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

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ABSTRACT

In the present investigation the antimutagenic effects of Phyllanthus fruit extract (PFE) has been evaluated against cyclophosphamide induced genotoxicity in bone marrow cells of mice. The administration of Phyllanthus fruit extract at various doses i.e. 170, 340 and 680 mg /kg. When treated individually did not induce chromosomal aberrations in somatic cells of mice in 24hrs. A single Intraperitoneal administration of 50mg/kg of cyclophosphamide induced significant increase in the percentage of micronuclei 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 two weeks and on sixteenth day CP (50 mg/kg/bw) was given intraperitonially as a single dose. For each experimental group control, animals were maintained. Two days after the administration of the last dose, the animals were sacrificed 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. Thus the results clearly indicated the protective role of PFE on cyclophosphamide induced genotoxic damage in somatic cells of mice.

KEYWORDS: Cyclophosphamide, Phyllanthus bone marrow cells.

INTRODUCTION

A number 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.[1-4]

Cyclophosphamide (CPM) is a well-known bifunctional alkylating agent, widely used in cancer chemotherapy and expresses 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 200 years ago. It has been widely used by traditional medical practitioners for the treatment of various diseases. It exhibits many properties like antiviral, antimutagenic, hepato protective activity, hypoglycemic activity etc.[10-13] In the present investigation, the studies were carried out on protective role of PFE on cyclophosphamide induced genetic damage in somatic cells of mice.

2. MATERIALS AND 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. 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 method[16] and it amounted to 685mg/kg body weight.

Animal treatment

The study was conducted after taking the approval of Institutional Ethical Committee on twenty adult male swiss albino mice 30 to 50 days old and weighing around to 30 to 40 g were maintained in plastic cages under controlled lighting conditions (12:12 light and dark cycle) relative humidity (50±5%) and temperature (37±2^{o}C) 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.

The dose protocols were as follows

Group I control group were treated with 5 ml of physiological saline.

Group II the animals were treated with CP 5 mg/animal/day intraperitoneally.

Group III control were treated with PFE 170, 340 & 680 mg/animal/day for two weeks daily.

Group IV Experimental batch were pretreated with 170, 340 & 680 mg/kg BW PFE for 15 days on the 16th day single intraperitoneal dose of cyclophosphamide 5 mg/kg/bw, were administered.

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

Group I: controls

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

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 killed 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 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 24 hrs the percentage of chromosomal aberrations for 170, 340 and 680 mg/kg body weights of fruit extract in the treated groups recorded were 2.00, 2.80 and 2.4% respectively when compared with that of controls 1.6% (Table- 1). The differences in the frequencies of chromosomal aberrations between controls and PFE treated mice for 24 were analyzed by X^2 test and the results were found to be insignificant ($P>0.05$, Table- 1).

In the present study various doses of the cyclophosphamide of 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.

At 24 hrs of the study the controls have shown 2.4% of the chromosomal abnormalities when compared to mitomycin C were 21.2% and the unprimded doses of 12.5, 25 and 50 mg/kg body weight of cyclophosphamide were recorded (Table 2). The uprimed mice with cyclophosphamide have shown the chromosomal aberrations were 18.40, 21.6 and 24.8% respectively. The highest dose has shown maximum abnormal metaphases. Priming with 170 mg/kg body weight of Phyllanthus fruit extract, the effect has decreased. There was decrease observed and the aberrations were 18.80% respectively. Similarly with 340mg/kg body weight the recorded values were 8.4% and with 680 mg/kg body weight there was a decrease for 50 mg/kg body weight of cyclophosphamide with 5.6% respectively at 24 hrs (Table-2). The differences in the frequencies of the chromosomal aberrations were analyzed by X^2 test and the results observed 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 24hrs interval.

Dose (mg/kg)	Duration of treatment 24 hr	
	Normal metaphases scored (%)	Abnormal metaphases scored (%)
Control	246(98.4)	4(1.6)
170 mg/kg	245(98)	5(2)*
340 mg/kg	243(97.2)	7(2.8)*
680 mg/kg	244(97.6)	6(2.4)*

*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 24 hrs.

Dose	Normal Metaphases (%)	Abnormal Metaphases (%)
Control	244(97.6)	6(2.4)
Mytomycin C	197(78.8)	53(21.2)*
CP 50mg/kg	188 (75.2)	62(24.8)
50+170mg/kg	203 (81.2)	47(18.8)
50+240mg/kg	229(91.6)	21(8.4)
50+680mg/kg	236(94.4)	14(5.6)

The values in parenthesis are percentages

*P<0.05

DISCUSSION

The actively proliferating cells from bone marrow provide maximum information on the effect of any test compound. 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] Dietary inhibitors of mutagenesis and carcinogenesis are of particular importance since they may have a role in cancer prevention. 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 decrese in the percentage of chrosomal 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. *In vitro* and *in vivo* 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[24-27] 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.[30-33] It has been exhibited antipyretic, anti tussive, dyslipidemia, snake venom neutralizer, anti microbial immunosuppression, anti mutagenic and anti carcinogenic properties[34-37] However the genoprotective 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.

From the above data, it can be concluded that antioxidants such as PFE protects the damage from free radicals. A number of medicinal plants, traditionally used for thousands of years are present in a group of herbal preparations of the Indian traditional health care system (Ayurveda) named rasayana identified for anti oxidant properties PFE have been supported for its anti oxidant activity. It contains tannoid principles of embliconin A, embliconin B, punigluconin and peunculagin, have been reported to poses antioxidant activity *in vivo* and *in vitro*.[38-39]

Further the PFE accounts for approximately 45-70% of the antioxidant activity. Further the phenolic compounds derived from plant exhibit a number of beneficial effects and potentially inhibited several stages of carcinogenesis. Phenolic compounds are the major components from the fruit juice of PFE and from the branches, leaves and roots showed stronger inhibition against P16F10 cell growth than against Hela and MK1 cell growth.[40] PFE and its medicinal preparations may prove beneficial as a component of combination therapy in phase I clinical trial in cancer patients under the cyclophosphamide treatment(Haque et al 2001). Ethanolic extract of PFE was experimentally evaluated for protection against genotoxicity induced by DMBA. PFE administrated orally at different concentrations (100, 250, 500mg/kg

body weight) for 7 consecutive days in Swiss albino mice prior to a single intraperitonial injection of DMBA decreased the frequency of bone marrow micronuclei.[42] These observations are in accord with our results where PFE has been showed to render protection against CP induced toxicity .When animals pre-treated with PFE and subsequently treated with CP, it was observed that M1 was equivalent to that of control group. Such observations indicate that when organisms. administered with fruit extract for a longer period of time than perhaps toxic agents such as anticancer drugs might not be effective in induction of chromosomal aberrations.

Earlier the protective effects of phyllanthus emblica in adriamycin, an anticancer drug and chromium induced genotoxicity has been reported from our laboratory.[43-44]

CONCLUSION

From the above studies, it is concluded that phyllanthus emblica was a potential candidate as protective agent to cyclophosphamide induced genotoxic effect in somatic cells of mice. The combined treatment of cyclophosphamide and PFE holds a promise as a safe and effective chemotherapeutic strategy.

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