

EVALUATION OF ANTIMUTAGENIC POTENTIAL OF RIPE *AEGLE MARMELLOS* FRUIT EXTRACT USING MICRONUCLEUS AND CHROMOSOMAL ABERRATION ASSAY IN MOUSE BONE MARROW

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ABSTRACT

The present study was designed to evaluate the antimutagenic potential of ripe *Aegle marmelos* fruit, using micronucleus and chromosomal aberration assay in mouse bone marrow. The antimutagenic effect of 50% methanolic extract of ripe *Aegle marmelos* fruit was assessed using cyclophosphamide induced micronuclei (MN) formation and chromosomal aberration (CA) in mouse. The animals were pre-treated with the AME given intraperitoneally (i.p.) at three test doses of 600, 900, 1200 mg/kg body weight. In MN test the three doses provided protection when given 24 hrs prior to a single ip administration of 50mg /kg body weight of cyclophosphamide and the efficacy of the test doses were compared with the positive control group. In CA assay the three doses provided protection when given 24 hours prior to a single ip administration (50mg/kg body weight) of cyclophosphamide followed by a single ip administration of colchicine (25mg/kg body weight), 2 hours before cell harvesting and efficacy of the test doses were compared with that of the positive control group. The percentage protection was increased in a dose dependent manner. These results demonstrate that AME has got antimutagenic potential.

KEYWORDS : *Aegle marmelos*, Antimutagenic, Cyclophosphamide, Chromosomal aberration test, Micronucleus assay.

INTRODUCTION

The use of plants is as old as history itself. The plant kingdom represents a rich storehouse of organic compounds, many of which have been used for medicinal purposes and could serve as lead for the development of novel agents having good efficacy in various pathological disorders in the coming years. Many of the modern day drugs were actually derived from plants. Traditional medicine involves the use of herbal medicine, animal parts, and minerals and about 80% of the world population is dependent (wholly or partially) on plant-based drugs.¹

Aegle marmelos (L.) Correa (Rutaceae), commonly known as Bael, is a sacred tree for Hindu Religion, native to northern India, but is found widely throughout the Indian peninsula and in Ceylon, Burma, Thailand and Indo-China². Leaves, fruits, stem and roots of this plant have been used in ethno medicines for several medicinal properties: astringent, antidiarrheal, antidysenteric, demulcent, antipyretic, anticourbutic, aphrodisiac and an antidote to snake venom³. Essential oil isolated from the leaves of the *Aegle marmelos* show antifungal activity⁴. The leaves of are astringent, laxative, and expectorant and are useful in the treatment of ophthalmia, deafness,

inflammations, cataract, diabetes, diarrhea, dysentery, heart palpitation, and asthma⁵. Leaves are claimed to have contraceptive property as well⁶. Fresh aqueous and alcoholic leaf extracts of *Aegle marmelos* were reported to have a cardio tonic effects in mammals^{7, 8}. Hypoglycemic and antioxidant activity of *Aegle marmelos* leaves against alloxan induced diabetic rats have been found to be useful in the long term management of diabetes⁹. The ethanolic extract of *Aegle marmelos* leaf possesses anti spermatogenic activity¹⁰ and aqueous extract of the leaf has antimotility action on spermatozoa in rats¹¹. Hepatoprotective activity of leaves *Aegle marmelos* have also been evaluated with positive results¹². Both fruit and leaves of *Aegle marmelos* have radio protective activity^{13,14}. *Aegle marmelos* fruit extract exhibits antihyperlipidaemic effect in streptozotocin-induced diabetic rats¹⁵. Unripe fruit extract of *Aegle marmelos* has shown gastro protective and antidiarrhoeal properties¹⁶. Like leaves, fruits have also shown hypoglycemic effect¹⁷. Modulation of doxorubicin-induced toxicity has been reported with *Aegle marmelos* extract¹⁸. Mutations are not only involved in carcinogenicity but also play an important role in the pathogenesis of other chronic degenerative diseases such

as atherosclerosis and heart diseases¹⁹. The major mechanisms of antimutagenesis are chemical or enzymatic inactivation, prevention of formation of active species, scavenging, antioxidant free radical scavenging²⁰. Cyclophosphamide a commonly used chemotherapeutic agent is also a well known mutagen and clastogen²¹.

