

Effect of *Bauhinia Variegata* Bark Extract on DMBA-Induced Mouse Skin Carcinogenesis: A Preliminary Study

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Abstract: *Bauhinia variegata* Linn. (Kanchnar/Rakta kanchan), is a widely used medicinal plant by the tribals throughout India and popular in various indigenous system of medicine like Ayurveda, Unani and Homoeopathy. In the present investigations, the anticarcinogenic activity of *Bauhinia variegata* bark extract was evaluated using two stage protocol in skin papilloma model in *Swiss albino* mice. The significant prevention of papillomas in DMBA + *B. variegata* bark extract (500 and 1000 mg/kg body weight) + croton oil treated group was found to be effective in decreasing the rate of tumor incidence in comparison to the control. Furthermore, cumulative number of papillomas, tumor yield and tumor burden were also found to be reduced. The depleted levels of glutathione were restored in *Bauhinia variegata* bark extract treated groups. The study has revealed the chemopreventive role of *B. variegata* bark extracts against DMBA-induced skin carcinogenesis in mice.

Key words: *Bauhinia variegata* • Papilloma • Skin carcinogenesis • Chemoprevention • Glutathione

INTRODUCTION

Bauhinia variegata (Family *fabaceae*, Genus *Bauhinia*) is an herbaceous plant, found throughout India. The plant is known as Kachnara in Sanskrit and Hindi. Its powdered bark is traditionally used for tonic, astrain; ulcers. It is also useful in skin disease [1]. The bark is alterative, anthelmintic, astringent and tonic. The juice of the bark is used in the treatment of amoebic dysentery, diarrhoea and other stomach disorders. A paste of the bark is useful in the treatment of cuts and wounds, skin diseases, scrofula and ulcers. It can also be used in cough conditions, asthma, abdominal distention, also act as a gargle for sore throats, prevent from skin diseases, or internally as a remedy for diarrhea. It is helpful in managing skin discoloration [2,3].

There are various types of the fatty acid compound found from *B. variegata* such as linolenic acid, oleic, steric, palmitic and myristic acid [4]. A new lectin from seeds of the *B. variegata* was purified and biochemical characterized [5] It also shows the Anti-inflammatory activity by the flavonol glycoside which is present in the *Bauhinia Variegata*. The antibacterial activity of all the extracts of *B. variegata* was reported [6]. An infusion

from its bark is used as an astringent, tonic and useful in scrofula, skin diseases and ulcers. Previous phytochemical studies on the stems [7,8,9] flowers [10,11] and seeds [12] of this species have led to the isolation of several flavonoids. The antitumor activity of the ethanolic extract of *B. variegata* also reported against Dalton's ascetic lymphoma (DAL) in Swiss albino mice [13] and in N-nitrosodiethylamine induced experimental liver tumour in rats and human cancer cell lines [14]. The sub chronic toxicity study was also reported on *albino* rats treated with alcoholic extract of *B. variegata* [15]. Therefore we have planned to carry out this study to see the chemopreventive effect in experimental animals.

MATERIALS AND METHODS

Animals: The study was conducted on random bred, 6-7 weeks old and 24- 28 gm body weight bearing, male *Swiss albino* mice (*Mus musculus*). Animals were maintained under controlled conditions of temperature and light (Light: dark, 10 hrs: 14 hrs.). They were provided standard mice feed (procured from Hindustan Levers Ltd., India) and water *ad libitum*. The study protocol is approved by the Departmental Animal Ethical Committee

and confirms to the guidelines set by World Health Organization, Geneva, Switzerland and Indian National Science Academy (INSA), New Delhi (India).

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.

Preparation of the *Bauhinia Veriegata* Bark Extract:

The identification of the plant *Bauhinia veriegata* (Kachnar) (family: *Leguminose*) was done by botanist Dr. S.S. Khan (Voucher Specimen No: SP/101/LGOB/2006), Department of Botany, Safia Science College, Bhopal, Madhya Pradesh (India). The non-infected stem barks of the plant were extracted with 95% methanol by refluxing for 36 hrs. at 50-60°C. The powder was treated with petroleum ether for 3 hours for defatting. 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 500 and 1000 mg/ kg body weight.

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 8 groups comprising six mice each. The treatment was provided topically on shaved area using the following protocol Berenblum, 1975.

Treatment Groups

Group 1 (Untreated control): No treatment

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

Group 3 (DMBA Alone): - 104 µg DMBA was dissolved in 100 µl acetone and single application was given.

Group 4 (Croton Oil Alone): - 1 % Croton oil was applied on skin 2 times a week up to 16 week.

Group 5 (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 week.

Group 6 (DMBA + *B. Veriegata* Bark Extract. + Croton Oil): - 104 µg DMBA was dissolved in 100 µl acetone and single application was given afterwards the 100 µl dose of *B. veriegata* bark extract at the dose of 500 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.

Group 7 (DMBA + *B. Veriegata* Bark Extract. + Croton Oil): - 104 µg DMBA was dissolved in 100 µl acetone and single application was given afterwards the 100 µl dose of *B. veriegata* bark extract at the dose of 1000 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.

Group 8 (*B. Veriegata* Bark Extract Alone): - was applied on skin 2 times a week up to 16 week.

The animals of all groups were kept under observation for gross and microscopic changes in skin.

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.* [16]. 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.* [17].

