

**ANTIMUTAGENIC PROTECTION OF *FICUS BENGHALENSIS* EXTRACT AGAINST CYCLOPHOSPHAMIDE INDUCED GENOTOXICITY IN RAT BONE MARROW**SUNNY SHARMA<sup>\*1</sup>, GYANDERA K SHARMA<sup>2</sup>, ANIL MEHTA<sup>3</sup><sup>1</sup>Department of Pharmacology, Sapience Bio-Analytical Research Lab (SBRL) Bhopal, M.P, India, <sup>2</sup>Department of Biotechnology, BDC Bhopal, M.P, India, <sup>3</sup>Department of Biotechnology. Govt. P.G. College Rajouri, J&k, India, Email: sunnysharma63@yahoo.com

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**ABSTRACT**

Herbs have always been used as a common source of medicines, the *Ficus benghalensis* is an important herbal plant used in Ayurveda as a traditional medicinal system of India. In the present investigations, the preventive effect of *Ficus benghalensis* bark extract was evaluated against cyclophosphamide induced of chromosomal Abbreviation and micronucleus formation in the rat bone marrow cells. The single p.o administration of *Ficus benghalensis* bark ext at the dose of 250, 500 and 800 mg/kg body weight, 24 hours prior the administration of cyclophosphamide (at the dose of 50 mg/kg) have significantly prevented the micronucleus formations and Chromosomal Abbreviation in dose dependent manner in bone marrow cells of rat as compared to cyclophosphamide group. It is seems to have a preventive potential against CP-induced mutagenic effect in rat bone marrow cells.

**Key words:** *Ficus benghalensis* bark extract, Mutagenicity, Micronucleus, Bone marrow, cyclophosphamide, chromosomal abbreviation.

**INTRODUCTION**

All chemicals that produce DNA damage leading to mutation or cancer are described as genotoxic. Toxicological studies have undergone a significant evolution during the past decade, with much greater emphasis being placed on chronic toxicity, carcinogenicity, teratogenicity and mutagenicity. The mutations in somatic cells are not only involved in the carcinogenesis process but also play a role in the pathogenesis of other chronic degenerative diseases, such as atherosclerosis and heart diseases, which are the leading causes of death in the human population (De Flora and Izzotti, 2007). Micronucleus test and chromosomal aberration test are used for studying antimutagenic activity of a drug. One of the best ways to minimize the effect of mutagens and carcinogens is to identify the anticlastogens, antimutagens (substances which suppress or inhibit the process of mutagenesis by acting directly on the mechanism of cell) and desmutagens (substances which somehow destroy or inactivate, partially or fully the mutagens, thereby affecting less cell population) in our diets and increasing their use. Nature has bestowed us with medicinal plants. There is a need to explore them for use as antimutagenic and anticarcinogenic food or drug additives. *Ficus benghalensis*, family of Moraceae is commonly known as Banyan tree in English and bad/vat in Hindi. Ficus has more than 800 species and 2000 varieties of *Ficus* species. Fresh juice (50-100 ml) of leaves of *Ficus racemosa* L. is given with water for about 10 days to treat gastrointestinal problems (Rout SD et al 2009). Bark of *Ficus arnottiana* and *F. hispida* shows hypoglycemic activity (Papiya Mitra et al 2009, Rajib Ghosh et al 2004). Rootsof *Ficus benghalensis* shows anthelmintic activity. The extracts also reported to inhibit insulinase activity from liver and kidney. Fruit extracts exhibits anti-tumor activity (Manoj Aswar et al 2008). The present investigation is aimed to study the antimutagenic activity of the methanolic extract of bark of *F. benghalensis*.

**ABBREVIATIONS**

**MN**, Micronuclei; **PCE**, Polychromatic erythrocytes; **NCE**, Normochromatic erythrocytes; **MFB**, Methnolic bark extract of *Ficus benghalensis*; **CAT**, Chromosomal aberration test.

**MATERIAL AND METHODS****Animals**

Swiss albino rats (150-200 g) were provided by Sapience Bio-analytical Research Laboratory, Bhopal, India. Each rat was housed in cage with dimensions of 7.5-in width, 11.5-in length and 5.0-in height, containing 2-cm wood shavings. The animals were housed in standard conditions of temperature (25±2 °C) & 12 hr light-dark cycle. The rats were fed with commercial diet and water *ad Libitum*.

