INCIDENCE OF CHROMOSOMAL ABERRATIONS BY DOXORUBICIN AND ITS PROTECTION BY AEGLE MARMELOS

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ABSTRACT
Adriamycin (ADR) (doxorubicin) is one of most effective chemotherapeutic agents and is the most commonly used anthracyclin antibiotic effective in treatment of various cancers. Adriamycin induces mutations and chromosomal aberrations in normal and tumor cells. Adriamycin has high affinity for cell nuclei and about 60% of total intracellular adriamycin is found in cell nucleus and binds to DNA polymerase and inhibits synthesis of nucleic acid and protein, results in DNA damage and free radical formation. As Adriamycin is widely used, it is important to reduce its toxicity to normal cells which can be achieved by concurrent administration of antioxidants. Herbs are gaining additional focus because of their less toxicity and high efficacy against a number of ailments. has been used Aegle Marmelos extract for broad spectrum of diseases and its isolated compound Aegle Marmelos extract found to be anticarcinogenic and a potential antioxidants. In the present investigation studies were carried out to observe the efficacy Aegle Marmelos fruit extrac(tAMFE) of against Adriamycin induced cytogenetic damage in bone marrow cells of mice. The animals treated with 10mg/kg, 15mg/kg and 20mg/kg of showed Aegle Marmelos fruit extract to be non mutagenic. Aegle Marmelos extract shows protective effects against the adriamycin induced genotoxicity in bone marrow cells of mice. Hence Aegle Marmelos fruit extract C supplementation is safer in chemotherapeutic s Aegle Marmelos stragey.

KEYWORDS: aeges marmelus extract, Adriamycin, Micronuclei, Bone marrow cells.

INTRODUCTION
Adriamycin(Doxorubicin) is an anthracycline antibiotic used as an antitumor agent against human malignancies such as leukemia, lymphomas and many solid tumors but which also has
a wide variety of toxic side effects, including cardio toxicity, cytotoxicity and the induction of chromosomal aberrations. The majority of antineoplastic drugs, besides their generic growth property, display genotoxic effects which in turn contribute to growth inhibition.\cite{1} These genotoxic effects may lead to initiation of unrelated tumours years after cessation of chemotherapy.\cite{2} Free radical mediated reactions are responsible for a wide range of chemotherapy-induced side effects and antioxidants are able to protect non-malignant cells and organs against damage caused by cytostatic agents.\cite{3} Most cancers can be controlled by adopting appropriate conventional treatments such as surgery, radiation and chemotherapy. However these treatments cause side effects. Hence the important of conventional therapies may decline. Alternative treatments founded in a back to nature approach might yield improved treatment avenues with fewer or no undesirable side effects. In the search of these new treatment, natural products are carving a path as prospective anticancer agents. Induction of chromosomal aberrations in somatic and germ cells in Swiss albino mice has been reported.\cite{4}

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.\cite{5,6} 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 diarrhoea, dysentery and dyspeptic symptoms.\cite{7, 8} Marmelosin, isolated from the AM, has been reported to have anti-helminthic, anti-bacterial, antioxidant activity and antitumorogenic.\cite{9-11} 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**

*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, 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 adlibitum.

**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 Antonio and Brito.[12]

**Preparation of extract**
Powdered plant material was repeatedly extracted in 4000 mL round bottom flask with 2000 mL methanol. The methanolic extracts were 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 AMF extract for seven days. In the secondary experiment Group I -Control,,Group II-200 AMF+16mg/kg DOX, Group III-400 AMF+16mg/kg DOX, Group IV-600AMF+16mg/kg DOX given interpertanlly 24 hrs prior to the administration of plant extract.

In the present study the air drying technique of Preston et al (1987)[13] was employed with slight modifications to study the effect of test compounds on somatic cells of mice. Sampling times were ranged from 48 hrs to cover short and long term effects on cells at different stages of cell cycle at the time of exposure to the test compound.

