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Antigenotoxic effects of *Aegle marmelos* Fruit Extract in Cyclophosphamide Induced Chromosomal Aberrations and Aberrant Sperms in Germ Cells of Swiss Albino Mice

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ABSTRACT

The present investigation was attempted to study the possible anti-genotoxic activity of crude Aegle marmelos (AMF) fruit extract in cyclophosphamide-induced genotoxicity in mouse by using chromosomal aberrations (CA's) in germ cells and sperm morphology essay (SMA). Three different doses of Aegles marmelos (200, 400, 600 mg/kg) were orally administered for their modulatory capacity on the mutagenecity exerted by CP (50 mg/kg, i.p.). AMF alone did not induce any significant variation in the incidence of CA's and frequency of aberrant sperms. Pretreatment of mice with AMF for 7 days and simultaneously with a single dose of cyclophosphamide significantly reduced the frequency of CA's and frequency of aberrant sperms, suggesting that the Aegles marmelos fruit extractmodulates the CP induced genotoxicity in a dose dependent manner. Thus the results clearly indicate the protective nature of Aegles marmelos fruit extract on cancer chemoprevative strategy.

Key words: *Aegle marmelos, Chromosomal Aberrations, Aberrant Sperms, Germ Cells.*

INTRODUCTION

Cyclophosphamide is an anti-cancerous alkylating agent. The metabolites of this compound can alkylate nucleophilic sites in DNA, RNA and protein[4,10]. It induces DNA single strand breaks at molecular level in rat embryos[19,24] in testicular cells[31]. Further cyclophosphamide is capable of inducing structural chromosomal aberrations in Chinese Hamster cells, in human chorionic villae and various stages of spermatogenesis in germ cells[11]. Further chromosomal damage and a decrease in mitotic index were reported in somatic cells[1,22,27] comet tail length in swissmice[26,28].

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[5,37]. *Aegle marmelos*, known as bael grows in tropical and subtropical parts of the world. Various parts of the *Aegles marmelos* are used in Indian system of medicine for treatment of many diseases, including diarrhoea, dysentery and dyspeptic symptoms[29,30].

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Marmelosin, isolated from the AM, has been reported to have anti-helminthic, anti-bacterial, antioxidant activity and anticarcinogenic[13,14,23]. Hence in the present investigation a study was undertaken to observe the efficacy of AMF extract against Cyclophosphamide induced chromosomal aberrations and aberrant sperms in germ cells of mice.

MATERIALS AND METHODS

Chemicals

Trisodium citrate (Merck), NaCl (Loba Chemie), Methanol (s.d fine chemie), acetic acid (Qualingens), and 2% Giemsa stain solution in phosphate buffer (pH 6.8) were all purchased from E. Merck, India. Cisplatin, garlic extract, ethanol, mitomycin-C and eosin were purchased from Cipla. However, all other chemicals used in the experiments were of analytical grade.

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.

Experimental animals

Eight weeks old random bred male Swiss albino mice (*Mus musculus*) average body weight of 25 ± 2 gms were purchased from National Institute of Nutrition, Hyderabad, were maintained in the departmental animal house under an absolute hygienic conditions as per the recommended procedures by fulfilling all the necessary ethical standards. They were housed in polypropylene shoe box type cages dimensions were 13.5" L x 7.0" W x 6.5" to 8.5"H cages, bedded with rice husk (rice husk procured locally and autoclaved to free from microorganisms) and kept in AC room at the temperature 25°C (± 2°C) and RH 65 ± 5% and a photo-cycle of 12:12 h light and dark periods, were fed with pelleted diet (from National Institute of Nutrition, Hyderabad)composed of 20.0% crude protein, 4.0% crude fiber, 1.0% calcium, 0.6% phosphorus, 8% fish meal, 20% ground nut cake and enriched with stabilized vitamins A, B, C, D3, K, thiamine, riboflavin, pantothenic acid, niacin, folic acid, minerals & trace elements and water.

Treatment

Aegle marmelos (200, 400 and 600 mg/kg) extractions were given in orally for 7 consecutive days and 50mg/ kg of cyclophmaide was administrated on day 7 one hour after regular exposure to antimutagen as a single intraperitonial dose. This is repeated for four weeks. Control (H_2O) and positive control (0.1 ml of mitomycin-C) group of animals were also maintained simultaneously. 5 animals were used in each treatment and control group. Slides were screened with Leica CW 4000 Image analyzer.

