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Evaluation of Antigenotoxic Effects of *Aeges Marmalos* Leaf Extract in Bone Marrow Erythrocytes of Mice

Ch. Prabhakar Reddy, K. Rudrama Devi*

**Human Genetics and Toxicology Laboratory,
Department of Zoology, Osmania University,
Hyderabad, INDIA – 500007.**

***Corres.author: rudramadevi_k@yahoo.com**

Abstract: In the present study the protective effects of *Aegle mamelos* leaf extract (AMLE) were carried out in somatic cells of mice against doxyrubicin induced genotoxicity. Three different doses of *Aegle Marmelos*leaf extract were tested for antimutagenicity using micronucleus test in bone marrow cells of mice. The test compound doxyrubicin(Dox) higher dose 16 mg/kg was injected intrapertioneally prior to the administration of plant extract. The *Aegle Marmelos* leaf extract did not induce micronuclei significantly by all the three doses tested where as doxyrubicin induced significant increase in the frequency of micronuclei in bone marrow erythrocytes of mice. Pre treatment of mice with AMLE for 7 days and simultaneously with single dose of doxyrubicin, there was significant inhibition in the percentage of micronuclei was found. Thus *Aegle Marmelos* leaf extract modulates the doxyrubicin induced cytotoxicity in dose dependent way. Thus the overall results suggest the protective nature of *Aegle Marmelos* leaf extract, it is safer dietary antioxidant as cancer chemopreventive therapy.
Keywords: Doxyrubicin, Genotoxicity, *Aegle Mamelos* Leaf Extract, Micronuclei, Mice

Introduction:

Agele marmelos (L.) Correa (Rutaceae), commonly known as Bael, is a sacred tree for Hindu Religion, native to northern India, but is a found widely throughout the Indian peninsula and in Ceylon, Burma, Thailand and Indo-China [1]. Leaves, fruits, stem and roots of this plant have been used in ethno medicines for several medicinal properties: astringent, antidiarrheal, antidysentric, demulcent, antipyretic, anticourbutic, aphrodisiac and an antidote to snake venom [2]. Essential oil isolated from the leaves of the *Agele marmelos* show antifungal activity [3]. 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 asthma [4]. Fresh aqueous and alcoholic leaf extracts of *Aegle marmelos* were reported to have a cardio tonic effects in mammals. 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 diabetes . The ethanolic extract of *Aegle marmelos* leaf possesses anti spermatogenic activity and aqueous extract of the leaf has antimotility action on spermatozoa in rats. Hepatoprotective activity of leaves *Aegle marmelos* have also been evaluated with positive results. Both fruit and elaves of *Aegle marmelos* have radio protective activity. *Aegle marmelos* fruit extract exihibits antihyperlipidaemic effect in streptozotocin induced diabetic rats. Unripe fruit extract of *Aegle marmelos* has shown gastro protective and antidiarrhoeal properaties. Like leaves fruits have also shown hypoglycemic effect. modulation of doxyrubicin induced toxicity has been reported with *Aegle marmelos* extract [5].

Antitumor agents are used for common therapy against many of human cancer. However as with many drugs that have mammalian toxicity as a target, physiological side effects can occur and genotoxic effect rise to secondary tumors [6]. The anthracyclin antibiotic adriamycin (doxorubin) is one of most effective chemo therapeutic agents against a wide variety of cancers. The tumor that respond better breast and esopgeal, carcinomas, asteosarcoma, soft tissue sarcomas, hodgkins and non-hodgkin lymphoma. Because of its beneficial effects it is used in gastric cancer, bile duct pancreatic and endometrial carcinomas[7].

