Chemopreventive Potential of *Bauhinia variegata* Flower Extract Against DMBA-induced Skin Papillomagenesis in Mice

Sonam Pandey* and R.C. Agrawal

Department of Research
Jawaharlal Nehru Cancer Hospital and Research Centre
Idgah Hills Bhopal, Madhya Pradesh, India

E.Mail : sonam_research@yahoo.com

Summary

The present investigation was undertaken to explore the Chemopreventive action of *Bauhinia variegata* flower extract on 2-stage skin carcinogenesis, induced by a single topical application of 7, 12-dimethylbenz(a)anthracene (DMBA) (104 µg/ 100 µl acetone), and one weeks later, promoted by repeated application of croton oil (1% in acetone/twice in a week) till the end of the experiment (16 weeks) in Swiss albino mice. Single topical application of *B. variegata* flower extract at a dose of 500 and 1000 mg/kg body weight along with DMBA + Croton oil was found to be effective in decreasing the cumulative number of papilloma, tumour incidence, tumour yield and tumour burden as compared to control (DMBA + Croton oil) group. The differences in the values of the results of experimental groups were statistically analysed and found to be significant in comparison to the control group ($p<0.05$). The depleted levels of glutathione were restored in *Bauhinia variegate* flower extract treated groups. The study has revealed the chemopreventive role of *B. variegate* flower extracts against DMBA-induced skin carcinogenesis in mice.

Keywords: Chemopreventive, DMBA, *Bauhinia variegate*, Papilloma, Skin carcinogenesis, 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 linolinic acid, oleic, steric, palmitic and myristic acid. (4) A new lectin from seeds of the *B. variegata* was purified and biochemical characterized. (5) also 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 (7-9) 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-notrosodiethylamine 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 chemoprivantive effects 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 (43, Ref. No. 670/225.IAEC/2008) and confirms to the guidelines set by World Health Organization, Geneva, Switzerland and Indian National Science Academy (INSA), New Delhi (India).

Chemicals

The initiator DMBA and croton oil (used as promoter) were procured from Sigma Chemical Co (St Louis, MO). DMBA was dissolved at a concentration of 100 µg/100 µl in acetone. Croton oil was mixed in acetone to give a solution of 1% dilution.

Preparation of the *Bauhinia variegate* flower Extract

The identification of the plant *Bauhinia verigata* (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 flowers of the plant were extracted with 50% methanol by refluxing for 36 hrs. at 50-60º C. Pellets of the drug were obtained and the required dose for treatment was prepared by dissolving the pellets in DDW 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) (15).

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. verigata* flower extract. + Croton Oil) - 104 µg DMBA was dissolved in 100 µl acetone and single application was given afterwards the 100 µl dose of *B. verigata* flower 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. verigata* flower extract. + Croton Oil) - 104 µg DMBA was dissolved in 100 µl acetone and single application was given afterwards the 100 µl dose of *B. verigata* flower 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. verigata* flower extract alone) - *B. verigata* flower 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 16^{th} week). The hepatic level of glutathione (GSH) was determined by the method of Moron *et al.* (1979) (17). 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) (18).

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 findings of the present study with the skin tumour model were show that 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. These were significantly reduced in the group which received the treatment of *B. variegata* flower extract additionally at the dose of 500 and 1000 mg/kg body weight all experimental groups (groups 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 9 & 7 and 1.5 & 1.1 and the tumour burden were found to be 2.2 and 2.3 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* flower extract by topical application. Vehicle Control, No treatment, *B. variegata* flower extract alone, Croton oil alone and DMBA alone groups did not induced any tumor incidence. The results are summarized in **Table 1.**

The significant fall in glutathione (GSH) level was observed in blood and liver of the animals which received DMBA + croton oil as compared to *B. variegata* flower extracts experimental groups, at the time of termination of the experiment (after 16 weeks). Treatment of *B. variegata* resulted in an enhanced level of GSH ($p<0.05$) in such groups. The results are summarized to **Table 2.**

TABLE 1 Effect of *B. variegata* (Kachnar*)* flower extract on DMBA- induced papillomas in *Swiss albino* mice

Groups	Cumulative No. of Papillomas	Tumour incidence	Tumour Yield	Tumour Burden	Average Latent Period
Vehicle alone	00	0/6	00	00	00
DMBA alone (1 application)	00	0/6	00	00	00
Croton oil alone	00	0/6	00	00	00
B. variegata flower extract alone	00	00	00	00	00
DMBA+ Croton oil	22	6/6 (100%)	3.6±0.42	3.6±0.42	7.43±0.29
DMBA + *B. variegata* flower extract (500 mg/kg)+ Croton oil	9	4/6 (66.66%)	1.5±0.56*	2.2±0.48*	8.44±0.47
DMBA+ *B. variegata* flower extract (1000 mg/kg)+ Croton oil	7	3/6 (50%)	1.1±0.54*	2.3±0.32*	9.94±0.16

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

Table 2 Variation in the glutathione level during DMBA-induced skin carcinogenesis with/without *B. verigata* flower extracts treatment

Treatment group	Glutathione level	
	Blood (µg/ml)	**Liver (µ mole/gm)**
Normal mice	3.49 ± 0.03	63.43 ± 0.59
Carcinogen (DMBA + Croton oil)	2.80 ± 0.06	55.98 ± 0.87
DMBA + *B. variegata* flower extract (500 mg/kg) + Croton Oil	3.22 ± 0.14*	61.85 ± 0.11*
DMBA + *B. variegata* flower extract (1000 mg/kg) + Croton Oil	3.30 ± 0.14*	62.52 ± 0.13*
Data are reported as mean ± s.e., n=6		

