

ASSESSMENT OF THE INHIBITORY IMPACT OF AEGLEMARMALOS LEAF EXTRACT ON ANTIGENOTOXIC ACTIVITIES IN BONE MARROW CELLS OF MICE

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ABSTRACT

In the present study the protective effects of *Aegle marmelos* 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 intraperitoneally 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. Pretreatment 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 marmelos* Leaf Extract, Micronuclei, Mice

Introduction:

Agelemarmelos (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-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 Agelemarmelos 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 exhibits antihyperlipidaemic effect in streptozotocin induced diabetic rats. Unripe fruit extract of *Aegle marmelos* has shown gastro protective and antidiarrhoeal properties. 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 esophageal, carcinomas, osteosarcoma, 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 been 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 deoxyrubin 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 Ranbaxy Laboratories, Hyderabad,

Animals: Six to eight weeks old male mice (*Mus Musculus*) of swiss albino mice weighing about 25-27gms 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 ad libitum.

Plant material: The plant material was procured from wholesale spice and herbs market Hyderabad. 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 (BuchiRotavapor).

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 whereas 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 interperantly 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

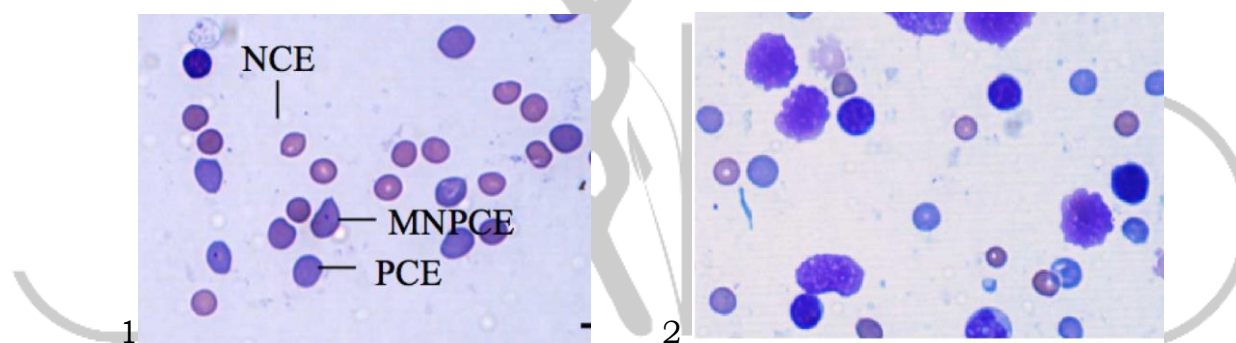


Fig. 1 The presence of micronucleus in Dox treated animals

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

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 intercalates 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 quinone 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⁺ doxorubicin free radical complex is produced. This iron doxorubicin complex can reduce oxygen to hydrogen peroxide and other active species [20, 21].

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 (N)	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)	54/18200(0.29)	0.78

*P>0.05

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

Groups	Micronuclei in polychromatic cells (P)	Micronuclei in normochromatic cells (N)	Micronuclei in total P+N cells	P/N ratio
Control	18/8000(0.22)	12/8060(0.15)	38/9880(0.38)	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

The animals were treated with methanol extract of *Aegle marmelos* of three doses showed an 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 a significant increase in the frequency of micronuclei from control (0.22%) to doxyrubicin treated groups (1.40%)

(Fig.1). Whereas the pretreatment with the methanolic extract of AMLE 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 a 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 streptozotocin 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 phyllanthusemblica 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 antioxidant 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 *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 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-2-naphthyl carboxy 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 marmelos* leaf extract can be used as a major chemopreventive agent against doxyrubicin induced mutagenicity.

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 marmelos* leaf extract can be used as a major chemopreventive agent against doxyrubicin induced mutagenicity.

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