Chromosome aberration analysis from germ cells

The mice were killed on 28th day, 24 h after administration of last dose of the drug. Seminiferous tubules from testis were collected in 5ml of isotonic 1.2% trisodium citrate solution and incubated at the temperature 37ºC for 45 min. The cell suspension was centrifuged in 120x17 mm conical centrifuge tubes for 10 min at 1000 rpm. To the pellet 5 ml of freshly prepared pre-chilled fixative (3:1 methanol and acetic acid) added and centrifuged. This process repeated for 4 to 5 times. The Chromosomal preparations were made by the air drying technique[6] and stained with 2 ml of 2% Giemsa (2 ml of 2% Giemsa in 46 ml of double distilled water plus 2 ml of phosphate buffer* pH 6.8) for 7-8 min. Approximately 500 meiotic metaphases screened for numerical (Autosomal Univalents, Sex- Autosomal Univalents, euploids and aneuploids) and structural (translocations) Aberrations.

Sperm morphology assay

All the control and treated animals were sacrificed on 35th day. This is because spermatogenesis takes about 34.5 days to complete in mice. Sperms were sampled from the caudal epididymis after the animals had been sacrificed by cervical dislocation. Sperm suspension was prepared from the caudal of each testis by mincing the caudal in physiological saline. To the suspension 2- 3 drops of 1% aqueous eosin was added and kept for about 20 min undisturbed. Smears were made on clean slides and allowed to dry in air.

1000 sperm cells/mouse were assessed for morphological abnormalities according to the criteria of Wyrobek and Bruce[35].

Statistical analysis

The significance for the differences between control and treated groups was statistically analyzed by using x 2 test. Data are expressed as mean ± SES in the Tables 1 and 2. Results were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Cyclophosphamide is widely used drug among alkylating agents in cancer chemotherapy. Acrolein and phosphoramide are active compounds of CP and further these compounds reduce the growth of cancerous cells by acting at DNA level[9]. There are many studies showing chemotherapeutic agents and CP cause gene mutations, CA and aneuploidy and rearrangements in somatic and germ cells of mice in vivo and in vitro test systems[2,18] and an elevated frequency of secondary treatment related tumors in human cancer survivors[15,16]. Earlier studies have shown that post meiotic germ cells are specifically sensitive to cyclophosphamide treatment[32,33]. The administration of low doses of cyclophosphamide to male rats for 6 week produced greater than 95% post implantation loss among their progeny[33]. This loss caused to male rat with cyclophosphamide was characterized by early pre implantation embryonic death[12]. Some abnormalities in progeny outcome caused by cyclophosphamide treatment persisted to a subsequent generation[7]. Thus the effects of cyclophosphamide exposure were both specific and heritable. Further chronic low dose exposure to cyclophosphamide produced adverse effects on progeny by altering sperm nuclear components[11]. The results of present study showed that there is a significant increase in the frequency of chromosomal aberrations when compared with control values ($p<0.05$). The mutagenicity of CP is clearly related to the formation of ultimate cytotoxic metabolite phosphoramide mustard through intermediate agent is hydroxyl cyclophosphamide and deschloroethyl cyclophosphamide which is responsible DNA cross links and strand lesions.

The present results clearly indicate the fruit extracts of *Aegle marmelos* showed antimutagenic in germ cells of mice. The percentage of CA in CP treated animals showed stastically significant when compared with controls (Table 2), whereas the frequency of CA's were very near to controls in meiotic cells of mice treated with AMF (Table 1 $P<0.05$). However when animals are co-administer with CP+AMF groups, the percentage of CA's decreased from 22.40(CP treated group) to 16.60, 10.60 and 7.50 in CP+AMF groups (Table 2 $P>0.05$). Further the AMF extract failed to induced sperm head abnormalities and values were near to control ($P>0.05$ Table 1). A significant increase in the percentage of sperm head abnormalities observed in cyclophosphamide treated group similar observations the incidence of aberrant sperms has been reported after treatment of male mice with irradiation[36] chemical agents[2,8]. Further the agents that accumulate in thetestes can cause alterations in testicular DNA responsible for disruption of spermatozoa differentiation. It is clear the obtained results showed protective nature of *Aegles Marmelos* fruit extract against CP induced DNA damage in germ cells of mice.