Doxyrubicin induces mutations and chromosomal aberrations in normal and tumor cells[8]. It has be proposed the capacity of doxorubin to inhibit DNA synthesis as a result of mode of action. Deoxyrubin has a high affinity of cell nuclei and about 60% of total intracellular of deoxryrubin is found in cell nucleus. It binds to DNA polymerase and inhibits nucleic acid synthesis, responsible for formation of protein – linked DNA double strand breaks[9]. Further cellular enzymes are capable of converting doxyrubin into free radical metabolites. For treatment of many types of cancer, Adriamycin is used in chemotherapy, it is important to reduce its toxicity to normal cells a goal can be achieved by concurrent administration of free radical scavenging agents such as antioxidants [10]. Further the consumption of fruits and vegetables can minimize to some extent the occurrence of some cancers[11]. Hence in the present investigation a study was undertaken to observe the efficacy of AMLE against doxyrubicin induced micronuclei in bone marrow erythrocytes of mice.

Materials and Methods:

Chemicals:

Doxyrubicin kindly provided by Director, MNJ Institute of oncology and Mytomycin from biochem pharma limited. The chemicals used in the study are purchased from Ranboxy Laboratories, Hyderabad, A.P.

Animals:

Six to eight weeks old male mice (*Mus Musculus*) of swiss albino mice weighing about 25-27 gms procured from National Institute of Nutrition, Hyderabad, were used in this study. The mice were housed in poly propylene cages in a well ventilated room and were provided with standard pellet diet(M/S Lipton India limited) and water adlabitum.

Plant material:

The plant material was procured from wholesale spice and herbs market Hyderabad. Professor Pratiba Devi, Medicinal Plant Division, Department of Environmental Botany, Osmania University, Hyderabad, verified the identity of plant material. The plant material was chopped and coarsely powdered to a mesh size of 1 mm as described by [12].

Preparation of extract:

Powdered plant material was repeatedly extracted in 4000 mL round bottom flask with 2000 mL methanol. The methanolic extracts was cooled at room temperature, filtered and evaporated to dryness under reduced pressure in a rotatory evaporator (Buchi Rotavapor).

Dosage schedule:

Two experiments were conducted. In the first experiment four groups were maintained to study whether the plant extract is toxic or not in bone marrow cells. Hence the group I received control saline where as group II, group III & group IV were orally administered with doses of 200mg /kg/bw, 400mg/kg and 600mg/kg/wt of AML extract for seven days.

In the secondary experiment Group I -Control, Group II-200 AML+16mg/kg DOX, Group III-400 AML+16mg/kg DOX, Group IV-600 AML+16mg/kg DOX given interpertanlly 24 hrs prior to the administration of plant extract.

Micronucleus test

All the animals were killed after twenty four of last treatment and bone marrow preparations were made. The control and experiment groups were killed by cervical dislocation femur bones were dissected out

and cells were flushed with total bovine serum into tubes. Smears were fixed with methanol and stained with Giemsa. The slides were screened for the presence of micronuclei in polychromatic erythrocytes of bone marrow cells in control and experimental group of animals. A total of 2000 polychromatic erythrocytes were examined for each animal under 100 x magnifications [13]. Student paired t test was used to detect statistical significance among the different groups. For each animal 2000 polychromatic erythrocytes (RBC) and corresponding normochromatic RBC were scored for the presence of micronuclei the appearance of micronuclei in polychromatic erythrocytes was used as an indicator of genetic damage. The ratio of polychromatic to normochromatic RBC was utilized to estimate the effect on the proliferative activity of bone marrow cells. The scoring was done separately for each animal and it was observed that there was no significant difference between individual animals of same group. The ratio of polychromatic to norm chromatic erythrocytes was used to estimate the effect on the proliferative activity of bone marrow cells

Results and Discussion:

The micronucleus test is an effective method for the genotoxicity of environmental mutagens and carcinogens. Since micronuclei (MN) are formed during cell division due to lagging of acentric chromosomes, chromatid fragments are entire chromosome, that are not included in the main daughter nuclei during metaphase, anaphase cell division can produced micronuclei [14,15]. The most frequently used genotoxicity test in mammals is the micronucleus test which provides simple and rapid indirect measure of structural and numerical aberrations [16] and it can performed only in dividing cells. A micronucleus is literally a small nucleus. The cell organelle contains the genetic material of fragmented DNA. During cell division the genetic material replicates divides between two daughter cells that are produced. If this process is disrupted, the chromosomes are broken or damaged by chemicals then the distribution of genetic material between the two daughter nuclei during cell division may be affected or formed new nuclei may be micronucleus clearly observed under microscope.