The present study was designed to assess the antimutagenic potential of ripe *Aegle marmelos* fruit against CP- induced MN formation and CA in mouse bone marrow.

MATERIALS AND METHODS

Preparation of Extract

The ripe fruits of *Aegle marmelos* were purchased from local market at Bhopal (M.P.) India. A voucher specimen has been kept in the herbarium of Research Centre of the Jawaharlal Nehru Cancer Hospital and Research Centre for future reference. The fruits were sliced and seeds were removed, they were then dried in shade and powdered. A sample of 50 gms fruit powder was extracted with 50% methanol in a separating funnel. The extract was dried in water bath at 60°C using water bath. The powder was treated with petroleum ether for 3 hours for defatting. Pellets of the drug were obtained and the required dose for the treatment was prepared by dissolving the pellets in double distilled water at different dose levels.

Chemicals

CP was purchased from Sigma chemical Co. (St. Louis, MO, USA.). Other Reagent Grade chemicals were purchased locally.

Animals

Male Swiss albino mice of 8-10 weeks old and weighing 20-25 grams body weight were collected from the animal house of Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal. Animals were housed in plastic cages and fed with standard pellet diet and water *ad libitum*. Experiments were performed in accordance with the current CPCSEA guidelines, India. All the Experiments were conducted according to the protocol approved by Institutional Ethics Committee (Approval No: 500/01/G/2001/19th/Proj-1/date-27/07/09 of IAEC, JNCH & RC, Bhopal)

Drug treatment protocol

Micronucleus Assay

In the MN assay the AME was given in different doses level such as 600,900,1200mg/kg at volume of 0.2 ml was injected 24hrs prior to a single i.p. administration of 50mg/kg body weight of cyclophosphamide to three groups. The positive controlled group received single i.p. injection of 50mg/kg body weight of cyclophosphamide in 0.9% saline. Another group received single i.p.

injection of 600 mg/kg body weight of AME at volume of 0.2 ml. All the above mentioned five groups contained six animals each. The slides were prepared essentially as described by Schmid²² and modified by Aron *et al.*,²³. After staining with May-Gruenwald and Giemsa, a total of 1000 were scored at a magnification of $\times 1000$ ($100 \times 10 \times$) for each the animal. The data was expressed as the average number of micronucleated cells / thousand polychromatid erythrocytes (PCE) cells/ animals (\pm 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 $P < 0.05$.

Chromosomal Aberrations Assay

In the CA Assay the AME was given in different doses level such as 600,900,1200mg/kg at volume of 0.2 ml was injected 24hrs prior to a single i.p. administration of 50mg/kg body weight of cyclophosphamide to three groups. Controlled mice were injected an equal volume of vehicle alone. The positive controlled group controlled group received single i.p. injection of 50mg/kg body weight of cyclophosphamide in 0.9% saline. Colchicine (4 mg/kg body weight) was administered i.p. 2 hours before the harvest of the cells. Animals were scarificied by cervical dislocation and the bone marrow cells were harvested. The slides were prepared essentially as per modified method²⁴. Femur bones were excised and the bone marrow extracted in 0.56% KCl. The harvested cells were incubated at 37° C for 20 minutes and then centrifuged for 10 minutes at 1000 rpm. Cells were fixed with 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 minutes and then put in xylene and mounted with DPX.

A total of 100 well spread metaphase plates were scored for chromosomal aberration at a magnification of 1000 X (100×10 X) for each group. Different types of chromosomal aberration such as chromatid breaks, gaps, fragmentations, polypoidy and centromic association, etc., were scored and the data was expressed as % chromosomal aberration. The % of chromosomal aberration of each group was calculated by using the following formula:

Percentage of Chromosomal aberration =

$$\frac{\text{Total no. of Chromosomal aberration seen} \times 100}{\text{Total no. of plates counted}}$$

Statistical significance was calculated using student's *t*-test at $P < 0.05$.

RESULTS

In the antimutagenicity studies, single i.p. administration of AME at the different doses 600. 900, 1200 mg/kg body weight, given 24 hours prior administration of

Cyclophosphamide 50 mg/kg body weight have significantly prevented the formation of micronucleus in a dose dependent manner as compared to Cyclophosphamide alone group. AME alone has not induced significant micronucleus formations in bone marrow cells as compared to control group (Table 1 and Fig 1). In CA Test different In CA test, different types of chromosomal aberrations such as chromatid gap, chromatid ring, centromeric association, chromatid fragment and chromatid break were prevented in a dose dependent manner, in the (AME + CP) treatment group as compared to the Cyclophosphamide alone group. The Percentage protection increased in a dose dependent manner (Table 2).