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[29].The protective effects of *Aegle marmelos* fruit and leaf extract against deoxyrubucin induced micronuclei in bone marrow cells has been reported[25].The present results are comparable with who reported the protective effects of *Aegle marmelos* in mouse bone marrow cells at CP induced CAs. Earlier we have reported to protective effects of *phyllanthus emblica* fruit extract on adriamycin induced genotoxicity in mice[17]. The protective against CP induced genotoxicity by *Aegle marmelos* may be due to inhibition of free radicals formed by Cyclophsphamide 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 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[3]. It is well known that consumption of fruits and vegetables is associated and are known to prevent chromosomal and DNA damage in animals[20,21]. Usually antimutagens acting in rodents are active in human too[34]. Our results have a practical decline of genotoxic effects of cyclophosphamide in drug exposed population and pharmaceutical lead plant workers handle this drug which may alternate the higher risks for development of secondary malignancy and for abnormal reproductive outcomes.

Table 1: Incidence of CAs in meiotic spermatocytes of mice treated with *Aegle marmelos* fruit extract

Treatment	Normal sperms	Changes in chromosome number				Structural changes	Abnormal metaphase scores%
		Autosomal urivalents	Sex chromo urivalents	Polyploidy	Aneuploidy	Translocation	
Control-I	477(95.40)	11(2.10)	8(1.60)	2(0.40)	4(0.80)	0(0.00)	27(4.60)
200mg/kg	476(95.20)	10(2.00)	8(1.60)	1(0.20)	5(1.10)	0(0.00)	24(4.80)*
400mg/kg	474 (94.90)	10(2.00)	8(1.60)	2(0.40)	6(1.20)	0(0.00)	26(5.10)*
600mg/kg	469 (93.80)	10(2.00)	11(2.20)	3(0.60)	7(1.40)	1(0.10)	31(6.20)*

The values in parenthesis are percentages *p>0.05

Table 2: Protective role of AMF extract in CP treated animal groups

Treatment	Normal metaphases	Changes in chromosome number				Structural changes	Abnormal metaphase scores%
		Autosomal	Sex chromo urivalents	Polyploidy	Aneuploidy	Translocation	
Control - II	476(95.20)	10(2.00)	8(1.60)	2(0.40)	3(0.60)	0(0.00)	24(4.80)
mitomycin	397(79.40)	32(6.40)	38(6.90)	20(4.00)	10(2.00)	3(0.60)	107(20.60)|*a
50mg CP	388(77.60)	38(7.60)	40(8.00)	21(4.10)	16(2.10)	2(0.40)	112(22.40)*a
50+200mg/kg	420(84.00)	30(6.00)	32(6.40)	10(2.00)	8(1.60)	1(0.10)	80(16.00)*b
50+400mg/kg	447(89.40)	18(5.65)	20(4.00)	8(1.60)	6(1.10)	1(0.10)	53(10.60)*b
50+600mg/kg	463(92.50)	14(2.80)	16(3.10)	4(0.800	3(0.60)	0(0.00)	37(7.50)*b

The values in parenthesis are percentages

a* p<0.05 CP treated group compared with control group

b* p<0.05 Primed group compared with CP treated group

Table 3: Antigenotoxic effects of *Aegles marmalos* fruit extract on the incidence of aberrant sperms in mice

Treatment	Normal sperms					Aberrant sperms %
		B	A	H	hookless	
Control -I	481(96.20)	8(1.60)	6(1.20)	4(0.80)	1(0.20)	19(3.80)
200mg/kg	480(96.90)	9(1.80)	5(1.00)	4(0.80)	2(0.40)	20(4.00)*
400mg/kg	479(95.70)	9(1.80)	6(1.10)	4(0.80)	2(0.40)	21(4.30)*
600mg/kg	478(95.60)	9(1.80)	7(1.60)	4(0.80)	1(0.20)	22(4.40)*

The values in parenthesis are percentages p>0.05

Table 4: Protective role AMF extract on CP induced aberrant sperms in mice

Treatment	Normal sperms	Changes in sperm morphology				Aberrant sperms %
		Banana	Amor	Hamma	hookless	
Control -I	482(96.40)	6(1.10)	4(0.80)	5(0.80)	3(0.60)	18(3.60)
Mitomycin-c	436(87.20)	26(3.80)	22(2.40)	10(2.00)	6(+.20)	64(12.80)*a
CP 50 mg/kg	427(85.40)	28(5.40)	26(5.10)	19(3.60)	10(2.00)	73(14.60)*a
50+200 mg/kg	458(91.60)	11(2.40)	16(3.10)	8(1.60)	6(1.20)	42(8.60)*b
50+400 mg/kg	466(93.20)	14(2.80)	10(2.00)	6(1.20)	4(0.80)	34(6.80)*b
50+600 mg/kg	474(94.80)	12(2.40)	8(1.60)	4(0.80)	2(0.40)	26(5.20)*b

