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Evaluation of Anticlastogenic Activity of *Aegle marmelos* Leaves Extract Against Cyclophosphamide Using *in vivo* Micronucleus Assay

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ABSTRACT

Aegle marmelos, a plant having tremendous therapeutic potential, the importance seems largely due to its medicinal properties and all the parts viz. roots, leaves, fruits, bark and seeds are used for curing human ailment. In the present investigation, the anticlastogenic potential of hydromethanolic *Aegle marmelos* (AM) leaves extract has been evaluated using *in vivo* Micronucleus assay in Swiss albino mice. Cyclophosphamide (CP), a well known mutagen was given intraperitoneal (i.p.) injection at the dose of 50 mg/kg bodyweight (b.w.). AM leaves extract at the doses of 450, 675, 900 mg/kg b.w. provided protection when given 24 hrs. prior to CP administration. In CP treated animals, a significant induction of micronucleus was recorded and in different AM extracts supplemented groups, a dose dependent significant decrease in CP induced clastogenicity was observed which was statistically significant ($p<0.05$) as compared to the cyclophosphamide group. It was also observed that *Aegle marmelos* leaves extract alone could not induce micronuclei formation at the test dose of 450 mg/kg b.w. Thus, the study revealed the antigenotoxic potentiality of AM leaves extract against CP induced micronuclei formation.

Keywords: Cyclophosphamide, Antigenotoxic, Anticlastogenic, Micronucleus, Intraperitoneal.

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INTRODUCTION

Cyto-geno analysis has been widely used in the genotoxicity assessment of test compounds under *in vitro* and *in vivo* conditions. Formation of micronuclei and chromosomal aberrations are 2 important cytogenetic endpoints that are routinely used in genotoxicity evaluation[8,14,15]. Among them, for assessing DNA damage in mammalian cells *in vivo*, the micronucleus test in mouse bone marrow erythrocytes has been widely used to elucidate the relationship between chemical substances and carcinogenesis. Micronuclei are formed from chromosome fragments or entire chromosome lagging behind during cell division[9]. A direct link between MN frequencies and early stages of carcinogenesis was also reported demonstrating that the frequencies of MN increases significantly in both low and high grade diagnostic categories of cervical carcinogenesis in women[13]. Several anticancer drugs used for the treatment of malignancies, possesses a wide spectrum of cytotoxicity to normal cells in humans and experimental animals. Thus, according to the possible applications of antigenotoxic drugs in cancer treatment, worldwide research has converge to a variety of plant extracts, food supplements or dietary products[5,19,6,4]. *Aegle marmelos* Corr. is a popular medicinal plant in the Ayurvedic and Siddha system of medicine and folk medicines, used to treat a wide variety of ailments. Various parts of the tree including the fruit possess medicinal properties[3]. The roots are used for treating diarrhoea, dysentry and dyspepsia[12]. The leaves and seed oil have antifungal, antiaflatoxigenic properties[7]. Several compounds such as aegelin, lupeol, cineole, citronellal, eugenol and marmesinin have been isolated from leaves extract and are responsible for various biological activities[10]. Considering the diverse medicinal properties of *Aegle marmelos* (AM), the present study was undertaken to evaluate the protective effect of AM leaves extract against Cyclophosphamide (CP) induced genotoxicity using micronucleus assay.

MATERIALS AND METHOD

Drugs and Chemicals

Cyclophosphamide, May-Gruenwald and Giemsa were obtained from Sigma Chemicals, Co. (St. Louis, USA). Colchicine was purchased from HiMedia Lab Pvt. Ltd, Mumbai. All other chemicals and solvents used were of analytical grade.

Animal

The Project was approved by Institutional Animal Ethical Committee (IAEC), Project no. 500/01/a/2001/19th/proj-1/27-7-09. Experiments were carried out on 6 weeks old mice (*Mus musculus*) weighing approximately 24-28 gm. Animals were maintained under controlled conditions of humidity, temperature (25±2^{0}C) and light (12hrs light: 12hrs dark). They were fed

standard mice pelleted diet (purchased from Hindustan levers ltd, India) and water *ad libitum*. Experimental animals were handled according to the University and Institutional legislation, regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Preparation of Hydromethanolic Extract of Leaves of *AEGLE MARMELOS*

The leaves of Aegle marmelos was dried in shade and pulverized. The powder was then treated with petroleum ether for defatting as well as to remove chlorophyll. The powder was cold macerated using 50% methanol as solvent. The extract was dried at 55-60^{0}C (yield-8% w/w) and then suspended in distilled water for further studies.

