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PROTECTIVE EFFECTS OF *AEGLE MARMELOS* EXTRACT AGAINEST DOXYRUBICIN INDUCED GENOTOXICITY IN SWISS ALBINO MICE

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ABSTRACT

In the present study the protective effects of *Aegle mamelos* fruit extract AMF were carried out in somatic cells of mice against doxyrubicin induced genotoxicity. Three different doses of *Aegle Marmelos* fruit extract were tested for the antimutagenicity using the micronucleus test in bone marrow cells of mice. The test compound doxyrubicin higher dose 16 mg/kg was injected intrapertioneally prior to the administration of plant extract. The *Aegle Marmelos* fruit 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 AMF for 7 days and simultaneously with a single dose of doxyrubicin, there was a significant inhibition in the percentage of micronuclei was found thus *Aegle Marmelos* fruit extract modulates the doxyrubicin induced cytotoxicity in a dose dependent way. Thus the overall results suggest the protective nature of *Aegle Marmelos* fruit extract, it is a safer dietary antioxidant as cancer chemopreventive therapy.

KEY WORDS: **Doxyrubicin, Genotoxicity, *Aegle Mamelos* Fruit Extract, Micronuclei, Mice**

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INTRODUCTION

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 rises to secondary tumors (Berelta, 1991). The anthracyclin antibiotic adriamycin (doxorubin) is one of most effective chemotherapeutic agents against a wide variety of cancers. The tumor that respond better breast and esophageal, 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 (Quiles et al., 2002).Doxyrubicin induces mutations and chromosomal aberrations in normal and tumor cells (Ge writez, 1999). It is 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(Evert et al., 2001). 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 (Amaramokrane et al., 1996).Further the consumption of fruits and vegetables can minimize to some extent the occurrence of some cancers (Dorai and Agarwal 2004). The plant extracts are gaining much importance for treatment of various diseases. Ziech et al (2011) and Birt et al (2001) have reported that fruits and vegetables, spice contains antioxidants which protects the process of carcinogenesis, DNA damage and lipid perodisation. The different parts of AM has been used for broad spectrum of diseases and its isolated compound marmelosin found to be antihelminitc antibacterial and anticarcinogenic (Shoba and Thomas, 2001; Sharma et al., 2007, Khan and Sultana, 2009; Patil et al., 2010; Khan and Sultana, 2011). Hence studies were carried out to observe the efficacy of AMF extract against deoxyrubin induced cytogenetic damage in bone marrow cells of mcie.

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 Antonio and Brito (1998).

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-600 AMF+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 was examined for each animal under 100 x magnifications (Schmid 1975). 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 the 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 (Lingberg et al., 2007, Rudrama Devi et al., 2011). The most frequently used genotoxicity test in mammals is the micronucleus test which provides simple and rapid indirect measure of structural and numerical aberrations (Heddle et al., 1991) 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 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 (Anderson et al., 1997, Prahalathan et al., 2005, Kusum Latha and Rudrama Devi 2010). 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 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 (Granados et al., 2010, Xu et al., 2005).

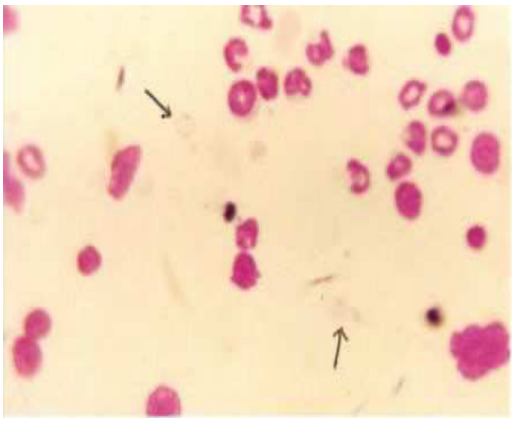

Figure 1
The presence of micronucleus in Dox treated animals

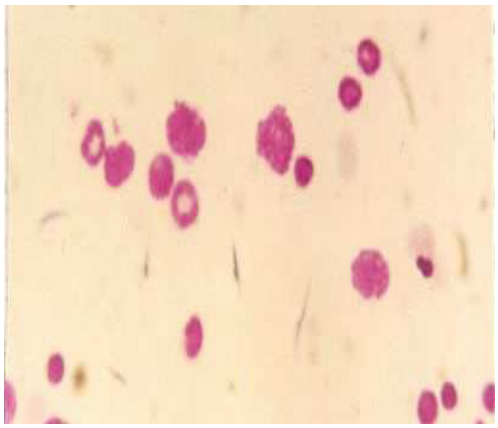

Figure 2
The absence of micronucleus in AMF extract treated animals

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 a significant increase in the frequency of micronuclei from in control(0.22%)to doxyrubin treated groups(1.58%)(Fig.1). Whereas the pre treatment with the methanolic extract of AMF 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 the AMF extract (MKL) brings the values to lower range (0.85%). This indicates the chemoprotective nature of the AMF extract. The difference in the frequency of micronuclei between the group III & Group IV showed statistically significant (P<0.01). Thus the data indicate AMF 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 (Geetha and Rudrama Devi 1992, Kusum Latha & Rudrama Devi, 2010, Rudrama Devi et al., 2010). The present results are comparable with (Venkatesh et al., 2007) who reported the protective effects of *Aegle Marmelos* in mouse bone marrow cells at 350 mg/kg dose level. Earlier we have reported on the protective effects of *phyllanthus emblica* fruit extract on adriamycin induced genotoxicity in somatic cells of mice (Rudrama Devi and Kusumlatha 2012). 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. 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 wood healing because of its medicinal properties. Lupeol, a compound present in *A. marmelos* possess antineoplastic effects on various human neoplastic cell lines (Baliga et al., 2012). Marmelin (1-hydroxy-5, 7-dimethoxy- naplhale 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 (Baliga et al 2012). It is well known that consumption of fruits and vegetables is associated and are known to prevent chromosomal and DNA damage in animals (Nerseyan et al., 2004, Miyata et al., 2004). Usually antimutagens acting in rodents are active in human too(Weishurger et al., 2001). 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.

Table 1
Results on the frequencies of micronuclei in bone marrow erythrocytes of mice administered with various doses of Aegle marmelos fruit 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)	10/8078(0.12)	20/16078(0.12)	0.99
200mg/kg AMFE	20/8000(0.25)	14/8600(0.16)	34/16600(0.20)	0.93
400mg/kg AMFE	22/8000(0.27)	16/9720(0.16)	38/17720(0.21)	0.82
600 mg/kg AMFE	24/8000(0.30)	18/9800(0.18)	42/17800(0.23)	0.81

P>0.05

Table 2
Frequency of micronuclei in bone marrow erythrocytes of mice with doxyrubin primed with Aegle marmelos fruit 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)	11/8020(0.13)	29/16020(0.18)	0.99
Doxyrubicin 16 mg/kg	126/8000(1.58)	20/8060(0.24)	146/16060(0.90)	0.98
200+16 mg/kg AMFE+Dox	70/8000(0.87)*	42/9200(0.45)	112/17200(0.65)	0.86
400+16 mg/kg AMFE+Dox	62/8000(0.76)*	38/9400(0.40)	100/17400(0.57)	0.85
600+16 mg/kg AMFE+Dox	58/8000(0.72)*	40/9420(0.42)	98/17520(0.55)	0.85

P<0.05

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

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