RESEARCH COMMUNICATION

Evaluation of Anticarcinogenic and Antimutagenic Potential of *Bauhinia variegata* Extract in Swiss Albino Mice

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Abstract:

Background: Infusions from the bark of *Bauhinia* is used to treat various diseases in the traditional medical system of India and decoction of the roots is used in dyspepsia and act as an antidote to snake poison. Its chemopreventive potential for cancer was the subject of the present study. Materials and methods: To evaluate the anticarcinogenicity and antimutagenicity of Kachanar extract a skin carcinogenesis and melanoma tumour model was used, along with micronucleus and chromosomal aberration tests, in Swiss albino mice. Results: In the skin papilloma model, significant prevention, with delayed appearance and reduction in the cumulative no. of papillomas was observed in the DMBA + Kachanar + croton oil treated group as compared to the DMBA + Croton Oil group. C57 Bl mice which received a 50 % methanolic extract of Kachanar extract at the doses of 500 and 1000 mg/ kg body weight for 30 days showed increase in life span and tumour size was significantly reduced as compared to controls. In antimutagenicity studies,a single application of Kachanar extract at doses of 300, 600 and 900 mg/kg dry weight, 24 hours prior the i.p. administration of cyclophosphamide (at the 50 mg/ kg) significantly prevented micronucleus formation and chromosomal aberrations in bone marrow cells of mice, in a dose dependent manner. Conclusions: Our results suggest that Kachanar extract exerts anticarcinogenic and antimutagenic activity.

Key words: Kachanar - chemoprevention - skin carcinogenesis - micronucleus test - mutagenicity

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Introduction

Herbal medicines are the oldest remedies known to mankind. Herbs had been used by all cultures throughout history but India has one of the oldest, richest and most diverse cultural living traditions associated with the use of medicinal plants (Brown, 1980). Plants have formed the basis for the treatment of diseases in traditional medicine systems for thousands of years and continue to play a major role in the primary health care of about 80% of the world's inhabitants (Davies,1994) *Bauhinia variegata* L (whose Hindi name is Kachanar) of the family Leguminosae is a medium-sized deciduous tree found on the rocky hills of Circars, Deccan, and Carnatic regions of South India (Fransworth et al.,1985).

An infusion from the bark is used as an astringent, tonic, scrofula, skin diseases, and ulcers. The decoction of the roots is used in dyspepsia and act as an antidote to snake poison (Frohne, 1999). Previous phytochemical studies on the stems (Gamble, 1956; Gupta et al., 1979; 1980), flowers (Gupta and Chauhan, 1984; Gordon and David, 2001), leaves (Sharma et al, 1968; Singh et al., 2006) and seeds (Hirano et al, 1989) of this species have led to the isolation of several flavonoids.

There are various types of fatty acid compound found in *B. variegata* such as linolinic acid, oleic, steric, palmitic and myristic acid (Jose et al, 2007). A new lectin from seeds of the *B.variegata* was purified and biochemically characterized (Montobrind, 2000). Anti-inflammatory and antibacterial activity of all the extracts of *B. variegata* was reported (Rajkapoor et al., 2006). There are also a few reports of antitumour activity of *B.variegata* ethanolic extract against Dalton's ascetic lymphoma (DAL) in Swiss albino mice (Rajkapoor, 2004) and N-nitrosodiethylamine induced experimental liver tumours in rats and human cancer cell lines (Rajkapoor, 2003). Subchronic toxicity was also reported in albino rats treated with an alcoholic extract of *B.variegata* (Rahman and Begum, 1966).

Since there is a paucity of information on the antimutagenicity and anticarcinogenicity of compounds widely used in Ayurvedic medicine, we have therefore undertaken to study the anticarcinogenicity of plant extracts using a skin papilloma and melanoma tumour model and antimutagenicity using micronucleus and chromosomal aberration tests in bone marrow cells of mice. Here we present our findings with a methanol extract of *B.variegata*.

Materials and Methods

Chemicals

Bauhinia veriegata fresh leaves were obtained from

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the local garden in middle of July 2007 and identified by R.K.Patil, botanist from herbarium of MFP processing & Research centre, Bhopal. Fresh leaves of Bauhinia were dried in shades and powdered using mixer and grinder. After that 50 % methanolic extract was prepared. using separating funnel. The project was approved by the institutional animal ethic committee of J.N.Cancer Hospital and Research Centre, Bhopal (Project no.43, Ref.No. 670/1225 IAEC/2008). 7,12,Dimethyl benz(a)nthracene (DMBA) croton oil and Cyclophosphamide were purchased from Sigma chemical Co., U.S.A. and other chemical were reagents grade and purchased locally.

Skin Bioassay Protocol

It was done as per the method described by Berenblum (1975) in his two stage skin carcinogenesis protocol. Male Swiss Albino mice of 15-20 gms body weight were used in the study. They were kept on synthetic pellet diet and water ad libitum. The animals were randomly divided in to 8 groups. Each group comprises of 6 animals. Mice were shaved in 2cm2 area with the help of hair removing cream in interscapular region initially and after every 2 weeks hair were removed with the help of scissors. The treatment was provided topically on shaved area using the following protocol.