Experimental Protocol

The animals were randomly divided into 6 groups consisting of 6 animals each. CP dissolved in saline, was injected as a single dose of 50 mg/kg body weight (bw). AM extract dissolved in distilled water and was administered as a single dose of 450, 675 and 900 mg/kg bw, 24 hrs prior to CP administration. AM was also given alone at a dose of 450 mg/kg bw. Vehicle treated groups served as negative control group and cyclophosphamide alone as positive control group. All solutions were freshly prepared and the Intraperitoneal route of administration was followed for all drugs.

Micronucleus Assay

The protocol was performed as per the method given by Schmid (1975)[17] and modified by Aron *et al* (1989)[2]. Twenty-four hours after CP treatment, bone marrow samples were collected from the mice killed by decapitation. Both femora were dissected and their bone marrow cells were flushed out with Hanks balanced salt solution (HBSS) and pipette several times. The cell suspension was centrifuged at 1000 rpm for 10 min. and the cell pellet was resuspended in a small amount of HBSS and smeared on a clean glass slide. The preparations were dried, fixed with methanol for 5 min and stained with May-Gruenwald and Giemsa sequentially. Total 1000 cells were scored at the magnification of ×1000 (100 × 10 ×) for each group. The data are expressed as the average no. of Micronucleated cells (MN)/ thousand polychromatic erythrocytes (PCE) cells /animal (±SE) for a group of 6 animals. The results were compared with the control group using Student's 't' test with significance determined at $p<0.05$. The percentage of reduction in the frequency of CP-induced DNA damage was also calculated according to Manoharan and Banerjee (1985)[11].

RESULTS AND DISCUSSION

The effects of intraperitoneal treatment with 450, 675 and 900 mg/kg bodyweight of AM leaves

extract on the frequencies of micronucleated erythrocytes in the bone marrow of normal and exposed mice to cyclophosphamide are described in Table 1.

Table.1. Results showing the protective effect of AML in micronucleus formation induced by CP.

Group	Description	Treatment	MNPCE Mean±SE	PCE/NCE Mean±SE	% reduction in the frequency of CP induced DNA damage
I	Treatment group (AML)	450 mg/kg AML	0.8±0.4	0.67±0.09	-
II	Experimental group (AML+CP)	450+CP	2.3±0.4*	0.57±0.06	50%
III		675+CP	1.5±0.02*	0.64±0.03	69%
IV		900+CP	1.16±0.30*	0.75±0.04	76%
V	Control	Negative (Solvent alone)	0.16±0.16	0.67±0.05	-
VI		Positive (CP alone)	4.5±0.4	0.51±0.02	-

Data presented as the mean and standard error (SE) among mice (n = 6).

(*) Denotes statistically significant value at $p<0.05$

The results showed that the crude extract of AM did not have a mutagenic effect on mouse bone marrow cells of treated mice i.e. no statistically significant difference in the frequency of MN between the negative control and the groups treated with AM leaves extract could be detected. As reported in the literature, our result clearly showed that CP is inducing significant ($p<0.05$) enhancement of MN, over the basal level of bone marrow cells. On the other hand, when the antimutagenicity profile for the extract was evaluated, significant decrease was observed in a dose dependent manner in the frequency of CP induced MN in all groups studied. Treatment with AM leaves extract at the dose of 450, 675 and 900 mg/kg body weight reduced the frequency of MN with 50%, 69% and 76% respectively. CP is a drug used to treat cancer malignancies and as an immunosuppressive agent. But, it is itself mutagenic and induces many physiological side effects including alteration of male spermatozoa leading to sterility and a possibility of induction of genotoxicity and apoptosis in non-tumor cells[18,22,1]. Our first observation was the absence of micronuclei in the bone marrow cells of normal mice intraperitoneally treated with a single dose of AM leaves. Intraperitoneal treatment was preferred over other because it maximizes the absorption and penetration of target cells[14]**.** Consistent with the literature, the results showed that CP exposition enhanced the frequency of MN in bone marrow cell over the basal level. On the other hand, administration of 450, 675 and 900 mg/kg of AM leaves extract 24 hrs prior to exposition of CP, reduced the frequency of micronucleated erythrocytes in a dose dependent manner. It may be attributed that the protective effect was due to the potential involvement of the phytomolecules of the extract to interfere with the enzyme participating in the biotransformation of CP to cytotoxic

metabolites. Free radical scavenging represents one of the important strategies in antimutagenesis and anticarcinogenesis and certain evidences suggests that *Aegle marmelos* extracts contain rich amount of antioxidants[21,20]. It was also reported that extracts of *Aegle marmelos* contain tannin, saponins, flavonoids, glycosides and terpenoids[23]. Antioxidant vitamins, flavonoids, glucosinolates and organo-sulfur compounds have been proven to have antimutagenic potential[16].