Doxyrubicin is a potent antitumor agents used for the treatment of many cancer. It is demonstrated that this drug has the potential for initiating genetic events in non-tumor cells in human and in animal systems. The results showed that doxyrubicin (Dox)induced micronuclei in polychromatic erythrocytes male and female mice. The results are in agreement with other reports of Doxyrubicin cytotoxicity [17-19]. The biochemical mechanism of adriamycin causes cytotoxicity is unclear. However when it intercates with DNA generates free radicals. Two pathway of mechanisms has been proposed. Two different pathways of free radical formation of Dox have been described. First is formation of semiquinone free radical the semi quinine can be transferred to a C7 radical that can also mediate cellular damage. The reduction of doxorubicin by 2 electrons generates a secondary alcohol metabolite doxorubicinol. The second pathway doxorubicin free radicals come from an enzymatic mechanism that involves reactions with iron. For example Fe3+reacts with doxorubicin in a redox reaction after which the iron atom accepts an electron and a Fe2+ deoxyribicin free radical complex is produced. This iron doxorubicin complex can reduce oxygen to hydrogen peroxide and other active species [20, 21].

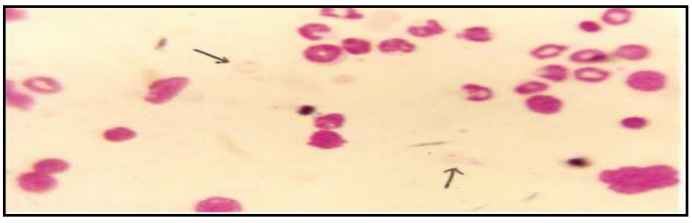

Fig. 1 The presence of micronucleus in Dox treated animals

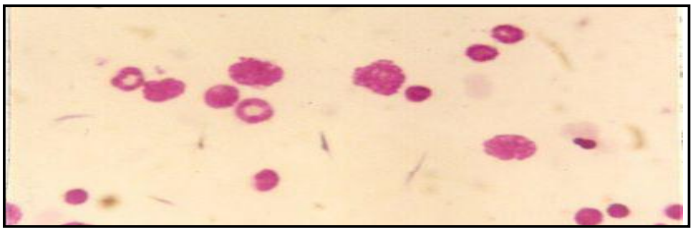

Fig. 2 The absence of micronucleus in AML extract treated animals.

The animals were treated with methanol extract of *Aegle mamelos* of three doses showed a increase at all dose levels in polychromatic erythrocytes of mice. However the differences in the frequency of micronuclei between control and treated groups were insignificant (P>0.05) (table 1). The P/N ratio is not changed and the values were observed equal to the control values.

There was significant increase in the frequency of micronuclei from in control(0.22%)to doxyrubin treated groups(1.40%)(Fig.1). Whereas the pre treatment with the methanolic extract of AML extract results showed a reduction in the induction of micronuclei when compared with Dox (table 2). The P/N ratio was decreased in Dox treated animals but concurrent administration of AMLE brings the values to lower range (0.85%). This indicates the chemoprotective nature of the AMLE. The difference in the frequency of micronuclei between the group III & Group IV showed statistically significant (P<0.01). Thus the data indicate AMLE extract supplementation reduced the cytotoxicity induced by doxyrubicin (fig. 2).

The *in vivo* micronucleus test is one of the best methods to screen the clastogenetic effects of chemicals and drugs. Using this procedure the mutagenecity of various alkylating agents, pesticides and drugs in swiss albino male mice has been reported [22-24].

