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Research Article

THE PROTECTIVE EFFECTS OF PHYLLANTHUS EMBLICA IN CYCLOPHOSPHAMIDE INDUCED GENOTOXICITY IN MICE

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ABSTRACT

In the present study the antimutagenic effects of *Phyllanthus fruit extract* (PFE) has been evaluated against cyclophosphamide induced genotoxicity in bone marrow cells of mice. when animals are treated with different doses of *phyllanthus fruit extract* i.e., 170,340 and 680 mg/kg to mice, the treated group has not showed any significant increase in the percentage of chromosomal aberrations in bone marrow cells of mice at 48 hrs treatment. A single Intra peritoneal of 50mg/kg of cyclophosphamide induced significant increase in the percentage of chromosomal aberrations in bone marrow cells of mice. However after co administration of three doses of PFE extract there was a dose dependent decrease in the % of micronuclei was observed. When animals were administered with *Phyllanthus Fruit Extract* PFE 170, 340 & 680 mg/kg/bw orally for seven days and on eightieth day CP (50 mg/kg/bw) was given intraperitonially. For each experimental group control, animals were maintained simultaneously. After the administration of the last dose, the animals were killed and air dried metaphase preparations were made and processed for identification of chromosomal aberrations in somatic cells of mice. In animals treated with single dose of CP, an increase was observed when compared with the values of control group. But when animals primed with PFE + CP group, there was a decrease in the frequency of chromosomal aberrations in somatic cells of mice. Thus the results clearly indicated the protective role of PFE on cyclophosphamide induced genotoxic damage in somatic cells of mice.

KEYWORDS: Cyclophosphamide, genotoxicity, *Phyllanthus emblica*

INTRODUCTION

A broad spectrum of antineoplastic drugs are in common use to combat various types of cancers. These are shown to be mutagenic in different test systems and these antineoplastic drugs such as Cyclophosphamide, Cisplatin, Tamoxifen, Gemcitabine and Paclitxel etc., have shown clastogenic effects in various test systems. Potential genetic damage due to drugs and other chemicals is well recognized. Extensive studies have been carried out on mutagenicity of various drugs in microorganisms, insects, mammals and in exposed population [1] [2] [3][4].

Cyclophosphamide (CPM) is a well-known bifunctional alkylating agent, widely used in cancer chemotherapy and showed its genotoxicity when metabolically activated [5]. It is extensively used for the treatment of various cancers as well as an immunosuppressant in organ transplantation, rheumatoid arthritis, systemic lupus erythromatosis, multiple sclerosis, and other benign diseases [6],[7]. According to the International Agency for Research on Cancer (IARC), CPM is widely used as reference mutagen and has been classified as carcinogenic for animals and humans [8].

According to believe in ancient Indian mythology, *Phyllanthus emblica* is the first tree to be created in the universe. It belongs to family Euphorbiaceae. It is also named as Amla, *phyllanthus Emblica* or Indian gooseberry. The species is native to India and also grows in tropical and subtropical regions including Pakistan, Uzbekistan, Srilanka, South East Asia, China and Malaysia. The fruits of PF are widely used in the Aryuveda and are believed to increase defense against diseases. It has its beneficial role in cancer, diabetes, liver, heart trouble, ulcer, anemia and various other diseases.

Diet can modify the pathological processes, because certain naturally occurring substances known as antioxidants are present in plants and other sources have shown to be protective against mutagens or carcinogens or endogenous mutagens [9]. Among the various phytonutrients, phyllanthus emblica posses good antioxidants. It was described in Indian Ayurvedic literature more than 200years ago. It has been regularly used by traditional medical practitioners for the treatment of various diseases. It exhibits many properties like antiviral, antimutagenic, hepato protective activity, hypoglycemic activity etc [10] [11] [12]13]. In the present investigation, the studies were carried out on protective role of PFE on cyclophosphamide induced genetic damage in somatic cells of mice.

MATERIALS & METHODOLOGY

PFE Extract preparation

Cameron and Puling [14] suggested the daily intake of vitamin C is 1-10g/day for human being. Data based on maximum ascorbate concentrations in human body suggest a maximum body pool of around 5000mg, which is approximately 70mg/kg body weight in man[15]. In the present study, a corresponding amount of an aqueous extract of PFE containing the same amount of vitamin C was used for mice, as calculated from daily 1 g intake for a 60kg Person [16]. The fruits were procured in bulk, cut into pieces and dried in sunlight. Known quantities weighed and kept in distilled water for 24hr. The AA content of the decoction was estimated b the 2, 6-dichlorophenol indophenol method15 and it amounted to 685mg/kg body weight.

Animal treatment

The animals used in the present study are purchased from national institute of nutrition. The ethical committee clearance was obtained from University College of science, Osmania University which is necessary for the use of animals. The mice were maintained in plastic cages under controlled lighting conditions (12:12 light and dark cycle) relative humidity (50±5%) and temperature (37±2oC) fed with mice feed and were given ad libitium access to water. A group of 5 mice per experiment were taken and treated with CP and PEF. The doses were prepared daily in distilled water and were administered by gastric gavage method for PEF and 26G needle intraperitoneal injection for CP treatment to all the experimental animals.