The animals were sacrificed at appropriate time intervals of 48 hrs. 2hours prior to sacrificing, 0.2ml of 0.05 % colchicine was injected to all the animals to inhibit spindle formation in order to get well spread metaphases. All the animals were killed by cervical
dislocation and hind limbs were dissected out for femur bones and freed from connective tissue and muscles with the help of gauge and immediately suspended in hypotonic solution (0.56% KC1).

**Methodology:** The bone marrow was flushed out into clean glass Petri dishes with a hypodermic syringe fitted with a 22-gauge needle and dispersed well in hypotonic solution (0.56% KC1 i.e. 0.75M KC1) to get a homogeneous cell suspension. The suspension was collected in clean centrifuge tubes and incubated at 37°C for 45 minutes. After the incubation the tubes were centrifuged for 10 minutes at 1000rpm. The supernatant was removed carefully with the help of Pasteur pipette leaving a small volume over the pellet. To the pellet 5ml of pre chilled fresh fixative (3:1 absolute methanol: glacial acetic acid; prepared freshly before use and preserved in refrigerator for chilled condition) was added drop wise from the sides of the centrifuge tubes and immediately dispersed the cell suspension by aspirating several times with a Pasteur pipette. The tubes were left undisturbed for 10 minutes at room temperature. After 10 minutes the suspension was centrifuged again and the supernatant was removed carefully leaving a small volume of the supernatant over the pellet and 5ml of chilled fresh fixative was added carefully and kept for 10minutes undisturbed. This process was repeated for 4 to 5 times to ensure proper fixation. In the final change the cells were resuspended in 0.5ml of fresh fixative.

Two to three drops of cell suspension were dropped on clean grease free, prechilled slides. The slide was blown once across and allowed to dry on a slide warmer. Two slides from each animal were prepared by air drying technique from control and treated animals. The slides were coded and stored in dust free chambers. The staining was done within 24hours after the slide preparation. The slides were stained with 2% Giemsa (2ml of Giemsa in 46ml of double distilled water plus 2ml of phosphate buffer* pH 6.8) for 7-8 minutes and later they were rinsed in double distilled water and allowed to dry.

**Scoring:** Finally the slides were soaked in Xylene for overnight and mounted in DPX. For each mice 100 well spread metaphases were examined randomly using Leica CW 4000 Image analyzer.
Table 1: Frequency of Chromosomal aberrations recorded in somatic cells of mice analyzed after 48hrs treatment with various doses of *Aegle marmelos* fruit extract

<table>
<thead>
<tr>
<th>Dose (mg/kg) and Duration of treatment</th>
<th>48hrs</th>
<th>Normal metaphases %</th>
<th>Abnormal metaphases %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>486 (97.20)</td>
<td>14 (2.80)</td>
<td></td>
</tr>
<tr>
<td>200 mg/kg AMFE</td>
<td>485 (97.00)</td>
<td>15 (3.00)</td>
<td></td>
</tr>
<tr>
<td>400 mg/kg AMFE</td>
<td>483 (96.60)</td>
<td>17 (3.40)</td>
<td></td>
</tr>
<tr>
<td>600 mg/kg AMFE</td>
<td>482 (96.40)</td>
<td>18 (3.60)</td>
<td></td>
</tr>
</tbody>
</table>

The values in parentheses are percentages

Table 2: Classification of various types of chromosomal aberrations in somatic cells of mice analyzed after 48hrs of *Aegle marmelos* fruit extract

<table>
<thead>
<tr>
<th>Dose (mg/kg) and duration</th>
<th>Structural aberrations</th>
<th>Numerical aberrations</th>
<th>Total of no. of aberrations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gaps</td>
<td>Breaks</td>
<td>fragments</td>
</tr>
<tr>
<td>48hrs Control II -</td>
<td>8</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>200 mg/kg AMFE</td>
<td>9</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>400 mg/kg AMFE</td>
<td>11</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>600 mg/kg AMFE</td>
<td>11</td>
<td>14</td>
<td>3</td>
</tr>
</tbody>
</table>

Gaps and polyploids are not included in total aberrations

The values in parentheses are percentages

Table 3: Frequency of CA recorded in somatic cells of mice treated with Dox+ and primed with *Aegle marmelos* fruit extract