The animal experimental protocol was approved by the Institutional Animals Ethical Committee (IAEC), Sapience Bio-analytical Research Laboratory (SBRL), Bhopal, (M.P). All experimental procedures were conducted in accordance to the ethical guidelines of International Association for the study of toxicity (Cyclophosphamide). The experiment was approved by the institutional ethics committee and as per CPCSEA guidelines (approval no. 1413/a/11/CPCSEA).

**Chemicals**

All chemicals used throughout this study were analytical grade and purchased form locally.

**Preparation of *Ficus benghalensis* bark extract**

The *F. benghalensis* barks were collected from the Bhopal and dry for few days. Then make powder with the help of mixer. Take 50 gms. *F. benghalensis* bark powder in a separating funnel and add 50% methanol, then mix it gently. After few hours two separate layers to be seen. Collect the upper layer in a beaker and collect until transparent form appears. The extract was dried into powder at 60°C using water bath. The total weight of powder was weighed.

**Preparation of for MFB oral gavages**

Before used, the extract was dissolved in 0.9% of sterile normal saline in the amount of 500 mg/ml for dose 2500 mg/kg bw and kept in cold room at temperature 8°C. This method was carried out according to Chattopadhyay (1999). The dissolved extract would be gavage to Swiss albino rats with ball tip needle no.16 connected to five-ml syringe.

**Micronucleus Assay**

the micronucleus assay, 20 male Swiss-albino rats at age 6-7-wk. old weighing 150-200 g were divided into 5 groups (4 rats per group), the extract at the volume doses level such as 250, 500 and 800 mg/kg body weight was injected 24 hours before the treatment of cyclophosphamide, to 12 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) and modified by Aron *et al.* (1980) . 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 treated and positive control group using Student's 't' test with significance determined at p<0.05.

### Chromosomal aberrations assay

For chromosomal aberrations 20 Swiss-albino rats were divided into 5 groups for each assay the extract at the volume doses level such as 250, 500 and 800 mg/kg body weight was injected 24 hours before the treatment of cyclophosphamide, to 12 animals. The positive control group received single i. p. injection of 50 mg/kg cyclophosphamide in 0.9% saline and a single treatment of colchicine (0.0250gm /100 ml ddw). The animals were sacrificed by cervical dislocation, Animal Dissected and femur bone was excised. Bone marrow was aspirated by flushing with normal saline in the centrifuge tube. Flush the suspension in the tube properly to get good cell suspension. Centrifuged for 10 min at 1000 rpm. Supernatant discarded. Pellet was treated with pre-warmed (37°C) KCl on cyclomixer. Left above suspension in a water bath (37°C) for 20 min. Centrifuged and supernatant discarded. Pellet was treated with freshly prepared Cornoy's fixative on cyclomixer. Centrifuged and supernatant discarded. Above step of treatment with Cornoy's fixative was repeated 3 times to get debris free white pellet. To pellet added Cornoy's fixative (quantity sufficient) to get a good cell suspension. Slides were made with Air Drop Method. Stained (Giemsa's -3 min, Methanol-3 min and DDW- 1 Dip) and observed under microscope in 40X10 xs and then in 100X10x magnifications. No. of cells having aberration and the particular aberrations were scored (Total 100 cells were counted). ( Julian Preston, R 1987).

### Statistical evaluation

The differences of MNPCEs and PCE: NCE ratio between the control and treatment group was compared by Mann-Whitney *u*-test. Statistical significant of difference between groups were taken at values of less than 0.01 ( $p < 0.01$ ).

**Table 2: Frequencies of chromosomal abbreviation in rat bone marrow after oral administration of 250, 500 and 800mg/kg bw of *F. Benghalensis* Bark extract.**

Treatments	Mean $\pm$ S.E.M (C. Ab.)	% C.B	% C.F	% C.G	% R.A	% C.A
MFB 250 mg + C.P i.p	40.3 $\pm$ 5.26	19.2	16.7	5.4	NIL	0.78
MFB 500 mg + C.P i.p	32.9 $\pm$ 5.29	16.4	11.2	3.0	NIL	NIL
MFB 800 mg + C.P i.p	30.6 $\pm$ 5.2	15.7	8.2	2.3	NIL	NIL
Cyclophosphamide i.p	49.8 $\pm$ 8.90	25	22	67.7	NIL	1.4
vehicle treated	18.6 $\pm$ 8.30	10.3	6.0	1.4	NIL	NIL