Dosage schedule

In the present study two experiments were conducted. The animals were feed orally with cyclophosphamide and PFE extract and categorized in to following groups

Group I : controls with 0.5ml of physiological saline.

Group II: PFE extract 170 mg/kg

Group III: PFE extract 340 mg/kg

Group IV: PFE extract 680 mg/kg

In the second experiment for modulation studies all the three groups as follows:

Group I : controls with 0.5ml of physiological saline.

Group II: Cyclophosphamide 50 mg/kg

Group III: PFE extract 170 mg/kg + Cyclophosphamide 50 mg/kg

Group IV: PFE extract 340 mg/kg + Cyclophosphamide 50 mg/kg

Group V: PFE extract 680 mg/kg + Cyclophosphamide 50 mg/kg

Analysis of chromosomal aberrations in somatic cells of mice

The animals were sacrificed two days after administration of the last dose. The bone marrow was flushed into clean glass Petri dishes with hypertonic solution (0.56% KCl) were used to get a homogeneous cell suspension. It was then collected in clean centrifuge tubes and incubated at 37oC for 45 minutes. Four slides for each group were prepared from control and experimental animals. The staining was done within 24 h of preparation according to the method [17]. The slides were screened for 50 well spread metaphases per animal for the presence of various types of chromosomal aberrations like gaps breaks, fragment, chromatid separations and polyploids in control and treated group of animals. The differences in the frequencies of chromosomal aberrations between control and treated groups were analyzed using Chi-Square test. For calculating mitotic index (MI) a minimum of 1000 cells were counted for each animal.

RESULTS

The doses selected for Phyllanthus fruit extract were 170, 340 and 680 mg/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 Table 1 At 48 hrs the frequencies (%) of chromosomal aberrations in the PFE treated mice 2.4, 3.2 and 3.20% respectively when compared to that of controls 2.40% there was no increase in the The differences in the frequencies of chromosomal aberrations between controls and PFE extract treated mice for 48hrs

were analyzed by X^2 test and the results were found to be insignificant (P>0.05), Table 2

In the present study various doses of the cyclophosphamide of and 50 mg/kg were primed with different doses of *Phyllanthus fruit extract* of 170, 340 and 680 mg/kg body weight and the results were presented in Table 2.The results for 48 hrs of exposure of drug of various doses on priming with different doses of Phyllanthus fruit extract have been recorded (Table 3) . At 48 hrs of treatment the frequencies (%) in the chromosomal aberrations in controls have shown 3.2 when compared to the drug exposed were 14.40, 16.8 and 20.00% respectively for 50mg/kg body weight of cyclophosphamide while mitomycin C recorded was 18.4%. priming with 170 mg/kg body weight the values were and 4.8% respectively. With 340mg/kg it was 4.4% whereas at 680mg/kg body weight chromosomal aberrations were 3.6% respectively. As the dose and duration of exposure increased, there was gradual decrease in the incidence of abnormalities. The difference in the frequencies of the chromosomal aberrations between the controls and treated or primed mice for 48 hrs have been analysed using X^2 test and the results were found to be significant (P<0.01) Table 2.

Table 1: Frequency of Chromosomal aberrations recorded in somatic cells of mice after treatment with various doses of Phyllanthus fruit extract for 48 hrs interval.

Dose (mg/kg)	**48 hr and duration of treatment (hr)**	
	Normal metaphases scored (%)	Abnormal metaphases scored (%)
Control	244 (97.6)	6(2.4)
170 mg/kg	244(97.6)	6(2.4)
340 mg/kg	242(96.8)	8(3.2)
680 mg/kg	242(96.8)	8(3.2)

P>0.05

Table 2: Frequency of chromosomal aberrations recorded in somatic cells of mice treated with Cyclophosphamide and primed with Phyllanthus Fruit Extract for 48 hrs

Dose	**Normal Metaphases**	**Abnormal Metaphases**
Control	245(96.8)	5(3.2)
Mitomycin	198(81.6)	52(18.4)*
CP 50mg/kg	189(80)	61(20)*
50mg+170mg/kg	238(95.2)	12(4.8)*
50mg+340mg/kg	239(95.6)	11(4.4)*
50mg+680mg/kg	241(96.4)	9(3.6)*

The values in parenthesis are percentages

*P<0.05

DISCUSSION

The actively proliferating cells from bone marrow cells gives maximum information on the toxicity of any test compound16. The transition from proerythroblast to erythrocytes takes about seven cell division cycles. Each cell cycle takes 10-11 hrs and the terminal mitosis is completed in about 10hrs before the transition of orthochromatid erythroblast to polychromatic erythrocytes. In view of the above to see the long and short term effect of test compound on cells, the sampling time ranged was from 6-72 hrs has taken in present observation There are different type of chromosomal aberrations observed in present analysis These aberration are classified into structural, numerical and other abnormalities. Structural aberration includes gaps, breaks, fragments, terminal deletion and centric fusion these end points serve as indicators for assessing the mutagenic effect of test substance.