<table>
<thead>
<tr>
<th>Grouping</th>
<th>Dose (mg/kg) and Duration of treatment</th>
<th>48hrs</th>
<th>Normal metaphases %</th>
<th>Abnormal metaphases %</th>
<th>% of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Control</td>
<td>487 (97.40)</td>
<td>13 (2.60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>Dox 16mg/kg</td>
<td>385 (77.00)</td>
<td>115 (23.00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>200 AMFE + 16mg/kg Dox</td>
<td>413 (82.52)</td>
<td>87 (17.40)</td>
<td></td>
<td>24.78</td>
</tr>
<tr>
<td>Group IV</td>
<td>400 AMFE + 16mg/kg Dox</td>
<td>431 (86.20)</td>
<td>69 (13.80)</td>
<td></td>
<td>40.00</td>
</tr>
<tr>
<td>Group V</td>
<td>600 AMFE + 16mg/kg Dox</td>
<td>442 (88.40)</td>
<td>58 (11.60)</td>
<td></td>
<td>49.56</td>
</tr>
</tbody>
</table>
Table 4: Classification of CA recorded in somatic cells of mice treated with Dox and primed with *Aegle marmelos* fruit extract

<table>
<thead>
<tr>
<th>Dose (mg/kg) and duration of treatment</th>
<th>Structural aberrations</th>
<th>Numerical aberrations</th>
<th>Total of no. of aberrations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gaps</td>
<td>Breaks</td>
<td>fragments</td>
</tr>
<tr>
<td>Control II</td>
<td>11</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Dox 16 mg/kg</td>
<td>76</td>
<td>81</td>
<td>27</td>
</tr>
<tr>
<td>200 AMFE + 16 mg/kg Dox</td>
<td>43</td>
<td>58</td>
<td>23</td>
</tr>
<tr>
<td>400 AMFE + 16 mg/kg Dox</td>
<td>40</td>
<td>47</td>
<td>18</td>
</tr>
<tr>
<td>600 AMFE + 16 mg/kg Dox</td>
<td>34</td>
<td>41</td>
<td>16</td>
</tr>
</tbody>
</table>

The values in parenthesis are percentages *p>0.05 **p<0.01

Gaps and polyploides are not included in total aberrations

RESULTS AND DISCUSSION

The doses selected for AMFE extract were 200, 400 and 600/kg body weight at various time intervals. The mutagenic effects of the extract were studied on somatic cells of mice for different time intervals. The results were recorded in table 1-4.

At 48 hrs the frequencies (%) of chromosomal aberrations in the AMFE extract treated mice 200, 400 and 600mg/kg were 3.00, 3.40 and 3.60% respectively when compared to that of controls 2.80% (Table -1). The differences in the frequencies of chromosomal aberrations between and AMFE extract treated mice for 48hrs were analyzed by X2 test and the results were found to be insignificant (P>0.05, Table- 3). The difference in the frequencies of chromosomal aberrations between controls and AMFE extract treated mice for 48 hrs were analyzed by X2 test and the results were found to be insignificant (P>0.05, Table -1). At 48 hrs the frequency was found to be 17.40, 13.80 and 11.60 in AMFE primed mice against 23.00 in dox alone administered mice with that of contro 2.60 %.

At 48 hrs the frequency was found to be 17.40, 13.80 and 11.60 in AMFE primed mice against 23.00 in dox alone administered mice with that of contro 2.60 %. The differences in the frequencies of chromosomal aberrations between controls and treated mice with 16 mg/kg body weight of doxorubicin on priming with 200, 400 and 600 mg/kg body weight were analyzed using X2 test (24,48 and 72 hrs) and the results were found to be significant (P<0.05, Table- 3).
Increasing concentrations of AMF extract GE (200, 400 and 600 mg/kg) was primed to mice and they were administered with 16 mg/kg of DOX to evaluate the mutagenic effect of AMFE extract. The results were tabulated in table- 17,18 and 19 and illustrated in graph- 8

Among the non primed groups the controls have shown only 3.80% of abnormal metaphases when compared to 21.20 in DOX alone administered mice. There was a significant decrease in the percentage of abnormal metaphases in mice primed with AMF extract (200, 400 and 600 mg/kg) as 15.00, 13.80 and 9.20 respectively for various concentrations (Table 17 and Graph 8).