MFB = Methanolic bark extract *Ficus benghalensis*, Normal saline 0.5 ml/kg bw, CP = Cyclophosphamide 50 mg/kg bw, i.p. = Intraperitoneal, C.Ab. - Chromosomal Aberration, C.B. - Chrometid Break, C.F. - Chrometid Fragment, C.G. - Chrometid Gap, R.F. - Ring formation, C.A. - Centromeric Association

### DISCUSSION

The bone marrow micronucleus test and chromosomal aberration tests are the most suitable genotoxicity tests. The antimutagenic activity of *Ficus benghalensis* was evaluated by measuring their inhibitory effect on cyclophosphamide induced mutagenesis. It is indicating in results cyclophosphamide induced chromosomal damage in mouse bone marrow cells. These fragmented chromosomes were condensed to form micronuclei which are not included in the main nucleus (Hayashi et al., 1994). *Ficus benghalensis* decreased the cyclophosphamide

induced formation of micronuclei in MNPCEs and PCE/NCE, which may be due to the inhibition of cyclophosphamide induced chromosomal damage. In chromosomal aberration test, there was a significant, time dependent rise in the total no. of chromosomal aberrations, of cyclophosphamide treated animals, when compared with normal control animals. Cyclophosphamide gets metabolized to phosphoramidate mustard and acrolein before it can act as a mutagenic agent to promote chromosomal aberrations (Hales, 1982). Chromosomal aberrations are due to lesions in DNA caused by phosphoramidate mustard which lead to discontinuities of the DNA helix. *Ficus benghalensis* significantly inhibits the cyclophosphamide induced chromosomal aberrations, which may be due to inhibition of cyclophosphamide induced chromosomal damage. *Ficus benghalensis* possess antimutagenic activity.

### RESULTS

The present antimutagenic studies data indicated that Methanolic extract of *Ficus benghalensis* bark may have good Antimutagenic activity against cyclophosphamide induced genotoxicity. The administration of *Ficus benghalensis* bark extract possesses the antimutagenic activity resulting on the significant inhibition of micronucleus formation and chromosomal aberrations inhibition against cyclophosphamide after 24 hr oral administration of 800, 500 and 250mg/ kg bw of bark extract.

**Table 1: Frequencies of micronucleated polychromatic erythrocytes MNPCEs And PCE/ NCE ratio in rat bone marrow after oral administration of 250, 500 and 800mg/kg bw of *F. Benghalensis* Bark extract.**

Treatment	MNPCE $\pm$ S.E.M.	PCE/NCE $\pm$ S.E.M
MFB 250 mg + C.P i.p	0.881 $\pm$ 0.5*	0.833 $\pm$ 0.28
MFB 500 mg + C.P i.p	0.751 $\pm$ 0.11*	0.715 $\pm$ 0.12
MFB 800 mg + C.P i.p	0.575 $\pm$ 0.11*	0.557 $\pm$ 0.19
Cyclophosphamide i.p	1.00 $\pm$ 0.50	0.947 $\pm$ 0.21
vehicle treated	0.45 $\pm$ 0.20	0.533 $\pm$ 0.16

MFB = Methanolic bark extract *Ficus benghalensis*, Normal saline 0.5 ml/kg bw, CP = Cyclophosphamide 50 mg/kg bw, i.p. = Intraperitoneal, MNPCEs = Micronucleated Polychromatic Erythrocytes PCE = Polychromatic Erythrocyte, NCE = Normochromatic Erythrocyte 1Mean  $\pm$  SD, n = 8, 1000 PCEs scored per animal; 2Mean  $\pm$  SD, n = 8, 300 erythrocytes (PCE/NCE) scored per animal. \*denotes statistically significant in student's t-test at  $p < 0.05$  when compared with positive control group.

### CONCLUSION

The present antimutagenic studies data indicated that Methanolic extract of *Ficus benghalensis* bark may have good Antimutagenic activity against cyclophosphamide induced genotoxicity. The administration of *Ficus benghalensis* bark extract possesses the antimutagenic activity resulting on the significant inhibition of micronucleus formation and chromosomal aberrations against cyclophosphamide after 24 hr oral administration of 800, 500 and 250mg/ kg bw of bark extract. The anti-mutagenicity of bark extract suggested an enhancement of detoxification enzymes against cyclophosphamide as shown in a reduction of micronucleus formation and reduced the chromosomal aberrations. It is possible that some compounds in *Ficus benghalensis* bark extract may play an important role in such enhancement of detoxification mechanism. This finding, therefore, confirmed health benefits of *Ficus benghalensis* bark as a medicinal plant to reduce mutagenicity.

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