DISCUSSION

Micronucleus Assay

The micronucleus study showed that the single application of the AME at the dose of 600, 900, 1200 mg/kg body weight prior to the administration of cyclophosphamide have significantly prevented the micronucleus formation in dose dependent manner.

Chromosomal Aberration

The Chromosomal aberration showed that the single application of the AME at the dose of 600, 900, 1200 mg/kg body weight prior to the administration of cyclophosphamide have significantly prevented the chromosomal aberrations in dose dependent manner and caused the antimutagenicity of this compound. The different types of chromosomal aberration such as chromatid gap, chromatid ring and centromeric association were prevented in drug treated (Aegle Marmelos + cyclophosphamide) group as compared to cyclophosphamide (control) group. The % protection was increased in dose dependent manner.

The results showed that AME exhibited effective degree of antimutagenic activity. This can provide a lot of scope for further research in this regard and isolation of chemicals from it may lead to the production of a variety of potent drugs. Aegle marmelos ripe fruit may also find its place as a nutraceutical supplement, to check mutagenic changes that may occur in the body.

CONCLUSION

It is therefore concluded that 50 % methanolic extract of Aegle marmelos fruit (AME) caused antimutagenicity in micronucleus and chromosomal aberration tests in bone marrow cells of mice. Mutation is one of the principle pathways that lead to cancer. The antimutagenic effects of AME may be an important contributor in the use ripe fruit of Aegle marmelos as a potential anticarcinogenic drug. Although a variety of work has been done to explore the various potential of the different parts of Aegle marmelos tree, but very few works are reported on

the ripe Aegle marmelos fruit. Thus, the present study on the hydromethanolic extract of ripe fruit of *Aegle marmelos* will provide a lot of scope for further research in this regard and isolation of chemicals from it may lead to the production of a variety of potent drugs.

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TABLE 1: EFFECT OF AME ON MICRO NUCLEUS FORMATION IN MOUSE BONE MARROW CELL

GROUP	MNPCE+SEM	PCE/NCE RATIO
Cyclophosphamide Alone (50mg/kg)	4.4 ± 0.244	0.513 ± 0.035
AME (600mg/kg) + CP (50mg/kg)	1.8 ± 0.374*	0.85 ± 0.05*
AME (900mg/kg) + CP (50mg/kg)	1.4 ± 0.244*	0.5 ± 0.040*
AME (1200mg/kg) + CP (50mg/kg)	1 ± 0.316*	0.63 ± 0.027*
AME (600mg/kg) alone	0.6 ± 0.244*	0.88 ± 0.071*
Solvent (DDW)	0.45±0.03	0.549±0.03

* denoted statistically significant in student's t-test at p<0.05 as compared with Positive Control treated group (Cyclophosphamide Alone).

TABLE 2: EFFECT OF AEGLE MARMELOS EXTRACT ON CHROMOSOMAL ABERATION IN MOUSE BONE MARROW CELL

S. No.	GROUP	Mean± S.E.M (C.Ab)	% C.B.	% C.F.	% C. G.	% R.F.	% C.A.	% Protection
1.	Cyclophosphamide Alone (50mg/kg)	50 ± 2.0	28	30	4	Nil	14	Nil
2	AME (600mg/kg) + CP (50mg/kg)	42.6 ± 1.65*	23	12	10	2	3	22.24
3	AME (900mg/kg) + CP (50mg/kg)	40.16 ± 1.902*	27	12	4	1	1	30.74
4.	AME (1200mg/kg) + CP (50mg/kg)	37.15 ± 1.511*	22	24	3	1	4	35.94
5.	AME (600mg/kg) alone	10 ± 0.40*	4	8	-	2	2	-
6.	Solvent (DDW)	10.0 ± 2.90*	5.37	6.25	-	-	-	-

1. C.Ab. - Chromosomal Aberration.
2. C.B. - Chromatid Break.
3. C.F. - Chromatid Fragment.
4. C.G. - Chromatid Gap.
5. R.F. - Ring Formation.
6. C.A. - Centromeric Association.