The inhibitory effects of AMFE extract against doxorubicin induced chromosomal aberration in germ cells of male mice were 29.24, 34.90 and 56.60% in III; IV & V grouped animals Table- 17 and graph.9. The differences in the frequencies of the chromosomal abnormalities between the controls and treated mice were analyzed by $X^2$ test and the results were found to be significant and represented (P<0.01, Table- 19)

The Inhibitory effects of AMFE extract against doxorubicin induced chromosomal aberrations in somatic cells of male mice were 48hrs the percentage of inhibition was 24.78, 40.00 and 49.56%. depicted in table-4.

In the results of Larramendy et al (1980) the frequency of chromatid-type aberrations exhibited a direct-correlation with the dose in mice treated for 6h but not for 12 h. On the other hand, chromosome-type aberrations detected 12 hrs after injection were directly correlated with the dose of adriamycin, the genotoxic effects of the metacentric-like chromosomes induced by adriamycin arise either from translocations involving entire chromosomes arms or from aberrations of the exchange type between 2 short arms of acrocentric chromosomes of doxorubicin induced a significant increase (p <0.01) the frequency of chromosome abnormalities, these results being consistent with those reported by other authors. In fact, according to Ling et al. (1996) doxorubicin could induce apoptosis by promoting cyclin B accumulation.

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 nontumor cells in human and 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 Doxycubicin cytotoxicity.\textsuperscript{[15,17,18]} The biochemical mechanism of adriamycin causes cytotoxicity is unclear. However when it intercates with DNA generates free radicals. Two pathway of mechanisms have been proposed. Two different pathways of free radical formation of Dox have been described. First is the formation of semiquinone free radical the semi quine 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.\textsuperscript{[19,20]}

It is important to reduce its toxicity in normal cells, a goal that can be achieved by concurrent administration of free radical scavenging agents, such as antioxidants.\textsuperscript{[21,22]} Natural antioxidants in the human diet can attenuate the effects of mutagens and genotoxic carcinogens. Some antioxidants, such as vitamins A, C and E, minimize the side effects of antineoplastic drugs and can improve cancer chemotherapy. An increase in the dietary content of antioxidants through the increased ingestion of fruits and vegetables rich in these compounds can decrease the oxidation of DNA by free radicals, thereby preventing cancer and other degenerative diseases. Oxidative damage to biomolecules caused by stress is one of the major risk factors for atherosclerosis, mainly through the oxidation of low density lipoprotein (LDL) in the blood.

The present results are comparable with\textsuperscript{[23]} who reported the protective effects of \textit{Aegle Marmelos} in mouse bone marrow cells at 350 mg/kg dose level. Earlier we have reported on the protective effects of \textit{phyllanthus emblica} fruit extract on adriamycin induced genotoxicity in somatic cells of mice.\textsuperscript{[24]} 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. The fruit of \textit{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 wood healing because of its medicinal properties. Lupeol, a compound present in \textit{A. marmelos} possess antineoplastic effects on various human neoplastic cell lines.\textsuperscript{[25]} Marmelin (1-hydroxy-5, 7-dimethoxy- naplhale necarboxy aldehyde) present in \textit{A. marmelos} inhibiting
growth of epithelial cancer cells, but not normal cells (mouse embryo fibroblasts) further it decreases cell survival, proliferation and invasiveness.\cite{25} It is well known that consumption of fruits and vegetables is associated and are known to prevent chromosomal and DNA damage in animals\cite{26,27}. Usually antimutagens acting in rodents are active in human too.\cite{28} Our results have a practical decline of genotoxic effects of doxorubicin 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 doxorubicin induced genotoxic effect in somatic cells of mice. It is concluded that *Aegle mameleos* can be used as a major chemopreventive agent against doxorubicin induced mutagenicity.

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**REFERENCES**