Group 1 (Untreated control) No treatment was given.

Group 2 (Vehicle control) 100µl acetone 2 times /week was given up to 8 weeks

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

Group 4 (DMBA + Croton Oil) 104 µg DMBA was dissolved in 100µl acetone and single application was given afterwards 1 % Croton oil was applied on skin 2 times a week up to 8 week .

Group 5 (DMBA + Kachanar ext. + Croton Oil) 104 µg DMBA was dissolved in 100µl acetone and single application was given after one week, the 100µl Kachanar extract at the dose of 500 mg/kg b. wt. was given one hour before the each application of 1 % croton oil 2 times a week up to 8 weeks.

Group 6 (DMBA + Kachanar ext. + Croton Oil) 104 µg DMBA was dissolved in 100µl acetone and single application was given after one week the 100µl Kachanar extract at the dose of 1,000 mg/kg b. wt. was given one hour before the each application of 1 % croton oil 2 times a week up to 8 weeks

Group 7 (Croton oil alone) 1 % Croton oil was applied on skin 2 times in a week up to 8 weeks.

Group 8 (Kachanar extract alone) The 100 µl Kachanar extract at the dose of 500 mg/kg b. wt. was given 2 times a week up to 8 weeks

The animals of all groups were kept under observation and tissues were fixed in neutral formalin for gross and microscopic changes.

Antitumour activities

Melanoma cell line were obtained from National Cell science centre, Pune, and maintained in our laboratory. The C57 Bl hybrid mice of both sexes of the mean weight of 25 gm and 6-7 weeks old were obtained from the animal colony of our institute. They were housed in good laboratory condition and given standard mouse pellet diet and water ad Libitum. All the mice were kept at controlled light and temperature condition. Cell suspension having total 0.5 million cell / animal were injected. After implantation of the melanoma cell line, animal were kept under observation and experiment was started after 10 days when the tumours were seen. The treatment of Kachanar extract was given orally for 30 days and tumour volume and survival time of each animal was recorded. The following groups were maintained.

Control Group: This group consisted of four mice. The melanoma cell line (B6F10) were injected subcutaneously (S.C.) in all four mice.

Test Group – This group was divided into two subgroups. Each group consisted of four animals. The melanoma cell line was injected by s.c. route. The tumour bearing mice were orally given dose of 500 and 1,000 mg/ Kg body weight in 50 % methanolic extract of Kachanar.

Micronucleus Assay and Chromosomal Aberrations Test

Animals and Treatment

Male Swiss albino mice of 15-20 g body wt. were obtained from the animal colony of our Research Centre and 4-5 animals were housed in each plastic cage. The animals were provided standard pallet diet and water ad libitum. For the chromosomal aberrations assay, the Kachanar extract at the volume of 0.2 ml was injected 24 hours before the treatment of cyclophosphamide. The positive control group received single i.p. injection of 50 mg/kg cyclophosphamide in 0.9% saline. Colchicine (4 mg / kg b.wt) was administered intraperitoneally 2 hours before the harvest of the cells. Animals were sacrificed by cervical dislocation and bone marrow cells were harvested. The slides were prepared essentially as per modified method of Preston et al (1987) for chromosomal aberrations and method of Schmid (1975) for micronucleus evaluations. For chromosomal aberration assay, the femur was excised and the bone marrow was extracted in 0.56 % KCl. The harvested cells were incubated at 370C for 20 minutes and then centrifuged for 10 minutes at 1000 rpm. Cells were fixed in Carney's fixative (Methanol: Acetic acid, 3:1) and burst opened on a clean slide to release the chromosomes. The slides were stained with 5 % Giemsa solution for 15 minutes and then put in xylene and mounted with DPX. A total of 100 well spread metaphase plates were scored for chromosomal aberrations at a magnification of 1,000 X (100 x 10 X) for each group. Different types of chromosomal aberrations such as chromatid breaks, gaps, pulverization, polyploidy, centromeric association etc. were scored and expressed as % chromosomal aberrations. About 1000 cells were counted and number of micronucleated cells was also scored.

The data are presented in MNPCE+SE. The statistical significance of differences was evaluated using the Student's t test.

