AEGLE MARMELOS HAS PROTECTIVE EFFECTS AGAINST GENOTOXICITY INDUCED BY LEAD IN THE BONE MARROW CELLS OF MICE

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ABSTRACT

In the present studies, the protective effects of *Aegle marmelos* fruit extract has been evaluated against Lead nitrate induced micronuclei in bone marrow cells of mice. Single I.P treatment of *Aegle marmelos* fruit extract of various test doses 200, 400 & 600 mg/kg had shown nontoxic nature in bone marrow cells of mice. In Lead nitrate treated groups, there is a significant increase in the frequency of micronuclei in polychromatic erythrocytes of mice when compared with control values. However, along with Lead nitrate when animals were primed with *Aegle marmelos* fruit extract there is an inhibition in the frequency of micronuclei was observed at all dose levels. Thus that clearly indicate protective effects of *Aegle marmelos* fruit extract against Lead nitrate induced genotoxicity in bone marrow cells of mice. Therefore the data showed *Aegle marmelos* fruit extract is a safer dietary component in heavy metal induced DNA damage in cancer chemotherapeutic strategy.

KEY WORDS: *Aegle marmelos*fruit extract (AMF), Micronuclei,Bone marrow cells.

INTRODUCTION

Lead (Pb) is a heavy metal and harmful even in small amounts. Nevertheless, humans get exposed to Pb through their environment and diet [1]. The manifestations of Pb poisoning in humans are nonspecific. They may include weight loss, anemia, [2,3] memory loss, [4] nephropathy, infertility, liver, testis and heart damages' [5, 6] , DNA damage [7] Herbs are gaining additional focus because of their less toxicity and high efficacy against a number of ailments. Epidemiological studies have shown that fruits, vegetables, spices, tea and medicinal herbs rich in antioxidants and other micronutrients protect against diverse forms of chemically induced carcinogenesis, inhibit DNA-damage, mutagenesis and lipid peroxidation [8,9]*Aegle marmelos*, known as bael, grows in tropical and subtropical parts of the world. Various parts of the AM are used in Indian system of medicine for treatment of many diseases, including diarrhea, dysentery and dyspeptic symptoms [10,11]. Marmelosin, isolated from the AM, has been reported to have anti-helminthic, anti-bacterial, antioxidant activity and anti-carcinogenic [12-14]. Hence in the present investigation a study was undertaken to observe the efficacy of AMF extract against Drug induced micronuclei in bone marrow erythrocytes of mice

MATERIALS AND METHODS

Chemical, Lead nitrate was purchased from Sigma Chemical Co. (St Louis, MO, USA). Other Reagent grades chemical were procured locally.

Extract Preparation: The identification of the plant *Aegle marmelos* was done by botanist Prof. Prathibadevi, Department of Botany, Osmania university, Hyderabad, Andhra Pradesh, India. The fruit *Aegle marmelos* were collected. The pieces of fruits were taken and cut in to small pieces. After that paste was taken in a separating funnel and added double distilled water and extracted with double distilled water by refluxing for 36 hrs. at 60°C. On the day of experimentation, the desired amount of powder was dissolved in double distilled water for the final administration.

The study was conducted on random breed, 6-7 weeks old and 24- 28 gm body weight male Swiss albino mice. They were maintained under controlled conditions of temperature and light (light: dark, 12 hrs: 12 hrs.). They were provided standard mice feed and water ad libitum. The study protocol was approved by the Institutional Animal Ethical Committee (IAEC, Ref. No. - 2157/225/2006). For micronucleus test, three dose of *Aegle marmelos* i.e. 200, 400 and 600 mg/kg body weight were administered. *Aegle marmelos* extract were dissolved in double distilled water and administered as single dose in 0.2 ml per mouse 24 hours prior to Lead nitrate administration. Swiss albino mice weighing about 22-24 g aged 810 weeks old were utilized in the present study. The drug was supplied by Reddy Labs Hyderabad. For each dose group of five animals were used. The animals were fed with 40 mg/kg Lead nitrate intraperitoneally in two installments within 24 hr interval. The control group of mice received 0.5 physiological saline simultaneously. The animals were scarified 6 hr after the last administration, bone marrow preparations were made by an air drying technique and stained with May Grunwald and Giemsa stains according to the method described by Maier and Schmid[15]. 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. The data obtained from these studies were analyzed using t-test adopted form Gold stein (1965).

