American-Eurasian Journal of Toxicological Sciences 2 (2): 112-114, 2010
ISSN 2079-2050

Clastogenic Analysis of *Bauhinia variegata* Bark Extract Using Micronucleus Assay in Mouse Bone Marrow Cells

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Abstract: Herbs have always been used as a common source of medicines, the *Bauhinia variegata* is an important herbal plant used in Aruveda as a traditional medicinal system of India. In the present investigations, the preventive effect of *Bauhinia variegate* bark extract was evaluated against cyclophosphamide induced micronucleus formation in the mouse bone marrow cells. The single ip administration of *B. variegate* bark ext at the dose of 125, 250 and 375 mg/kg body weight, 24 hours prior the administration of cyclophosphamide (at the dose of 50 mg/kg) have significantly prevented the micronucleus formations in dose dependent manner in bone marrow cells of mice as compared to cyclophosphamide group. However, *B. variegate* bark ext alone has not induced micronucleus formations in bone marrow cells as compared to control group. Therefore seems to have a preventive potential against CP-induced micronucleus formation in *Swiss* mouse bone marrow cells.

Key words: *Bauhinia variegata* bark extract · Mutagenicity · Micronucleus · Bone marrow · cyclophosphamide

INTRODUCTION

Micronuclei are cytoplasmic chromatin-containing bodies that appears in the cell like a small satellite nucleus around the cell nucleus, due to chromosome fragments or entire chromosomes that are not incorporated in the main nucleus after cell division. The presence of micronuclei (MN) in cells is considered as a biomarker of damage to the DNA. The micronucleus test, is an *in vivo* and *in vitro* short-time screening cytogenetic test, introduced by Heddle [1] and Schmid [2] is a widely used method for assessing genotoxicity of chemicals in organisms [3]. *Bauhinia variegata* (Family *fabaceae*, Genus *Bauhinia*) is a commonly found plant in moist waste ground and open plantations. It is cultivated throughout India in the forest lands in central India. According to Ayurveda, *Bauhinia variegata* Linn is used as a tonic for liver, in treatment of leprosy, menorrhagia, impurities of blood, tuberculous glands, wounds, ulcers, asthma etc. [4, 5]. The bark powder of the plant in a major ingredient of the herbal tonic Kachanar guggul, an ayurvedic remedy prescribed to increase the white blood cells. Phytochemical characterization shows the presence of tannins, stroids, alkaloids, flavonoids and saponin in the stem bark of *Bauhinia variegata* Linn [6]. The antitumor activity of the ethanolic extract of *B. variegata* also reported against Dalton's ascetic lymphoma (DAL) in *Swiss albino* mice [7] and in N-notrosodiethylamine induced experimental liver tumour in rats and human cancer cell lines [8]. The sub chronic toxicity study was also reported on *albino* rats treated with alcoholic extract of *B. variegata* [9]. The ethanolic extract of stem bark of *Bauhinia variegata* Linn contains â- sitosterol, lupeol vitamin C, kaempferol, flavonone and quercitin. Some studies have reported its antiulcer, antibacterial and antifungal activities [10]. The present investigation is amied at studying the Antimutagenicity activity of the methanolic extract of stem bark of *B. variegata* Linn in order to justify the traditional claims endowed upon this herbal drug as a rasayana.

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

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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) (Project No. 43, Ref. No. 670/225.IAEC/2008).

Chemicals: The Cyclophosphamide was purchased from Sigma chemical Co. U.S.A. and other chemical were reagents grade and purchased locally.

Preparation of the *Bauhinia veriegata* Bark 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, India. The non-infected bark of the plant was extracted with 50% methanol by refluxing for 36 hrs. at 50-60º C. The powder was treated with petroleum either for 3 hours for defatting. Pellets of the drug were obtained and the required dose for treatment was prepared by dissolving the pellets in double distilled water at the different dose levels.

