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"PROTECTIVE EFFECTS OF CURCUMIN AGAINST ADRIAMYCIN INDUCED MICRONULEI IN BONE MARROW CELLS OF MICE."

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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. *Cucuma longa* has been used for broad spectrum of diseases and its isolated compound curcumin found to be anticarcinogenic and a potential antioxidants. In the present investigation studies were carried out to observe the efficacy of *curcumin* against Adriamycin induced cytogenetic damage in bone marrow cells of mice. The animals treated with 10mg/kg, 15/kg and 20mg/kg of Curcumin showed to be non mutagenic. Curcumin shows protective effects against the adriamycin induced genotoxicity in bone marrow cells of mice. Hence Curcumin supplementation is safer in chemotherapeutic strategy.

KEYWORDS: *Curcumin* 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 sideeffects, 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.[1] These genotoxic effects may lead to initiation of unrelated tumours years after cessation of chemotherapy.[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.[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.[4]

Curcumin is phytochemical extracted from the rhizome of curcuma longa L. and has been reported to have anti-inflammatory, antioxidant and anticarcinogenic effects.[5,6] In vivo curcumin exposure studies suggest no genetoxicity or clastogenicity due to its antioxidant activity, evaluated on the basis of chromosomal aberrations.[7,8] So far no studies were carried on the protective nature of curcumin against adriamycin induced genotoxicity in male mice.

MATERIALS AND METHODS

Drugs and Chemicals

Adriamycin was bought from Apollo Pharmacy,Hyderabad and Mytomycin from biochem pharma limited. Methanol and Acetic acid(Merck),Giemsa 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.

Preparation of the extract

Curcuma longa (Turmeric) roots were collected from the local Super market. Dry spices (100 gm each) were crushed and sieved through mesh cloth to get the fine powder. Powdered spices were soaked in 200ml of distilled water and were kept at room temperature for 24 hours, then were filtered using Whatman no. 1 filter paper. The filtrate was heated at 40-50°C using water bath, until thick paste is formed. The thick paste was considered as 100% concentration of extract. These extracts were stored at 4°C in refrigerator.[9]

Animals and Treatment

The study was conducted on random breed, 8-10 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 doses of *Curcumin* i.e. 10, 15 and 20 mg/kg body weight were administered. *Curcumin* extract was dissolved in double distilled water and administered as single dose in 0.2 ml per mouse 7days prior to Adriamycin administration. The drug was supplied by Apollo Pharmacy, Hyderabad. For each dose group of five animals were used. The animals were fed with 16 mg/kg Adriamycin intraperitonally in two installments within 24 hr interval. The control group of mice received 0.5 physiological saline simultaneously.

Micronucleus test

All the animals were killed after twenty four hours of last treatment and bone marrow preparations were made according to the method described by.[10]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 May-Grunwald-Giemsa. According to the method described by Schmid(1975) 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 magnification. 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

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 0.29% and the values were 0.33%, 0.36% and 0.40% after the administration of 10,15 and 20mg/kg *Curcumin* extract respectively (Table-1). Hence, the results clearly indicate the non mutagenic nature of *Curcumin* extract, in Adriamycin treated group, there was a significant increase in the percentage of micronuclei(1.20%) in bone marrow cells of mice when compared to control –II value (0.36) (Table-2). However the frequency of micronuclei decreased to 0.78%, 0.62% and 0.52% after the co-administration of 10, 15 and 20mg/kg of *Curcumin* 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 Adriamycin treated group were found to be significant ($P< 0.01$), Table-2. The percentage of micronuclei between Adriamycin treated group and *Curcumin* extract primed group were found to be significant.