An aqueous extract of *Aegle marmelos* leaves exhibited significant hypoglycemic activity in stereptozotocin diabetic rats. It also significantly (0.05%) increased the plasma insulin levels of diabetic rats. The extract did not show any signs of toxicity and the LD50 was greater than 10.0 g/kg when given orally in rat. In the liver with *Aegle marmelos* indicated that the treatment may neutralize H2O2 toxicity by its increased decomposition by CAT [25]. Veerappan *et al*[26] reported that chronic administration of *A.marmelos* leaf extract at a dose levels of 50,70,90 and 100 mg/kg b.w for 14 consecutive days to male and female Wistar rats did not induce any short term toxicity collectively and reported that the extracts of the leaves have a high margin of drug safety Latica versa *et al.*[27] evaluated the anti cancer potential used in Bangladesh folk medicine, extract of *Aegle marmelos* were tested for cytotoxicity using brain shrimp lethality eggs assay, and MTT assay using tumor cell lines.

The present results are comparable with Venkatesh et al.,[28] who reported the protective effects of *Aegle Marmelos* in mouse bone marrow cells at 350 mg/kg dose level. Earlier we have reported to protective effects of *phyllanthus emblica* fruit extract on adriamycin induced genotoxicity in somatic cells of mice [29]. The protective against DOX induced genotoxicity by AME may be due to inhibition of free radicals formed by DOX in cytoplasm of cells and increased antioxidiant status by addition of fruit extract. More than 30 identified compounds from the leaves of *Aegle marmelos* have been reported. The bioactive compounds of leaves of *Aegle marmelos* including – skimmianine, aegelin, lupeol, cineole, citral, citronellal, marmesinin, marmelosin, aurapten, marmelide and more specifically eugenol and marmesinin are found to possess potent antioxidant property and reported to inhibit lipid peroxidation. The antioxidative phytochemicals such as flavonoids, alkaloids, sterols, tannins, phlobatannins present in the leaf extract also possess free radical scavenging activity[30]. The fruit of *Aegel marmelos* contains marmelosin, luvangetin, aurapten, psoralen, marmelide, tannins and phenols. The AMF extract has been used in for treating diarrhea, diabetic , constipation heart disease, ulcers woodhealing because of its medicinal properties. Lupeol, a compound present in *A. marmelos* possess antineoplastic effects on various human neoplastic cell lines. Marmelin (1-hydroxy-5, 7-dimethoxy-2naplhale necarboxy aldehyde) present in *A. marmelos* inhibiting growth of epithelial cancer cells but not normal cells (mouse embryo fibroblasts) further it decreases cell survival, proliferation and invasiveness [31].

It is well known that consumption of fruits and vegetables is associated and are known to prevent chromosomal and DNA damage in animals [32, 33]. Usually antimutagens acting in rodents are active in human too[34]. Our results have a practical decline of genotoxic effects of doxyrubicin in cancer patients some health care workers as nurse and pharmaceutical plant workers handle this drug which may alternate the higher risks for development of secondary malignancy and for abnormal reproductive outcomes.

Conclusion:

From the above studies it is concluded that AMF Extract as protective agent against doxyrubicin induced genotoxic effect in somatic cells of mice. It is concluded that *Aegle mamelos* leaf extract can be used as a major chemopreventive agent against doxyrubicin induced mutagenicity.

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Table 1:Results on the frequencies of micronuclei in bone marrow erythrocytes of mice treated with various doses of *Aegle marmelos* leaf extract

Groups	Micronuclei in polychromatic cells (P)	Micronuclei in normochromatic cells	Micronuclei in total P+N cells	P/N ratio
Control	20/8000(0.25)	6/8020(0.07)	26/16800(0.15)	0.99
200AMLE	22/8000(0.27)*	16/8800(0.18)	40/16800(0.23)	0.90
400AMLE	28/8000(0.32)*	20/9200(0.21)	48/17200(0.27)	0.86
600AMLE	30/8000(0.37)*	24/10200(0.23)	04/18200(0.29)	0.78

*P>0.05

Table 2: Frequency of micronuclei recorded in bone marrow erythrocytes of mice treated with doxyrubin primed with *Aegle marmelos* leaf extract