Results

The anticarcinogenic activity of Kachanar extract was evaluated using two stage protocol in skin papillioma model in Swiss albino mice and melanoma model in C57 Bl tumour bearing mice. In the skin papilloma model, significant prevention of papilloma development was observed in the DMBA + Kachanar + Croton oil group (50 and 67 % tumours in groups 5 and 6, respectively) as compared to DMBA + Croton Oil group (100 % tumour). The first appearance of papilloma was also delayed in DMBA + Kachanar + Croton oil group (53 and 45 days in group 5 and 6 respectively) as compared to DMBA + Croton Oil group (27 days) The cumulative no. of papillomas was also reduced in DMBA + Kachanar + Croton oil group (6 and 3 in groups 5 and 6 respectively) as compared to DMBA + Croton Oil group (12 papillomas). In another experiment, the C57 Bl mice which received extract of Kachanar at the dose of 500 and 1,000 mg/ kg body weight for 30 days showed increase in life span of animals and tumour size was significantly reduced in Kachanar extract treated mice as compared to control. The tumour volume was significantly reduced to 35 % and 48 % in Kachanar extract treated mice as compared to 135 % in untreated control animals.

In antimutagenicity studies, single application of Bauhinia ext (Kachanar ext.) at the doses of 300, 600 and 900 mg/kg dry weight, 24 hours prior the i.p. administration of Cyclophosphamide (at the dose of 50 mg/kg) have significantly prevented the micronucleus formations and chromosomal aberrations in dose dependent manner in bone marrow cells of mice as compared to Cyclophosphamide group (Table 1). Dose dependent protection was observed in chromosomal aberrations in bone marrow cells of mice in *Bauhinia* extract treated mice as compared to known mutagen, Cyclophosphamide treated groups (Table 2).

Table 1. Effects of Kachanar Extract on Micronucleus Formation in Mouse Bone Marrow Cells

Group	MNPCE+SE	PCE/NCE Ratio
Cyclophosphamide (50 mg/kg)	3.30 ±0.56	0.455±0.219
Kachanar + Cyclophosphamide		
(a) 300mg/kg	0.800±0.84*	1.780±0.681
(b) 600 mg/kg	1.166±0.654*	1.326±0.395
(c) 900 mg/kg	0.667±0.632 *	1.361±0.254
Kachanar alone	0.000±0.000	1.338±0.150
Solvent (Water)	0.45 ±0.03	0.549±0.080

*denotes statistical significance as compared to cyclophosphamide at P<0.05

Table 2. Protection against Chromosomal Aberrations by Kachanar Extract

%Chromo.	Chromatid Aber.	Break	Fragment	Associ	Ring	Protection %
Cyclophosphamide (50 mg/kg)	47.8 ±3.16	18.6	3.00	11.0	5.08	-
Kachanar + Cy						
(a) 300 mg/kg	31.5±0.22*	11 .0	0.79	6.29	0.79	21.6
(b) 600 mg/kg	30.4±0.15*	8.69	7.82	12.1	-	36.3
(c) 900 mg /kg	20.9±0.10*	9.09	1.81	3.63	1.81	56.3
Kachanar alone	0.00±0.00	-	-	-	-	-
Solvent (Water)	10.0±2.90	5.37	6.25	-	-	-

*denotes statistical significance as compared to cyclophosphamide at P<0.05

Discussion

Chemoprevention is currently an important strategy for controlling the process of cancer induction. Therefore, there is a need to explore medicinal plants or other natural agents that can work as chemopreventive agents. The present study demonstrates the chemopreventive potential of Kachanar leaves extract on DMBA-induced skin tumorigensis in male Swiss albino and C57 BL mice. The skin model in experimental animals has been found to be a useful system for investigating the influence of dietary chemopreventors both mechanistically and operationally (Morse and Stoner, 1996).

The present study demonstrated that topical application of the Kachanar leaves extract (500 and 1000 mg/kg body weight) at the pre promotion phase showed a significant reduction in tumor incidence, tumor burden, tumor weight, tumor size, cumulative number of papillomas, in Kachanar treated groups as compared to the carcinogen treated control. The antitumour activity of ethanolic extract of *B.variegata* was reported in Dalton's ascetic lymphoma (DAL) in Swiss albino mice and in liver tumour in rats (Rajkumar et al., 2006). The exact mechanisms of anticarcinogenic and antimutagenic effects of kachnar are not yet known. However, significant effects were achieved; implying that the plant extract may have either inhibited the metabolism of DMBA to its active form, delayed the promotion phase of carcinogenesis, or down regulated reactive oxygen species formation. Such depletion of tumorigenesis owing to similar factors and in various plants has been reported by others (Kausar et al., 2003; Sancheti et al., 2005; Kumar et al., 2006). Evidence has accumulated to suggest that this is perhaps due to reactive oxygen species, which play an important role in tumor initiation by enhancing or facilitating the metabolic activation and/or initiating effects of carcinogens (Ather, 2002). The methonolic extract of Bauhinia racemosa also produced a protective effect by decreasing the level of serum enzymes, bilirubin and increased the protein and uric acid levels (Kumar et al., 2007). The flavonoids which are present in the extract and have been reported to posse's antimutagenic and anticarcinogenic activity may be possible the activity of Kachanar extract (Brown, 1980; Hirano et al., 1989). The present work suggests further evaluation of the efficacy of this well-known plant is warranted.

In conclusion, the present results suggest anticarcinogenic and antimutagenic activity of Kachanar extracts.

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