The values in parenthesisare percentages

a* $p<0.05$ CP treated group compared with control group

b* $p<0.05$ Primed group compared with CP treated group

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REFERENCES

1. Antunes, L.M., Araujo, M.C., Darin, J.D. and Bianchi, M.L.,The Molecular Targets and Therapeutic Uses of Curcumin in Health and Disease.*Mutat. Res.*, **465:** 131–137 (2000).
2. Anuradha, S. and Rudrama Devi, K.,Cytogenetic analysis of impact of Cisplatin on germ cells of mice.*The Bioscan.,***4(4):** 641-644(2010).
3. Baliga, M.S., Thilakch and, K.R., Rai, M.P., Rao, S. and Venkatesh, P., *Aegle marmelos* (L.) Correa (Bael) and its phytochemicals in the treatment and prevention of cancer.*Integr Cancer Ther.*,**12(3):** 187-196 (2012).
4. Benson, A.J., Martin, C.N. and Garne, R.C., N-(2-hydroxyethyl)-N-[2-(7-guaninyl)ethyl]amine, the putative major DNA adduct of cyclophosphamide in vitro and in vivo in the rat. *Biochem. Pharmacol*., **37:**2979-2985 (1988).
5. Birt, D.F., Hendrich, S. and Wang, W., Prediction of Molecular Targets of Cancer Preventing Flavonoid Compounds Using Computational Methods. *Ther*., **90:** 157-177 (2001).
6. Evans, E.P., Breckon, G., Ford, E.C., Air-drying method for meiotic preparations from mammalian testes. *Cytogenetics.*, **3:** 289-294 (1964).
7. Hales, B.F., Crosman, and Robaire, B., Adverse effects of cyclophosphamide on progency outcome can be medicated through post-testicular mechanisms in the rat.*Teratology*, **45:**671-678 (1992).
8. Hassan, N.H.A., Fahmy, M.A. and El Dawy, H.A., Effects of malathion and sevin on spermatocyte chromosomes and sperm morphology of mice. *Egypt. J. Med. Sci.*,**16(1):** 95-104 (1995).
9. Haubitz, M., Acute and long-term toxicity of Cyclophosphamide. *Tx Med*., **19:**26-31 (2007).
10. Hemminki, K., Binding of Metabolites of Cyclophosphamide to DNA in a Rat Liver Microsomal System and *in vivo* in Mice. *Cancer Res.*, **4 5:** 4237-4243 (1985).
11. Jianping, Q.I.U., Barbara, F., Hales, and Bernard Barbaire., Protective effects of solanum lycopersicum fruit extract on cyclophosphamide induced micronuclei in bone marrow cells of mice.*Biology of Reproduction*,**52:**33-40 (1995).
12. Kelly, S.M., Robaire, B. and Hales, B.F., Paternal cyclophosphamide treatment cause post implantation loss via inner cell mass specific cell death. *Teratology*, **45:** 313-318 (1992).
13. Khan, T.H. and Sultana, S., Apigenin induces apoptosis in hep G2 cells, possible role of TNF-α and IFN – 7 J. Enzyme. Inhib. *Med. Chem*., **24:** 320-327 (2009).
14. Khan, T.H. and Sultana, S., Nephroprotective efficacy of chryrin against cisplatin induced toxicity via attenuation of oxidative stress. *Toxicol. Mech. Methods.*, **21:** 453 462 (2011).

