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Evaluation of genetic potential of *Bacopa monnieri* extract in Mouse bone marrow cells by chromosomal analysis test

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Herbs have always been used as a common source of medicines, the *Bacopa monnieri* is an important herb used in Aruveda as a traditional medicinal system of India. In the present investigations, the genotoxic potential of *monnieri* Hydromethanolic extract (BMH) was evaluated employing Chromosomal analysis assay *invivo*. BMH was administered to Swiss Albino mice as i.p. dose of 80mg/kg, 160mg/kg, 240mg/kg body wt., 24 hours prior the administration of cyclophosphamide (CP) (positive control) at the dose of 50 mg/kg body wt. A dose-dependent, significant decrease in chromosome aberration was observed with respect to control. Result suggested that BMH have a preventive potential against CP induced chromosomal aberration in Swiss albino mouse bone marrow cells at the dose tested. Therefore seems to have a preventive potential against Chromosomal aberrations in *Swiss Albino* mouse bone marrow cells.

Keyword: *Bacopa monnieri* Hydromethanolic Extrrtract (BMH) , Cyclophosphamide, Chromosomal Aberration.

1. Introduction

Bacopa monnaieri (L.) Pennell belonging to the family Scrophulariaceae is an amphibious plant of the tropics and normally found growing on the banks of rivers and lakes. It is commonly called Brahmi in Sanskrit and in Hindi & water hyssop in English. *Bacopa monnieri* has been used in the Ayurvedic system of medicine for centuries (Mukherjee and Dey 1966). *Bacopa* is a small succulent creeper that thrives in warm climates throughout the world, growing in moist places and along waterways. It is a plant, native to both India and Australia. *Bacopa*'s botanical name has numerous synonyms, commonly encountered ones include: *Bacopa monniera* Wettst., *Bacopa monniera* Linn., and *Herpestis monniera* (Morgan & Bone 1999; Russo & Borrelli 2005). *Bacopa monnieri* is referred to as one of the greatest multipurpose miracle herb of oriental medicine capable of improving memory and treating several

neurological disorders. *Bacopa monnieri* rich in pharmacological activities. The plant, plant extracts and isolated bacosides have been extensively investigated in several laboratories for their neuropharmacological effects, the active principles apart from the facilitating learning and memory in normal rats, inhibited the amnesic effects of scopolamine, electroshock and immobilization stress.

2. Material & method

2.1. Collection of plant & extraction

The plant of *Bacopa monnieri* collected from Herbal garden of NBRI, Lucknow (U.P.). After authentication, the aerial parts of plant material were shade dried & powdered by a mechanical grinder. Powdered material weighed & soaked in petroleum ether for ½ an hr. & then the extract was taken out and dried and then again it was soaked in 50% methanol in a pear shaped separating funnel.

Mixture was agitated at regular intervals for 24 hours. The extract was filtered using what man filter paper (No.1) and then concentrated in a vacuum at 45 °C in Waterbath. The extracts were stored at about 2-8 °C.

2.2. Chemicals

Cyclophosphamide was purchased from Sigma Chemical Co. USA. All other chemicals were analytically grade & were procured locally.

2.3. Animals & treatment

Male Swiss albino mice 7 week old weighing 18-20 gm were used in present study. The experiment was done in the JNCH & RC, animal house in wide cages & well aerated rooms & they will be fed on special diets & sterilized water ad libitum.

2.4. Experimental Protocol

The Chromosomal aberration assay was performed as per the method reported by Schmidt [11] and modified by Aron *et al.* (1989) and standardized [1]. The requisite dose was dissolved in DDW and administered to Swiss albino mice before 24 hrs of Cyclophosphamide (CP) administration to 6 animals intraperitoneally (i.p.). The animals were sacrificed 24 hrs after the CP (50 mg/kg b. wt.) administration. The animals were sacrificed by cervical dislocation after the 24 hrs treatment. Colchicine (Metaphase arresting agent) (0.25gm/50ml DDW) was administered i.p. at 2 hrs before killing the animals. The slides were prepared essentially as per modified method of Preston [8]. Briefly, femur bone were excised & the bone marrow extracted in 0.56% KCl. The harvested cells were incubated at 37 °C for 20 min & then centrifuged for 10 min at 1000 rpm. Cells were fixed in Carnoy's fixative (methanol : acetic acid =3:1) and burst opened on a clean slide to release chromosomes. The slides were stained with 5% Giemsa solution for 15 min & mounted with DPX. A total of 100 well spread metaphase plates were scored for the chromosomal aberration at a magnification of 1000 (100×10x) for each group. Different types of chromosomal aberrations such as chromatid breaks, gaps, centromeric association etc were scored and expressed as % chromosomal aberrations.

2.5. Experimental Groups

Swiss albino mice were divided into 5 groups for the present study.

I Group: (CP Alone): This group served as a positive control group. These animals received Cyclophosphamide (CP) i.p. at the dose of 50mg/kg body wt.

II Group: (BMH extract +CP): These animals received BMH extract (80mg/kg body wt) i.p. After 24 hours, Cyclophosphamide (CP) (50mg/kg body wt.) was given i.p.

III Group (BMH extract +CP): These animals/ received BMH extract (160mg/kg body wt) i.p. After 24 hours, Cyclophosphamide (CP) (50mg/kg body wt.) was given i.p.