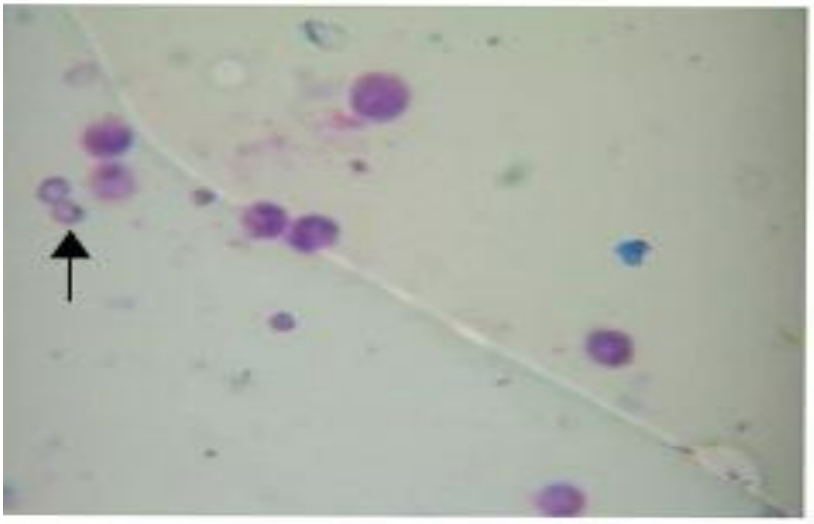

Fig. 1 The presence of micronucleus in Adriamycin treated animals

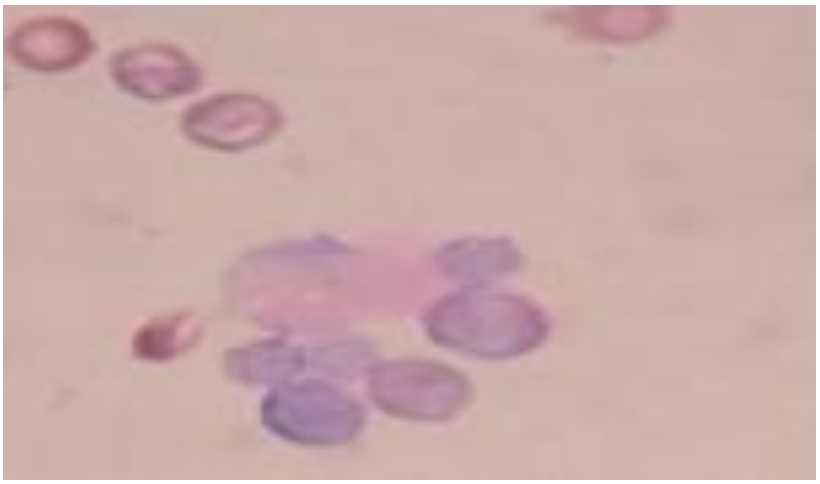

Fig. 2 The absence of micronucleus in Curcumin extract treated animals.

Table 1: Incidence of micronuclei in bone marrow erythrocytes of mice treated with curcumin extract.

Dose group	Micronuclei in polychromatic erythrocytes(P)	Micronuclei in normochromatic cells(N)	Micronuclei in total P+N cells	P/N ratio
Control I	35/12000 (0.29)	15/12200 (0.12)	47/24200 (0.19)	0.98
Curcumin				
10 mg/kg	48/14500(0.33)*	36/15200 (0.23)	86/29700 (0.28)	0.95
15 mg/kg	55/15000 (0.36)*	43/16200 (0.26)	96/31200 (0.30)	0.92
20 mg/kg	60/14900 (0.40)*	48/16600 (0.28)	105/31500(0.33)	0.89

P >0.05

Table: 2 Protective effects of Curcumin on Adriamycin induced micronuclei in bone marrow erythrocytes of mice.