Groups	Micronuclei in polychromatic cells (P)	Micronuclei in normochromatic cells	Micronuclei in total P+N cells	P/N ratio
Control	18/8000(0.22)	12/8060(0.15)	30/16060(0.37)	0.99
Doxyrubicin 16 mg/kg	112/8000(1.40)	52/9400(0.55)	164(17400(0.94)	0.85
200+16 mg/kg AMLE+Dox	90/8000(1.12)*	42/9700(0.43)	132/16700(0.79)	0.82
400+16 mg/kg AMLE+Dox	76/8000(0.95)*	40/9620(0.41)	116/17620(0.65)	0.83
600+16 mg/kg AMLE+Dox	64/8000(0.80)*	38/9880(0.38)	102/17880(0.57)	0.80

* P<0.05

References:

1. Bailey LH: How Plants Get Their Names. Dover publications, Inc., New York, New York: 1963
2. Khare CP, *Aegle marmelos* In: Indian Herbal Remedies. Khare, C.P. (Ed.). Springer, USA 2004; 27-29.
3. Rana BK, Jain AK, Evaluation of anti-fungal activity of essential oil isolated from leaves of the *Aegle marmelos*. J. Ethnopharmacology 1997. 57 (1); 29-37.
4. Kirtikar KR, Basu BD: Indian medicinal plants 2nd ed. Periodical Experts Book agency, New Delhi 1993; 499-505.
5. Das Supriya, Agarwal Rakesh Chandra, Sarkar Ashish and Gupta Manish K (2011). "Evaluation of antimutagenic potential of ripe *Aegle Marmelos* fruit extract using micronucleus and chromosomal aberration assay in mouse bone marrow." International Research Journal of pharmacy. 187-191.
6. Berelta G, (1991). Cancer treatment medical Guide 10th ed. Formitalia Carfo Erba Milan.
7. Quiles JL, Huertas JR, Battino M, Matrix J and Ramirez-Tortosa MC, (2002). Antioxidant nutrients and adriamycin toxicity. Toxicology, 180: 79-95.
8. Ge writez DA, (1999). A critical review of mechanism of action proposed for the anti tumor effects of anthracycline antibiotics adriamycin and duoxyribcin Bio Chem. Pharmaecology 57: 727-741.
9. Evert L, De Beer, Antonia E.B. and Emile E.V, (2001). Doxorubin and mechanical performance of cardiac trabeculae acute and chronic treatment a review. Europian Journal of pharmaecology 415(1): 1-11