***** Significance level among different groups 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 *variegata* flower extracts on DMBA-induced skin carcinogenesis in *Swiss albino* mice. The literature suggests that 1 subminimal dose of carcinogen initiates the process of carcinogenesis, and the treatment with croton oil promotes this process to a visible tumor stage (19). 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 (20). 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. verigata* flower extracts was observed. The phytochemical study indicated the presence of flavonoids and lectin in *B. variegata* extract. Flavonoids which have been shown to anticarcinogenic activity (21-22) and lectins reported to produce structural variation of the cell envelope (23). 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 (15). These reports support our finding. Since *B. variegata* is an important herbal drug used as a tonic in Aruveda a traditional medical system of India. From the present study, it has been found that the Indian plant *Bauhinia variegata* is a source of many antioxidants agents, which may be useful for the prevention of cancer. The present work suggests further evaluation of the efficacy of this well-known plant.

References

1. Manandhar, N.P. Plants and People of Nepal Timber Press. The Text book Oregon. 2002; 88(6); 192-227.
2. Gordon M.C., David J.N. (2001) Natural Product Drug Discovery in the next millennium, Pharm Blol. 2001; 39; 8-17.
3. Vileges J.H., DeMarchi E., Lancas E.M. Phytochemical activity of Natrural plant (Anal). 1997; 8;266-270.
4. Zaka, S.H., Saleem, M., Shakir, S., Khan, A. Fatty acid composition of *Bauhinia variegata* and *Bauhnia malabarica* seed oils-comparsion of their physico-chemical properties. 2006;85;4(2) ;169-170.
5. Jose A. Silva,Daniela C., Damico, S., Paulo A., Baldasso, marcelo, A., Mattiolo, F.V.W., Leonardo, F.F., Jose, C. Novello and Sergio Marangoni, Isolation and Biochemical characterization of a galactoside Binding lecting from *Bauhinia variegata* candida (BvcL) seeds The Protein Journal, 2007;26 (3);193-201.
6. Davies J. Inactivation of antibiotics and the dissemination of resistance genes. Science Direct. 1994;26(4);375-382.
7. Gupta, A.K., Chauhan, J.S., Constituents from the stem of *Bauhinia variegata.* National Academy of Science Letters. 1984;7;15–16.
8. Gupta, A.K., Vidyapati, T.J., Chauhan, J.S. 5,7-Dihydroxyflavanone-40-O-l-rhamnopyranosyl-d-glucopyranoside from *Bauhinia variegata.* Indian Journal of Chemistry. 1979; 18B; 85–86.

9. Gupta, A.K., Vidyapati, T.J., Chauhan, J.S., Chemical examination of the stem of *Bauhinia variegata*. Planta Medica. 1980; 38: 174–176.
10. Rahman, W., Begum, S.J., Flavonoids from the white flowers of *Bauhinia variegata.* Natur wissenschaften, 1966; 53;384-385.
11. Wahab, S.M.A.E., Wassel, G.M., Ammar, N.M., Hanna, T., Flavonoid constituents in the different organs of selected Bauhiniaspecies and their effect on blood glucose. Herba Hungeria. 1987; 26: 27–39.
12. Yadava, R.N., Reddy, V.M.S., A new flavone glycoside, 5-hydroxy-7,30,40,50-tetramethoxyflavone5-O-_-d-xylopyranosyl(1!2)-l-rhamnopyranoside from *Bauhinia variegata*. Journal of AsianNatural Products Research. 2001; 3; 341–346.
13. Rajkapoor B., Jayakar B. and Murgesh N., Antitumor activity of *Bauhinia variegata* on Dalton's ascetic lymphoma. Journal of Ethnopharmacology, 2003a; 89:09.
14. Rajkapoor B., Jayakar B. and Murgesh N., Sub chronic toxicity of plant extract *Bauhinia varigata* on rats. Journal of Ecotoxicology and Environmental Monitoring, 2003b;14(1): 71-74.
15. Rajkapoor B., Jayakar, B., Murgeshand, N.D., Akthisekaran Chemoprevention and cytotoxic effect of *Bauhinia variegata* against N-nitrosodiethylamine induced liver tumors and human cancer cell lines.,Journal of Ethnopharmacology, 2006; 104:407-409.
16. Berenblum I, Sequential aspects of chemical carcinogenesis: in skin cancer A Comprehensive treatise. Plenum Press new york. 1975; 1 :323-344.
17. Moron MS, Depiere JW and Mannervik B (1979). Levels of GSH, GR and GST activities in rat lung and liver. Biochemica Biophysica Acta. 582: 67-78.
18. Beutler E, Duron O and Kellin BM Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 1963; 61: 882-888.
19. Berenblum I. The co-carcinogenic action of croton oil. *Cancer Res*. 1941; 1:44-48.
20. Ather M. Oxidative stress and experimental carcinogenesis. *Indian J Exp Biol*. 2002; 40:656-667.
21. Brown, JP (1980). A review of the genetic effect of occurring flavonoids, anthraquinones and related compounds. Mutation Research 75,:243–277.
22. Hirano T, Oka K, Akiba M (1989). Antiproliferative effect of synthetic and naturally occurring flavonoids on tumour cells of human carcinoma cells lines. Research Communication in Chemistry, Pathology and Pharmacology. 64:69 -78.
23. Sammour RH, Elshanshoury AR (1992). Antimicrobial activity of legume seed proteins. Botanical Bulletin of Academia Sinica. 33:185–190.