RESULTS

The results on the induction of micronuclei in bone marrow erythrocytes of mice are depicted in Table-1 and the photographs of micronuclei are shown in Fig-1 & 2. The frequencuy of micronuclei in control was 0.25% and the values were 0.32%, 0.36% and 0.37% after the administration of 200,400 and 600mg/kg *Aegle marmelos* (AMF) extract respectively (Table1). Hence, the results clearly indicate the non mutagenic nature of AMF extract in Lead nitrate treated group, there was a significant increase in the percentage of micronuclei(1.12) in bone marrow cells of mice when compared to control –II value (0.26) (Table-II). However the frequency of micronuclei decreased to 0.76%, 0.61% and 0.50% after the coadministration of 200, 400 and 600mg/kg of *Aegle marmelos* extract. The P/N ratio in bone marrow cells showed a decrease when compared with control values. The differences in the frequency of micronuclei in control and lead treated group were found to be significant ($P< 0.05$), Table-II. The percentage of micronuclei between lead treated group and AMF extract primed group were found to be significant.

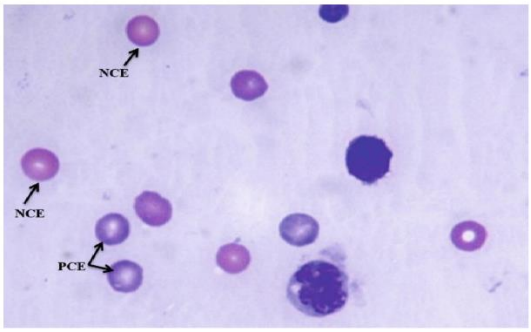

Fig. 1 The presence of micronucleus in Lead nitrate treated animals

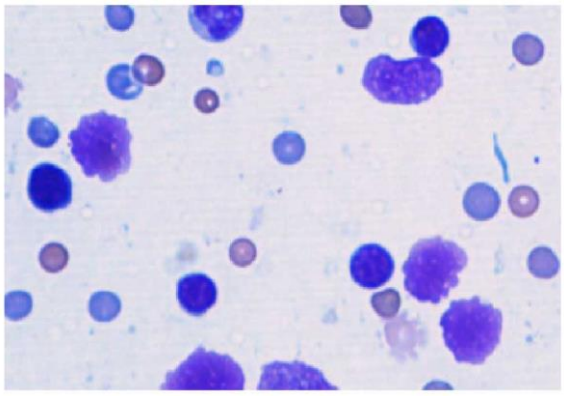

Fig. 2 The absence of micronucleus in AMF extract treated animals.

Table 1: Incidence of micronuclei in bone marrow erythrocytes of mice treated with*Aegle marmelos* fruit extract

Dose group	Micronuclei in polychromatic erythrocytes	Micronuclei in normochromatic cells	Micronuclei in total P+N cells	P/N ratio
Control I	40/16000 (0.25	20/16100 (0.12)	60/32100 (0.18)	0.99
Aegle marmelos				
200 mg/kg	58/16000 (0.36)*	40/16710 (0.23)	98/32710 (0.29)	0.95
400 mg/kg	60/16000 (0.37)*	46/17210 (0.26)	106/33210 (0.31)	0.92
600mg/kg	64/16000 (0.40)*	50/17800 (0.28)	114/33800 (0.33)	0.89

The values in parenthesis are percentages * P<0.01

Table 2: Protective effects of *Aegle marmelos* fruit extract in Lead nitrate induced genotoxicity in mice.

Dose group	Micronuclei in polychromatic erythrocytes	Micronuclei in normochromatic cells	Micronuclei in total P+N cells	P/N ratio
Control II	42/16000 (0.26)	18/16200 (0.11)	58/32200 (0.18)	0.98
Lead nitrate (40mg/kg)	180/16000 (1.125)*	194/16800(0.55)	274/32800(0.83)	0.95
200 mg/kg	124/16000 (0.76)*	62/17200 (0.38)	186/33200(0.56)	0.93
400 mg/kg	98/16000 (0.61)*	40/17280 (0.24)	138/33280(0.41)	0.93
600mg/kg	80/16000 (0.50)*	54/17682 (0.30)	134/33682(0.39)	0.90