Treatment Groups mg/Kg /b/w	Micronuclei in polychromatic cells of Mice(P)	Micronuclei in normachromatic cells of mice(N)	Micronuclei in P+N cells	P/N Ratio
I Controls	36/10000 (0.36)	12/10200 (0.11)	48/20200 (0.23)	0.98
II Adriamycin(ADR)	120/10000 (1.20) *	71/11600 (0.61)	191/21600(0.88)	0.86
Curcumin + ADR				
III 10mg/Kg+16mg/Kg	78/10000 (0.78) *	42/10900 (0.38)	120/20900(0.57)	0.91
IV 15mg/Kg+16mg/Kg	62/10000 (0.62) *	28/10600 (0.26)	90/20600 (0.43)	0.94
V 20 mg/Kg +16mg/Kg	52/10000 (0.52) *	33/10600 (0.30)	85/20700 (0.41)	0.93

The values in the 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[10] using this procedure the mutagenicity of various alkylating agents

drugs[11-13], [14] was also established. Naturally occuring antioxidants have been extensively studied for their capacity to protect organisms and cells from oxidative damage.

Curcuma longa is a mandatory food additive and an individual in his diet can consume 1-5g/day of powdered form of Curcuma (Turmeric).Which acts as a cleaning agent renders to protect against any diseases.[15] Several *in vitro* and *in vivo* studies showed the therapeutic potential of curcumin and protective effects of curcumin. It is anti inflammatory, anti hepatoxic, scabies, cancer, Alzimeir's disease.[16,17] However in our study we aimed to assess the protective effect of curcumin against Adriamycin induced genotoxic damage.

The chromosomal aberrations and a decrease in mitotic index are the most sensitive of bone marrow damage.[18,19] An effort has been made in the present investigation to assess whether such toxic effects induced by Adriamycin are neutralized or counter balanced by administration of curcumin. In addition to its preservative, flavoring or coloring properties in the diet, turmeric has been used in Asian medicine for generations for the treatment of many disorders including inflammation hepatic, biliary disorder cough and certain tumors based on short term studies conducted in animals and humans that curcumin is a safe agent when administered orally.[20]

No treatment toxicity was reported in 25 patients taking curcumin at concentrations upto 8000mg/day for a period of 3months. Curcumin has also been shown as an immunostimulant and immunorestor in *in vivo* this mechanism may also participate in cancer preventive activity[21] antimutagenic.[17] Our research group showed that curcumin protects cyclophosphamide induced genotoxicity in animal model.[22]

However the geno-protective nature of curcumin has not been evaluated against Adriamycin induced genotoxic damage and protection by curcumin. Hence it can conclude that antioxidant such as curcumin protects the body from damage to free radicals. Further curcumin, a hydrophobic polyphenol has a wide spectrum of biological and pharmacological activities. It is a bis- α, β– saturated, β- diketone (diferuloy/methane) which exhibits keto-enol *tautomerism* having a predominant keto form to acidic and neutral solutions and stable enol form in alkaline medium.[23] Due to polyphenolic structure β – diketone functional group, curcumin is able to scavenge or neutralize free radicals by interacting with oxidative cascade, quenches oxygen and by chelating some metal ions and inhibits peroxidation of membrane lipids there by maintaining membrane integrity and their function.[24] Curcumin

has been shown strong antioxidant activity and studies have shown curcumin reduce oxidative stress. Curcumin protects islets against streptozotocin induced oxidative stress by scavenging free radicals. Many authors have proved anti carcinogenic effects of curcumin on the inhibition of tumor formation in laboratory animals.[25,26]

The results are also comparable with that of.[27] who showed curcumin reusing the genotoxicity induced by copper ions by micronucleus and comet assay.Oral curcumin administration has been shown to prevent the development of cancers of the skin, soft plate, stomach, duodenum, colon ,liver, lung and Breast of rodents.[28] Topical application has been showed to inhabit the initiation and promotion stages of chemically induced skin cancer.

CONCLUSION

Animals when treated with various doses of curcumin showed non mutagenic and the percentage of micronuclei in bone marrow cells of mice were equivalent with that of control values.There was an increase in the incidence of micronuclei in adriamycin treated group when compared to the control Group. There was a significant decrease in the percentage of micronuclei in the bone marrow cells of mice when adriamycin was primed with various doses of Curcumin. Thus Curcumin showed protective effects against the adriamycin induced genotoxicity in bone marrow cells of mice. Hence Curcumin supplementation is a safer dietary componenet in chemotherapeutic strategy.

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