10. Amaramokrane Y.A., Lebucher M.P, Balansrad G, Dumeni G, Bolta B, (1996). Protective effects of alpha hedoin chlorophillin and ascorbic acid towards induction of minuclei by doxyrubicin human lymphocytes.
11. Dorai and T., Agarwal, B.B, (2004). Role of chemopreventive agents in cancer therapy cancer letters, 215: 129-140.
12. Antonio, M.A. and A.R.M.S. Brito, 1998. Oral anti-inflammatory and anti-ulcerogenic activities of a hydroalcoholic extract and partitioned fractions of *Turnera ulmifolia* (Turneraceae).J. Ethnopharmacol., 61: 215-228.
13. Schmid, W., (1975). The micronucleus Test. Mut. Res. 31. 9-12.
14. Lingberg, H.K. Wang, X and Jarventaus H, (2007). Origin of nuclear buds and micronuclei in normal and folate deprived human lymphocytes. Mut. Res. Fund Mol. M. 617: 33- 45.
15. Rudrama Devi K., Kiranmai N., Yamini C.H., Anusha A., Srinidhi Y., Dwija A., Mahesh G., Venkat Reddy K., (2011). Genotoxic effects of adriamycin in bone marrow erythrocytes of mice. International journal of agricultural biological research. vol. 27(1)1-5,.
16. Heddle J.A., C. Immino M.C. Hayashi, M. Romagana, Shelby, N.D. Tucker J. D Vanparys, P. H and Mac Gregar J. T., (1991). Micronuclei as an index of cytogenetic damage past presentand future. Env. Mol. Mutagen 18:1 277-291.
17. Anderson D, Yu T.W., Browne MA, (1997). The use of same image analysis system to detect genetic damage in human lymphocytes treated with duoxyribcin comet assay and insitu hybridization assay Mut. Res. 390(1-2) 69-77.
18. Prahalathan C, E. Selvakumar and P. Varalaxmi, (2005). Lipoiic acid ameliates adriamycin induced testicular mito chndriopathy. Repord. Toxicol. 20: 111-116.
19. Kusum Latha C.& Rudrama Devi K., (2010). Cyto genetics Effects of Adriamycin in Bone Marrow Cells of Swiss Albino mice. Bioscan Vol 5(2); 317-320
20. Granados- principal S, Quiles JL, Ramirez- Tortosa CL, Sanchez-Rovira P and Ramirez-Tortosa MC, (2010). New advances in molecular mechanism and prevention of Adriamycin toxicity by antioxidant nutrients. Food and chemicals toxicology, 48:1425-1438.
21. Xu X, Persson H L, and Richerdson D R, (2005). Molecular pharmacology of interaction of anthracyclins with iron. Mol. Pharmacol, 68: 261-271.
22. Geetha K. Y.' and Rudrama Devi K, (1992). Evaluation of cypermethrin for mutagenicity in somatic and germ cells of mice. Trends in Life Sciences, 7(2), P 99-104.
23. Kusum Latha C. & Rudrama Devi K, (2010). Cyto genetics Effects of Adriamycin in Bone Marrow Cells of Swiss Albino mice, Bioscan (Vol 5(2); 317-320).
24. Rudrama Devi K., Koushik A., Venkat Reddy K, (2010). Dose response relationship for cisplatin induced micronuclei in bone marrow erythrocytes of swiss albino mice. BioScan 5(4) :567-569.
25. Sabu, M. C and Kuttan, R (2004); "Antidiabetic activity of Aegle marmelos and its relationship with its antioxidant properties", Indian J Physiol Pharmacol; 48 (1), Page No.81–88.
26. Veerappan A, Miyazaki S, Kadarkaraisamy M, Ranganathan D (2007) "Acute and subacute toxicity studies of Aegle marmelos Corr., an Indian medicinal plant". Phytomedicine. 14(2-3), Page No.209-15.
27. Latica, V, and Costa, L. (2005)"Evaluation of anticancer potential used in Bangladeshi folk medicine", J. Ethnopharmacol., 99(1), Page No. 21-38.
28. Venkatesh P., Shantala B., Jagetia GC., Rao KK., Baliga MS., 2007. "Modulation of doxorubicin-induced genotoxicity by Aegle Marmelos in mouse bone marrow: a micronucleus study".
29. Rudrama Devi K. & Kusum Latha Chamyal (2012). "*Protective effects of Phyllanthus fruit extract in Adriamycin induced genotoxicity in bone marrow cells of mice"*, International Journal of Pharma and Bio Sciences Vol3(1), p 133-140.
30. Maity P, Hansda D, Bandyopadhyay U, Mishra DK. Biological activities of crude extracts and chemical constituents of Bael. Indian J Exp Biol. 2009;47:849–61.
31. Baliga MS, Thilakchand KR, Rai MP, Rao S, Venkatesh P. Aegle marmelos (L.) Correa (Bael) and its phytochemicals in the treatment and prevention of cancer. Integr Cancer Ther 2012; 12(3): 187-196.
32. Nerseyan A, Muradyan R. Seabuckhorn, (2004). Juice protects mice against genotoxic action of cisplatin. Exp. Oncol. 26: 153-5.
33. Miyata M, Takano H, Guo LQ, Nagata K, Yamazoe Y,(2004). Grape furit Juice intake does not enhance but rather protects against aflatoxin B, induced liver DNA damages through reduction of hepatic CYP3A activity carcinogenesis: 25: 203-9.
34. Weishurger JH, Hosey JR, Larios E PiH man B, Zang E, Hara Y, Kutcheremx G,(2001). Investigation of commercial mitolife as an antioxant and antimutagen. Nutrition: 17 3 22 - 25.

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