Micronucleus Assay: For the micronucleus assay, the extract at the volume of 0.2 ml at different doses level such as 125, 250 and 375 mg/kg body weight was injected 24 hours before the treatment of cyclophosphamide, to six animals. The positive control group received single i. p. injection of 50 mg/kg cyclophosphamide in 0.9% saline. The animals were sacrificed by cervical dislocation and bone marrow cells were harvested. The slides were prepared essentially as described by Schmid (1975) [2] and modified by Aron *et al.* (1980) [11]. After staining with May-Gruenwald and Giemsa, a total 1000 cells were scored at the magnification of x1000 (100 x 10x) for each group. The data are expressed as the average number of micronucleated cells/thousand polychromatied erythrocytes cells (PCE) cells/animals (±SE) for a group of six animals. The results were compared with the vehicle control group using Student's't' test with significance determined at $p<0.05$.

RESULTS

In antimutagenicity studies, single application of *B. verigata* bark extract at the dose of 125, 250 and 375 mg/kg body weight, 24 hours prior the i.p. administration of cyclophosphamide (at the dose of 50 mg/kg) have significantly prevented the micronucleus formations in dose dependent manner in bone marrow cells of mice as compared to Cyclophosphamide group. However, *B. verigata* bark extract alone has not induced significant micronucleus formations in bone marrow cells as compared to control group (Table 1 & Fig. No. 1).

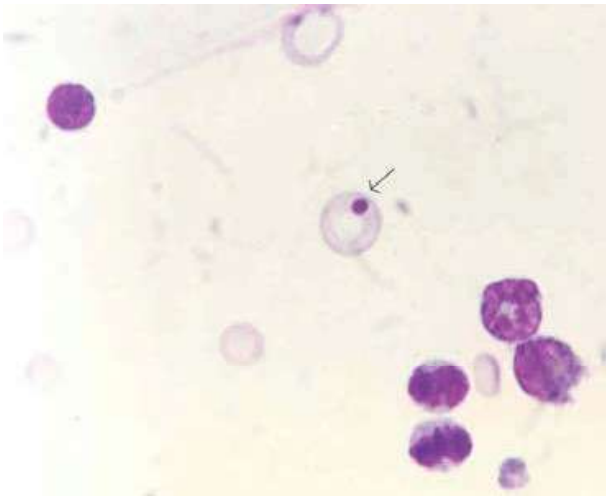

Fig. 1: Shows the micronuclei in bone marrow cell

Table 1: Effect of *B. verigata bark* extract on micronucleus in mouse bone marrow cells.

Group	MNPCE ± SE	PCE/NCE RATIO
Cyclophosphamide (CP)	3.33 ± 0.926	0.475 ± 0.023
B. verigata bark ext. (125mg/kg) + CP (50 mg/kg)	1.80 ± 0.603	0.685 ± 0.005
B. verigata bark ext. (250mg/kg) + CP (50 mg/kg)	1.33 ± 0.495	0.746 ± 0.027
B. verigata bark ext. (375mg/kg) + CP (50 mg/kg)	0.83 ± 0.268	0.826 ± 0.029
B. verigata bark ext (125 mg/kg) alone	0.50 ± 0.22	0.921 ± 0.055
Solvent (DMSO)	0.45 ± 0.20	0.980 ± 0.046

* Denotes Statistical Significance at $P<0.05$ in't' test. When compared with respective positive control group. Each group consists of six animals.

DISCUSSION

This preliminary study of the clastogenic effect of *B. variegata* bark extract using micronucleus revealed that there is a significant induction of micronucleus in formation as compared to Positive control group. The phytochemical study indicated the presence of flavonoids, lectin and albumin in *B. variegata* extract. Flavonoids which have been shown to posse's antimutagenic and anticarcinogenic activity [12, 13] and lectins reported to produce structural variation of the cell envelope [14]. Since *B. variegata* is an important herbal drug used as a tonic in Aruveda a traditional medical system of India. The mechanism underlying the antimutagenic 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. The present investigation therefore reveals that methanolic extract of B. variegata certainly possesses antimutagenic properties. However, further studies are warranted to elucidate the exact mechanism of action.

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