Chromosomal damage by low doses of radiation: protection by combinations of dietary antioxidants

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Mice which were fed antioxidants, consisting of a combination of β-carotene, α-tocopherol and ascorbic acid, or curcumin, ascorbic acid and chlorogenic acid are substantially protected against γ-ray induced micronuclei in polychromatic erythrocytes obtained from bone-marrow. In this context, the relevance of a more balanced *intake of food material especially* those with anticarcinogens/antimutagenic principles for human health care needs no over-emphasis.

An important issue of public concern today is the possible health effects of protracted exposure, over days and months, to small amounts of physical and chemical pollutants in our environment. Physical biohazards such as ionizing radiations originating from outer space or from the earth's crust or even from the nuclear power industry, cause great concern because exposure to these, in most circumstances, is unavoidable. Scientific opinion on the consequences of a lifetime exposure to low doses of radiation is quite divided. It is however to be noted that epidemiological studies on the incidence of cancer mortality, gross congenital abnormalities, hereditary diseases and fertility among the inhabitants of regions with high natural background radiation, such as the monazite areas of coastal Kerala and Tamil Nadu, do not support such a harmful effect of low-dose radiation[1,2]. Among the several known causes for inconsistencies in experimental data, the modulatory role of diet consisting of compounds of plant origin, is beginning to be appreciated. Many common foodstuff and beverages are known to reduce radiation- and carcinogen-induced DNA damages[3–6]. In fact, it is practically impossible for anyone to consume a diet which is devoid of antimutagens/anticarcinogens because of their diversity and widespread occurrence. Two recent studies from our laboratory have demonstrated that some of these naturally occurring dietary antioxidants like ascorbic acid, α-tocopherol, β-carotene, chlorogenic acid and curcumin can exert dose-related protective effects against *in vivo* chromosomal damage induced by a high dose of γ-radiation[7,8]. It should, however, be noted that ascorbic acid is known to counteract the protective action of aminothiols. Therefore, the present study involved an assessment of the influence of ascorbic acid on the radioprotective effects of other dietary compounds, such as α-tocopherol, β-carotene, curcumin and chlorogenic acid, having known radioprotective properties.

Experiments were carried out with 8–12 week old male Swiss albino mice, weighing 24–30 g. The animals were bred and maintained at 25 ± 2°C on standard mouse diet (Lipton India Limited) and water *ad libitum*. Ascorbic acid (AA), α-tocopherol (AT), β-carotene (BC), curcumin (CR) and chlorogenic acid (CGA) were purchased from Sigma Chemical Company (USA). Irradiation was carried out using the Eldorado 78 teletherapy unit obtained from the Atomic Energy of Canada and located in the Institute of Nuclear Medicine and Allied Sciences (INMAS), New Delhi. The test compounds were administered by gavage either in double distilled water (AA and CGA) or in peanut oil (AT, BC and CR). Animals receiving the same amount of water or oil alone served as controls. Selection of the time and frequency of administration of each compound was based on earlier studies involving a higher radiation dose[7,8]. The micronucleus test was carried out according to Schmid (1975). Six mice were used for each dose of the compound under investigation and 2500 PCEs were scored per animal. Bone-marrow cells were sampled 24 h after irradiation. Other details on the experimental protocol are given in Table 1.

It is clear from Figure 1 that a significant increase in the frequency of micronuclei is observed even with doses as low as 0.05 Gy. At 0.25 and 0.50 Gy, the frequency of micronuclei is fairly high; any increase or decrease due to chemical treatment can be assessed without ambiguity, at these doses. The effects of combined treatment with dietary antioxidants are shown in Table 1. In both combinations ascorbic acid, α-tocopherol, β-carotene, *curcumin and chlorogenic acid* bring about a reduction in the frequency of radiation-induced

Table 1. Reduction in the frequencies of radiation-induced Mn PCEs by combined treatment with dietary antioxidants

Combination of dietary antioxidants tested[c]	Radiation[b] (Gy)	MnPCEs/1000 PCEs[a] Without dietary antioxidants	With dietary antioxidants
β-carotene +			
α- tocopherol +	0.25	8.1 ± 0.4	5.0 ± 0.4**
ascorbic acid	0.50	16.2 ± 0.8	10.1 ± 1.1**
Curcumin +			
ascorbic acid +	0.25	8.8 ± 0.5	5.8 ± 0.5**
chlorogenic acid	0.50	16.9 ± 0.9	10.5 ± 0.8**

[a]Values are mean ± SE for groups of 6 animals.