The values in parenthesis are percentages *P<0.01

DISCUSSION

The in vivo micronucleus test is one of best methods to screen the clastogenic effects of chemicals and drugs [15] using this procedure the mutagenicity of various alkylating agents and drugs [16-19] was also established. Naturally occurring antioxidants have been extensively studied for their capacity to protect organisms and cells from oxidative damage. The present results are comparable with [20] who reported the protective effects of *Aegle marmelos* in mouse bone marrow cells at CP induced 350 mg/kg dose level. Earlier we have reported to protective effects of phyllanthusemblica fruit extract on adriamycin induced genotoxicity in somatic cells of mice (Kusumlatha and Rudrama Devi 2010). The protective effect against Lead nitrate induced genotoxicity by *Aegle marmelos* may be due to inhibition of free radicals formed by Lead nitrate in cytoplasm of cells and increased antioxidiant status by addition of fruit extract. The fruit of Aegelmarmelos contains marmelosin, luvangetin, aurapten, psoralen, marmelide, tannins and phenols. The AMF extract has been used in for treating diarrhea, diabetic, constipation, heart disease, ulcers woundhealing 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-dimethoxy2naplhale 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 [22]. It is well known that consumption of fruits and vegetables is associated and are known to prevent chromosomal and DNA damage in animals [23,24]. Usually antimutagens acting in rodents are active in human too [25]. Our results have a practical decline of genotoxic effects of Lead nitrate in heavy metal exposed population and pharmaceutical lead plant workers handle this metal which may alternate the higher risks for development of secondary malignancy and for abnormal reproductive outcomes. *Aegle marmelos* (L.) Corr. commonly known as Bael (Family: Rutaceae) is described in the Ayurveda for its use in various illness such as fever, hyperlipidemia, hypertension, analgesic, anti-inflammatory, heart disease, etc. It is an important medicinal plant and compounds purified from Bael have proven biologically active against several major diseases in experimental animal models and have shown activities including antispermatogenic, antimicrobial, and antioxidant. 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.[20]

REFERENCES

1. Gidlow DA (2004). Lead toxicity. Occup. Med. (Lond). 54: 76-81.
2. Khalil-Manesh F, Tartaglia-Erler J, Gonick HC (1994). J. Trace Elem. Electrolytes Health Dis. 8: 13-19.
3. Waldron HA (1966).. Br. J. Ind. Med. 23: 83-100.
4. Gurer-Orhan H, Sabir HU, Ozgüneş H (2004). Toxicology, (195): 147-154.
5. Hopkins A (1970). Experimental lead poisoning in the baboon. Br. J. Ind. Med., 27: 130140.
6. Patocka J, Cerný K (2003). ActaMedica (Hradec Kralove), 46: 65-72.
7. Dilip Reddy K ., Pramod Kumar and K Rudrama Devi; Int. Journal environmental Scivol (3).
8. Ziech, D., R. Franco, A. Pappa and M.I. Panayiotidis, 2011. Mutation Res., 711: 167 173.
9. Birt, D.F., S. Hendrich and W. Wang, 2001. Ther., 90: 157-177.
10. Shoba, F.G. and M. Thomas, 2001. J. Ethnopharmacol., 76: 73-76.
11. Sharma, B., S.K. Satapathi and P. Roy, 2007. Int. J. Pharmacol., 3: 444 452.
12. Khan, T.H. and S. Sultana, 2009. J. Enzyme. Inhib. Med. Chem., 24: 320-327.
13. Patil, D.N., A.R. Kulkarni and B.S. Patil, 2010 Int. J. Pharmacol., 6: 68-71.

14. Khan, T.H. and S. Sultana, 2011. Toxicol. Mech. Methods, 21: 453 462.
15. Maier, P. and Schmid, W. (1976). Mut. Res. 40(4): 325-327.
16. Rudrama Devi, K. and Reddy, P. P. (1985). IRCS Med. Sci. and Bio. 12: 125-1246.
17. Rudrama Devi, K. and Reddy, P.P. (1986). Cell and chromosome Res. 9(2): 39-41.
18. Rudrama Devi, K. and Reddy, P.P. (1987). Agri. and Biological Res. 4: P 6-9.
19. Rudrama Devi and Reddy G. M. (1995). Cell and Chromosome Research. 18: 91-94
20. Venkatesh P., Shantala B., JagetiaGC., Rao KK., Baliga MS., 2007.
21. KusumLatha C. & Rudrama Devi K, (2010). Bioscan (Vol 5(2); 317-320).
22. Baliga MS, Thilakchand KR, Rai MP, Rao S, Venkatesh P. Integr Cancer Ther 2012; 12(3): 187-196.
23. Nerseyan A, Muradyan R. Seabuckhorn, (2004). Exp. Oncol. 26: 153-5.
24. Miyata M, Takano H, Guo LQ, Nagata K, Yamazoe Y,(2004). Carcinogenesis: 25: 2039. 25
25. Weishurger JH, Hosey JR, Larios E PiH man B, Zang E, Hara Y, Kutcheremx G,(2001). Nutrition: 17 322-25.