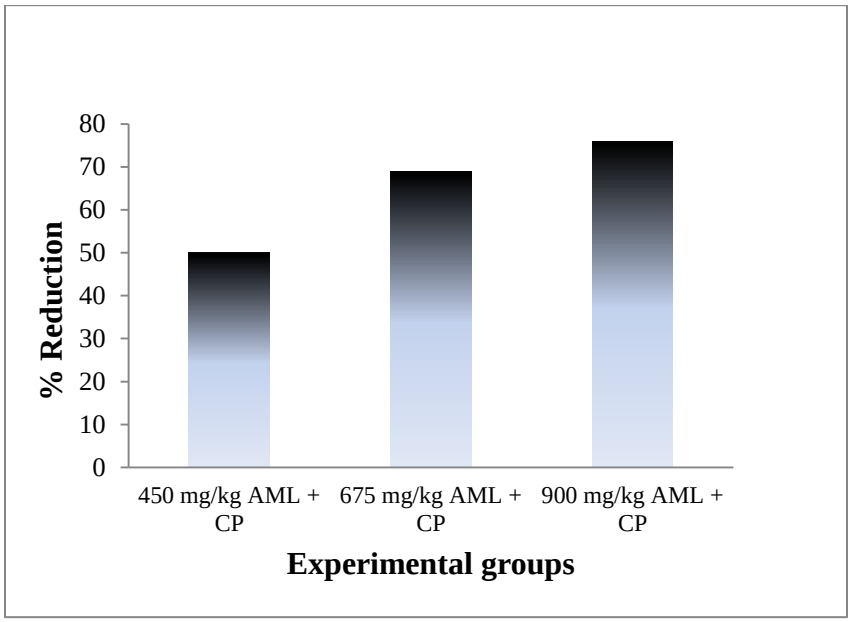

Figure 1: Showing the % Reduction in the frequency of CP induced DNA damage by *Aegle marmelos* leaves extract.

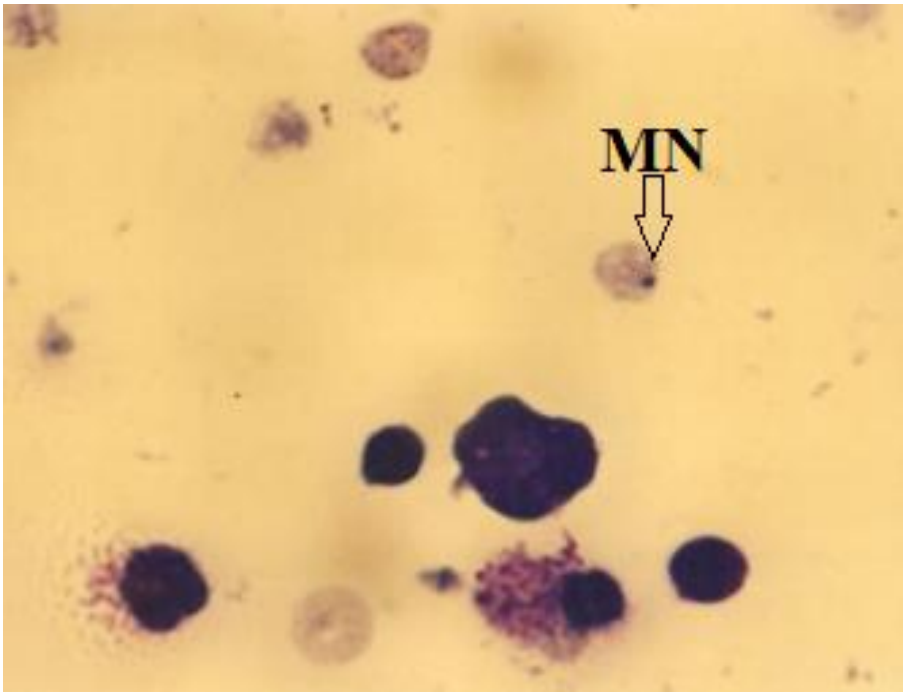

Figure 2: Showing Micronucleus (MN) in Polychromatic erythrocytes

CONCLUSION

The present investigation reveals that the hydromethanolic leaves extract of *Aegle marmelos* certainly possesses anticlastogenic activity against CP induced micronuclei formation. While the *Aegle marmelos* leaves extract alone could not induce micronuclei formation at the test dose of 450 mg/kg b.w. Thus a dose dependent significant reduction in CP induced genotoxicity was observed.

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