15. Kruawanand, K. and Kangsadalampai, K., Antioxidant activity, phenolic compound contents and antimutagenic activity of some water extract of herbs. *Thai J. Pharm. Sci*., **30:** 28-35(2006).
16. Kunpati Sundramoorthy, P.K., Sathiyavedu, T.S. and Rabandi, R., Interactive effects of saffron with garlic and curcumin against cyclophosphamide induced genotoxicity in mice. *Asia Pac. J. ClinNutr.*, **13:** 292-294 (2004).
17. Kusum Latha, C. and Rudrama Devi, K., Adriamycin Induced Chromosomal Aberrations in Germ Cells of Mice. *Bioscan*., **5(2):** 317-320 (2010).
18. Lakshmi Sowjanya, B., Rudrama Devi, K. and Madhavi, D., Modulatory effects of garlic extract against the cyclophosphamide induced genotoxicity in human lymphocytes *in vitro. Journal of Environmental biology*, **30(5):** 663-666 (2011).
19. Little, S.A. and Mirkes, P.E., (Teratogen-induced activation of caspase-6 and caspase-7 in early postimplantation mouse embryos.*Cancer Res*., **47:** 5421-5426 (1987).
20. Miyata, M., Takano, H, Guo, L.Q., Nagata, K. andYamazoe, Y.,Inhibitory effects of citrus fruits on cytochrome P450 3A (CYP3A) activity in humans. *Carcinogenesis*., **25:** 203-9 (2004).
21. Nerseyan, A. And Muradyan, R., Seabuckhorn, Sea-buckthorn juice protects mice against genotoxic action of cisplatin. Exp. *Oncol.*, **26:**153-5 (2004).
22. Oda, Y., An ENU-induced mutation in the Ankrd11 gene results in an osteopenia-like phenotype in the mouse mutant Yoda. *Mutat. Res*, **348:** 67–73 (1995).
23. Patil, D.N., Kulkarni, A.R. and Patil, B.S., Release of Drug Neomycin from Cordia Dichotoma Transdermal Film. *Int. J. Pharmacol*., **6:** 68-71 (2010).
24. Pillans, P.I., Ponzi, S.F. and Parker, M.I., Effects of ascorbic acid on the mouse embryo and on cyclophosphamide-induced cephalic DNA strand breaks *in vivo.Carcinogenesis.*,**10:** 83–85 (1989).
25. Prabhaker Reddy, C.H. and Rudrama, K., Protective effect sofaegesmarmales fruit extract inlead induced genotoxicity inmice. *International journal of pharma tech*., (2014).
26. Premkumar, K., Protective effects of saffron (Crocus sativus Linn.) on genotoxins-induced oxidative stress in Swiss albino mice. *Phytother Res*., **17(6):** 614-617 (2003).
27. Rudrama Devi, K., Madhavi, D. and Keshava Rao, K., Drug Metabolism Reviews, Suppliment1, *Journal of International Society on study of Xenobiotics*., **34:** 146 (2003).
28. Rudramadevi, K. and Keshava Rao, K., Cyclophosphamide induced chromosomal aberrations in germ cells of mice. *Indian journal of multi displnary research.*, **33(1):** 169 -172 (2007).
29. Sharma, B.,Satapathi, S.K. and Roy, P., Hypoglycemic and Hypolipidemic Effect oïAeglemarmelos (L.) Leaf Extract on Streptozotocin Induced Diabetic Mice. *Int. J. Pharmacol*., **3:** 444 452 (2007).
30. Shoba, F.G. and Thomas, M., Studies on the anti-diarrheal properties of leaf extract of Desmodium puchellum. *J. Ethnopharmacol*., **76:** 73-76 (2001).
31. Skare, J.A. and Schrotel, K.R., Cytotoxicity, DNA cross-linking, and DNA single-strand breaks induced by cyclophosphamide in a rat leukemia in vivo.*Mutat Res*., **130:** 295-303 (1984).
32. Trasler, J. M., Hales, B.F. and Robaire, B., Materinal treatment of cyclophaside and foter loss. *Nature.*, **316:** 144-146(1985).
33. Trasler, J.M., Hales, B.F. and Robaire, B., A time course study of chronic paternal cyclophosphamide treatment in rats: effect on pregnancy outcome and the male reproductive and hemopoitic studies. *Biology of Reproduction*., **37:** 317-326 (1987).
34. Weishurger, J.H., Hosey, J.R., Larios, E., PiH man, B., Zang, E., Hara, Y. and Kutcheremx, G., Protective Role of Murraya Koenigii Leaf Extract on Adriamycin Induced Micronuclei in Mice Bone Marrow Erythrocytes. *Nutrition*., **17:** 322-25 (2001).
35. Wyrobek, A.J. and Bruce, W.R., Chemical induction of sperm abnormalities in mice. *Proc. Natl. Acad. Sci*., **72:** 4425-4429 (1975).
36. Wyrobek, A.J. and Bruce, W.R., Induction of sperm shape abnormalities in mice and humans. In: Hollaender A, editor. Chemical Mutagens: principles and Methods for their detection. Vol. 5. Plenum Press, New York,(1978). pp 257-285.
37. Ziech, D.R., Franco, A., Pappa and Panayiotidis, M.I.,Reactive Oxygen Species (ROS)—Induced genetic and epigenetic alterations in shuman carcinogenesis. *Mutation Res*., **711:** 167 173 (2011).