The present results are comparable with that of Asita el al, [18] who investigated the intraperitoneal injection of mice with a single dose of 40 mg/kg body weight of Cyclophosphamide induced a significant increase in the frequency of MNPCE, 24 h after injection, when compared with animals that received water treatment. The present results are comparable to Santos Renato et al., [19] who reported that Cyclophosphamide at 135mg/kg dose induced a significant increase in the frequency of micronuclei in polychromatic erythrocytes of male mice Further, the percentage of chromosomal aberrations was 59.33 in 50mg/kg body wt. Cyclophosphamide treated mice [20].

The results are comparable with that of Dhir [21]who reported that Aqueous extract of edible dried fruits of *Phyllanthus emblica,* a well-known medicinal plant, the cytotoxic effects induced by low doses of nickel, at the higher doses it was ineffective. The greater efficacy if the fruit extract could be due to the interaction of its various natural components rather than to any constituent. Furthermore, the protective effects were more pronounced in the garlic-administered groups compared to curcumin and/or saffron administered groups. Protective effects of saffron against genetic damage induced by CP in mice were reported [22]. There was a significant decrease in the percentage of chromosomal aberrations in bone marrow cells of mice when CP primed with garlic extract. [23].

Phyllanthus emblica enjoyed a hallow position in Ayurveda an Indian system o medicine. It is a first tree to be created in the universe. Its fruit juice contains highest vitamin C contains as 478.56mg/100ml. It is used in the preparation of Indian pickles. The fruit when blended with other fruits boosted their nutritional quality in terms of vitamin C content . It is often used as Triphala which is a herbal formulation containing fruit of Terminalia chebula and Terminlia belerica in equal proportions. It has important medicinal value against various diseases. Invitro and invivo animal studies suggested wide range of potential therapeutic or preventive effects has been reported. Such effects in humans have not conformed so far. PFE when prepare in the Triphala delayed the development of fore stomach Papillomagness, breast cancer, skin tumors, liver fibrosis, diabetic cataract, Alzheimer's diseases [24] [25] [26] [27]. Hence in our study we aimed to access the protective effects of PFE against the Cyclophosphamide induced genotoxicty. Chromosomal aberrations and a decrease with mitotic index are the most sensitive indicators of bone marrow damage [28] [29]. In the present study an effort has been made to observe whether such toxic effects induced CP or neutralized or counter balanced by the treatment of PF fruit extract, primarily contains tannins alkoliods, phenolic compound, amino acids, carbohydrets and vitamin C. the PFE is prepared in formulations as Triphala, kalpaamrutha and chyavana prash were showed therapeutic beneficial for infected wounds, coronary artery disease, arthritis, an opthcare in number of inflammatory and degenerative opthalamic disorders [30] [31] [32] [33]. It has been exhibited antipyretic, anti tussive, dyslipidemia, snake venom neutralizer, anti-microbial immunosuppression, anti-mutagenic and anti-carcinogenic properties [34] [36]. However the geoprotective effect of PFE has not been evaluated against anticancer drug CP. Hence, it is of interest to assess the genotoxicity of CP and also the protection rendered my PFE against such genetic damage.

The present results are comparable with the reports of other investigators. When cadmium chloride administered orally 3mg/kg in a single dose, co-treatment with phyllanthus fruit extract at dose of 500mg/kg showed decreased mortality in rats. Further there are histopathological changes reduced peroxidation in liver, kidney and testis after acute cadmium exposure [37]. The protective effects of *phyllanthus* fruit extract aganist adriamycin and chromium induced genotoxicity in bone marrow cells of mice has been reported [38] [39]. The crude extract of *phyllanthus emblica* decreased the percentage of chromosomal aberration induced by Cesium chloride and aluminium etc., [40] [41]. In the present study pretreatment of phyllanthus fruit extract was shown to be more effective in reducing the genotoxicity of cyclophosphamide. The protective nature of *phyllanthus emblica* is because of presence of Vitamin C, tannins, polyphenolic compounds and elligic acid.[42] Ascorbic acid (vitamin c) polyphenolic compounds such as elligic and tannic acids are inhibitors and blocking agents aganist carcinogens on direct acting N- Nitroso compounds. Elligic acid protects DNA attack of electrophyilic species of free radicals by binding to nitcleophyilic sites [43][44].

From the above studies, it is concluded that *phyllanthus emblica* was a potential candidate as protective agent in Cyclophosphamide induced genotoxic effect in somatic cells of mice. The combined treatment of Cyclophosphamide and PFE is useful antioxidant for treatment of various types of cancer.

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