[b]Dose-rate 0.28 Gy/min.

[c]Compounds were administered by gavage as follows:
β-carotene (0.5 mg/kg/day) was given in peanut oil for 7 days and the animals were irradiated on day 8. α-tocopherol (3 mg/kg) and Curcumin (5 mg/kg), dissolved in peanut oil, were given 2h before irradiation; chlorogenic acid (50 mg/kg) and ascorbic acid (20 mg/kg) were given in double-distilled water 1h before irradiation

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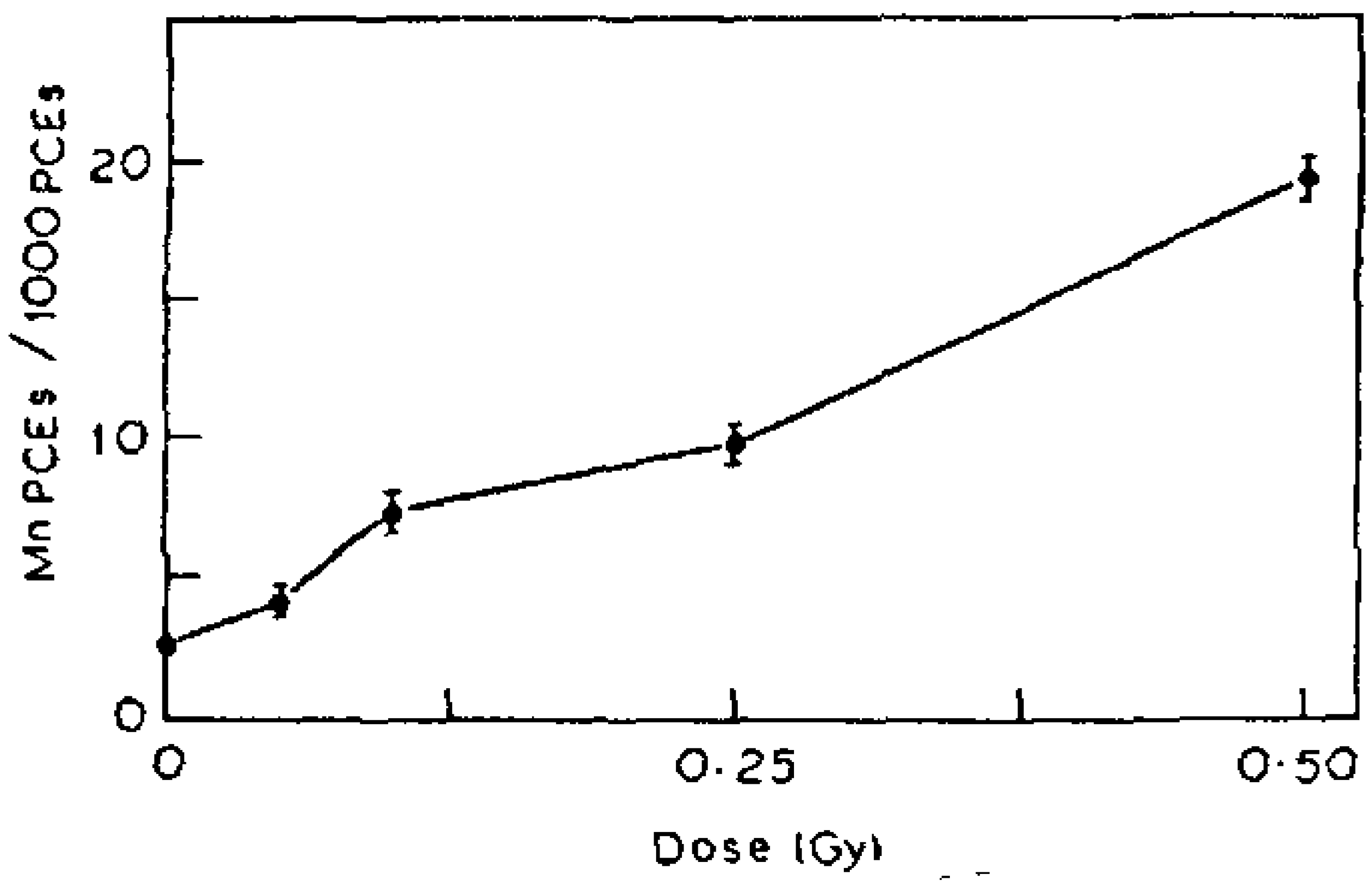