IV Group (BMH extract + CP): These animals received BMH extract (240mg/kg body wt) i.p. After 24 hours, Cyclophosphamide (CP) (50mg/kg body wt.) was given i.p.

V Group: (BMH extract Alone): These animals received BMH extract i.p. at dose of (80mg/kg body wt).

2.6. Statistical Analysis

The effect of different doses of extract were compared by one way ANOVA & all other data were analyzed by student 't' test using graph PAD InStat software. A value of $P < 0.05$ was considered statistically significant.

3. Result

In genotoxic studies, single application of BMH at the dose of 80, 160 and 240 mg/kg body weight, 24 hours prior the i.p. administration of cyclophosphamide (at the dose of 50 mg/kg) have significantly prevented the Chromosomal aberrations in dose dependent manner in bone marrow cells of mice as compared to Cyclophosphamide group. However, BMH alone has not induced Chromosomal aberrations in bone marrow cells as compared to control group (Table 1 & figure. No. 2 & 3).

Table 1. Effect of *B. monnieri* Hydromethanolic (BMH) extract on Chromosomal Aberrations induced by Cyclophosphamide (CP) in bone marrow cells of Swiss Albino mice.

S. No.	Groups	Treatment	Mean \pm S.E.	Diff. Aberrations in %					% Inhibition
				CB	CF	CG	RF	CA	
1.	Group I	CP Alone (50 mg/kg body wt.)	57.5 \pm 4.457	15	13	3	11	14	-
2.	Group II	BMH extract (80 mg/kg body wt.) + CP (50 mg/kg body wt.)	35.5 \pm 2.236*	10	13	3	5	5	37.3
3.	Group III	BMH extract (160 mg/kg body wt.) + CP (50 mg/kg body wt.)	29.9 \pm 1.178*	9	7	5	6	2	48.0
4.	Group IV	BMH extract (240 mg/kg body wt.) + CP (50 mg/kg body wt.)	24.08 \pm 2.10*	6	5	8	2	3	58.2
5.	Group V	BMH extract Alone (80 mg/kg body wt.)	15.4 \pm 1.873	3	3	2	2	5	-

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



Fig 1: showing the Normal Chromosomes in S. Albino mice

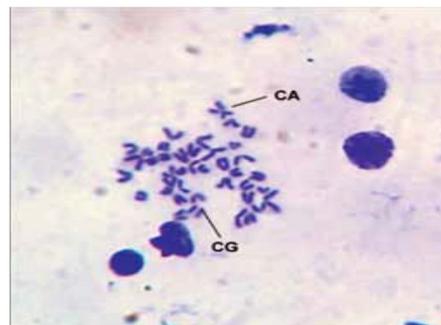


Fig 2: showing CH Aberration

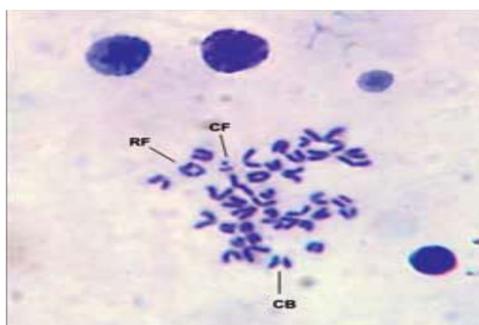


Fig 3: showing CH Aberration

Where:

CA- Centromeric Association
 CF- Chromatid Fragment
 CB- Chromatid Break

CG- Chromatid Gap
 RF- Ring Formation

4. Discussion

Many conventional drugs that are available today also originate from plant sources. For example, Aspirin is derived from Willow bark [12]. Alteration in gene function occurring as a result of several different kinds of cancer [13], indicating the probable involvement of chromosomal aberrations in carcinogenesis. Consistent with this relationship, chromosomal aberrations are induced by many known mutagens and/or carcinogens [4,8]. The chromosomal aberration assay in rodent bone marrow nucleated cells can detect a wide spectrum of changes, which result from breakage of one or more chromatids as the initial event. Breakage of chromatids or chromosomes can result into micronucleus formation if an acentric fragment is produced. Therefore, assays detecting either chromosomal aberration or micronuclei are acceptable for detecting clastogens [3]. *In vivo* chromosomal aberration assays assess the potential of a test chemical to cause DNA damage that may affect chromosome structure, or interfere with the mitotic apparatus causing changes in chromosome number. CAs were classified according to Savage [10] as gaps, breaks, deletions, fragments, rings, and dicentric chromosomes. Structural chromosome aberrations (gaps, breaks, ring, fragments, association). This preliminary study of the genotoxic potential of BMH revealed that the aberrations induced by CP were found to be chromatid break, fragments, gaps, ring formation & centromeric association. Extract alone failed to induce Ch. Aberra. significantly confirming its non mutagenicity. The BMH extract treated experimental groups (Group II, III & IV) the bone marrow cells of mice showed a prevention of chromosomal aberrations in dose dependent manner as compared with that of the Cyclophosphamide positive control group (Group I). The mechanism underlying genotoxic potential of *BMH* is not clear; the beneficial effect of *BMH* may be due to either individual or combined effects of its constituents.

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