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

RESULTS

The results of the present investigation have been summarized in Tables 1 and 2. Single topical application of DMBA followed by croton oil, produced skin papillomas, which started appearing from the sixth week onward. The tumor incidence in the DMBA + croton oil treated mice (carcinogen control) reached 100% by the end of the experiment (16 weeks). The cumulative number of papillomas in these mice was recorded as 22. The average number of papillomas per mouse (tumor yield) as well as the papillomas per papilloma-bearing mice (tumor burden) was found to be 3.6 (Fig. 1). These were significantly reduced in the group which received the treatment of *B. variegata* stem bark extract additionally at the dose of 500 and 1000 mg/kg body weight (groups VI and VII).

Table 1: Effect of *B. variegata* (Kachnar) stem bark extract on DMBA-induced papillomas in *Swiss albino* mice

S.N.	Groups	Cumulative No. of Papillomas	Tumour Incidence	Tumour Yield	Tumour Burden	Average Latent Period
I	Vehicle alone	00	0/6	00	00	00
II	DMBA alone (1 application)	00	0/6	00	00	00
III	Croton oil alone	00	0/6	00	00	00
IV	<i>B. variegata</i> bark extract alone	00	00	00	00	00
V	DMBA+ Croton oil	22	6/6 (100%)	3.6±0.42	3.6±0.42	7.34±0.31
VI	DMBA + <i>B. variegata</i> bark extract (500 mg/kg)+ Croton oil	11	4/6 (66.66%)	1.8±0.65*	2.7±0.47*	8.29±0.29
VII	DMBA + <i>B. variegata</i> bark extract (1000 mg/kg)+ Croton oil	8	3/6 (50 %)	1.3±0.61*	2.6±0.19*	9.21±0.53

* Significance level among different groups at p< 0.05.

Table 2: (B) Variation in the glutathione level during DMBA-induced skin carcinogenesis with/without *B. variegata* stem bark extracts treatment

S.N.	Treatment group	Glutathione level	
		Blood (µg/ml)	Liver (µ mole/gm)
I	Normal mice	3.49 ± 0.03	63.43 ± 0.59
II	Carcinogen (DMBA + Croton oil)	2.80 ± 0.06	55.98 ± 0.87
III	DMBA + <i>B. variegata</i> bark extract (500 mg/kg) + Croton Oil	2.98 ± 0.17*	59.81 ± 0.06*
IV	DMBA + <i>B. variegata</i> bark extract (1000 mg/kg)+ Croton Oil	3.02 ± 0.05*	61.17 ± 0.05*

Data are reported as mean ± s.e., n=6

* Significance level among different groups at p< 0.05



Fig. 1: Photograph showing the skin tumour induced by DMBA + Croton oil for 16 weeks



Fig. 2: Photograph showing the skin tumour which received the treatment of DMBA + *B. variegata* bark extract + Croton oil for 16 weeks

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 1.3 respectively. The average latency period (i.e. time lag between the application of the promoter and the appearance of 50% of tumors) was also greater with *B. variegata* bark extract by topical application (Fig. 2). Vehicle Control, No treatment, *B. variegata* stem bark extract alone, Croton oil

alone and DMBA alone groups did not induced any tumor incidence.

A significant fall in glutathione (GSH) activity was noticed in blood and liver in the carcinogen control animals as compared to *B. variegata* bark extract experimental (groups VI- VII), at the time of termination of the experiment (i.e., 16 weeks). Treatment of *B. variegata* resulted in an enhanced level of GSH (p<0.05) in such groups.

DISCUSSION

The present study demonstrates 100% tumor incidence in the carcinogen control group. Topical application of TPA (active constituent of croton oil) has been reported to increase production of free radicals [18]. This is perhaps due to the free radical oxidative stress that has been implicated in the pathogenesis of a wide variety of clinical disorders [19]. Glutathione is one of the antioxidant enzymes that act as the first line of defense against prooxidant stress. One of the mechanisms by which *B. variegata* rendered protection against carcinogen can be an elevation in the glutathione level that could have been mediated through the modulation of cellular antioxidant level.

The anticarcinogenic effect in skin papilloma model in *Swiss albino* mice of *B. variegata* bark extracts were observed. The phytochemical study indicated the presence of flavonoids, lectin and albumin in *B. variegata* extract. Flavonoids which have been shown to anticarcinogenic activity [20,21] and lectins reported to produce structural variation of the cell envelope [22]. Thus, antitumour effect produced by the *Bauhinia* extract may be due to its flavonoid and lectin the antitumour activity of ethanolic extract of *B. variegata* was also reported against Dalton's ascetic lymphoma (DAL) in *Swiss albino* mice [13] and in experimental liver tumour in rats [14]. These reports support our finding. Since *B. variegata* is an important herbal drug used as a tonic in Arurveda a traditional medical system of India.

The major proposal for action of *B. variegata* bark extract seems to be the effectiveness to intercept the free radicals and protect cellular molecules from oxidative damage. Further, it modulates glutathione level and is found to inhibit in liver and blood. The mechanism underlying the chemopreventive action of *B. variegata* bark extract and its active principles is not clear; the beneficial effect of *B. variegata* bark extract may be due to either individual or combined effects of its constituents. All these data point to the possibility of developing an extract of *Bauhinia variegata* as a novel, potential agent in the area of cancer chemotherapy. Further research is required in this direction.

Single application of DMBA was given at the dose of 104 mg/kg body wt. and followed 1 week later by 1% croton oil was given 1 hour after the each applications of *B. variegata* leaf extract 2 times/week until the end of the experiment (i.e. 16 weeks); *B. variegata*

stem bark extract 500 and 1000 mg/kg/2 cm²/week/mice treatment starting with the application of DMBA until the end of the experiment.

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