Figure 1. Frequencies of MnPCEs in bone marrow erythrocytes following whole-body exposure to low doses of γ-radiation at a dose rate of 0.28 Gy/min.

micronuclei. In particular, the presence of ascorbic acid does not abolish the radioprotective action of the combination(s) of the dietary agents. This indicates that these compounds continue to exert radioprotection when present together, as in a normal human diet. The magnitude of radioprotection afforded at the two radiation doses, 0.25 and 0.50 Gy is quite comparable. It would be even more interesting if these dietary constituents abolish the small but significant increase in the frequency of micronuclei observed in animals exposed to as small as 0.05 Gy. At such low doses, fluctuations in the frequency of MnPCEs are much greater, and therefore, the sampling size would have to be increased. Such studies are in progress. The doses of various dietary antioxidants used in the present study are those which can be achieved through the diet. Carrots, spinach, cabbage, guava and lemon are rich sources of ascorbic acid and β-carotene while almonds and peanuts contain large amounts of α-tocopherol. Chlorogenic acid is present in high concentrations in coffee. Curcumin is the main colouring principle of turmeric, an edible spice which is widely used in Indian foods. Therefore the present finding which suggests that the intake of a diet rich in 'natural' antioxidants can reduce the chromosomal damage induced by exposure to ionizing radiation has implications from the point of risk assessment.

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Tree ring evidences of the 1991 earthquake of Uttarkashi, Western Himalaya

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Tree-ring analyses of 'kail' pine (*Pinus wallichiana*) from Agora in Uttarkashi, Western Himalaya, were carried out to evaluate the effect of the 1991 earthquake event on tree growth. Tree rings were commonly found to be narrow in 1992, one year after the earthquake in most of the trees studied. The tilted trees showed eccentric growth, i.e. narrow rings on the upper side of the stem opposite to the lean in comparison to the wider contemporary rings of the lean side. The growth eccentricity was found to be synchronic in many trees and could be related to the 1991 earthquake event. Such tree ring studies along the active fault zones would help in the dating of palaeoseismic events.

In India reliable records of past earthquakes are limited to 200 years including 67 years of instrumental record. Proxy records could be utilized to date the past earthquake events. Longer records of earthquakes would help in seismic zoning by delimiting the areas experiencing frequent shocks.

Ring sequences of trees growing along the active fault zones offer the potential of dating the past earthquake events with the precision of actual year of their occurrence. Even in some cases tree rings have been found to provide the information to the level of months or seasons if the earthquake occurred in the growing season[1]. Strong seismic shocks cause intensive physical damage to trees such as severing of roots, tilting and sometimes even complete destruction. The trees surviving such shocks record the event in their annual growth rings with features such as narrow rings, growth eccentricity and increased proportion of reaction wood. Several studies[2–10] have demonstrated the applicability of tree rings in dating of palaeoseismic events.