* denoted statistically significant in student's t-test at p<0.05 as compared with Positive Control treated group.

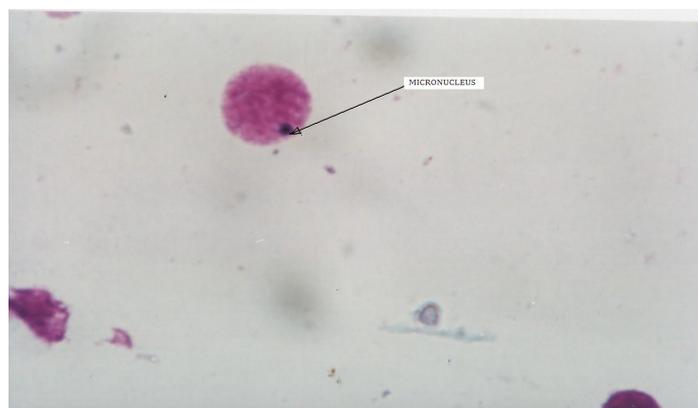


FIGURE 1: PHOTOGRAPH SHOWING MICRONUCLEUS IN PCE CELL

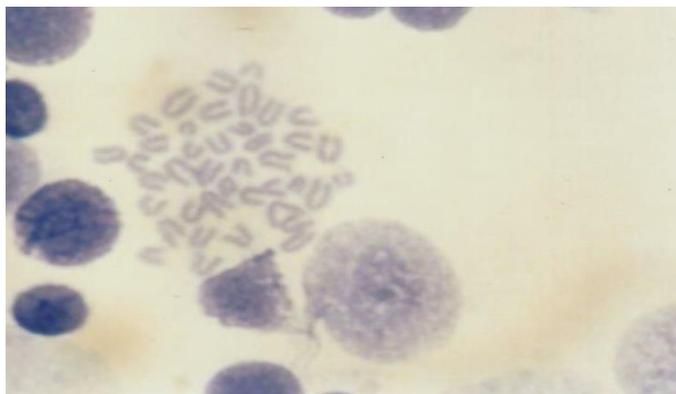


FIGURE 2: NORMAL CHROMOSOME

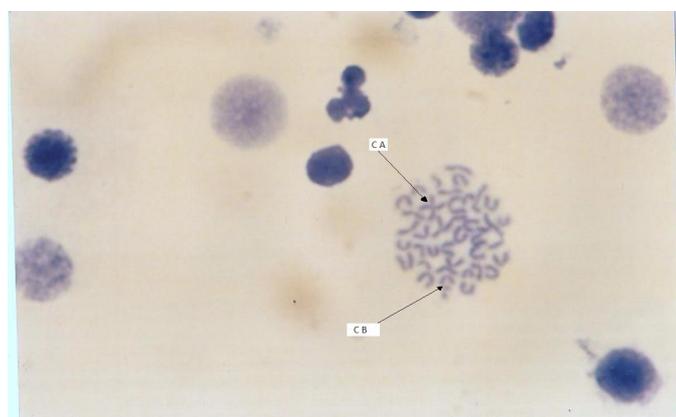


FIGURE 3: ABNORMAL CHROMOSOME [SHOWING CHROMATID BREAK (CB) & CENTROMERIC ASSOCIATION. (CA)].

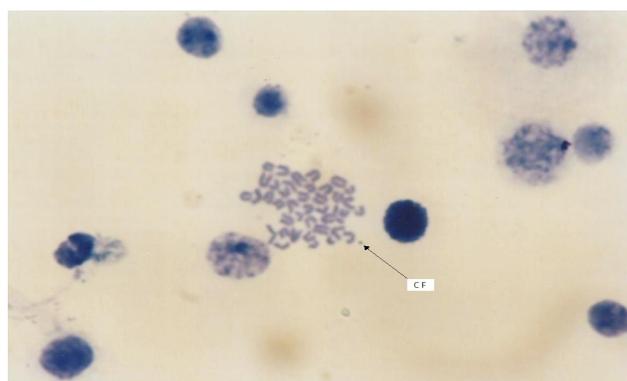


FIGURE 4: ABNORMAL CHROMOSOME [SHOWING CHROMATID FRAGMENT (CF)].

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