6–10) might have played a synergistic role in the inhibition of tumorigenesis as observed in the present investigation.

The cancer chemopreventive efficacy is assessed by its 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 (19–20) which are scavenged by plant products possessing anti-oxidant property (23, 24). A significant increase in the activities of hepatic phase I (i.e., cytochrome P₄₅₀ and cytochrome b₅), phase II (i.e., Glutathione-S-transferases or GSTs) system enzymes and antioxidant enzymes (Glutathione peroxidase, Glutathione reductase, Superoxide dismutase, Catalase and Glutathione level) were observed when mice were fed by oral gavage with *Boerhaavia diffusa* extract at a dose level of 125 mg and 250 mg/kg body weight for a period of 14 days in our laboratory (25). This leads to the supposition 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 *Boerhaavia diffusa*.

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