Consumption of antioxidant dietary agents, curcumin and vitamin C, protects cellular DNA from gamma-radiation

C.K.K. Nair* and A. Menon

Pushpagiri Institute of Medical Sciences and Research Centre, Thiruvalla - 689101, Kerala, India

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**** Corresponding author:***
Dr. Chreupally Krishnan Krishnan Nair
Fax: *+91 469 2731005*
E-mail: *ckknair@yahoo.com*

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ABSTRACT

Background: Exposure to ionizing radiation results in genotoxicity and the unrepaired lesions in cellular DNA results in cell cycle arrest, reproductive death, interphase death, division delay, chromosome aberrations, mutations, etc. leading to the intensive destruction of cells and violation of their proliferative capacity there by adversely affecting the mammalian system. Since ionizing radiation is widely used in medicine, industry, agriculture and research, an effective radioprotector is a must to protect living beings. This work aims to identify the ability of dietary supplements, curcumin and ascorbic acid in offering protection from radiation induced genomic insult to human peripheral blood leukocytes. ***Materials and Methods***: Alkaline single cell gel electrophoresis was performed on human peripheral blood leukocytes exposed to 2 doses of gamma radiation in presence and absence of curcumin and ascorbic acid (*in vitro*). The same technique was also applied to blood leukocytes of volunteers before consumption of these supplements and 5 days after (*ex vivo*). The comet parameters such as % DNA in tail, tail length, tail moment and olive tail moment were determined. ***Results***: The comet parameters of leukocytes increased upon exposure to ionizing radiation indicating DNA damage, *in vitro* or *ex vivo*. The extent of DNA damage was found significantly decreased either in the presence or following the intake of curcumin and ascorbic acid. Curcumin offered higher protection than ascorbic acid. ***Conclusion***: Intake of dietary antioxidants such as curcumin or ascorbic acid could offer protection against ionizing radiation induced cellular DNA damage in peripheral blood leukocytes.

Keywords: *Radiprotection, antioxidant dietry, curcumin, vitamin C, DNA damage.*

INTRODUCTION

Ionizing radiation induced cellular damages, such as reproductive death, interphase death, division delay, chromosome aberrations, mutations etc. are attributed to irreversible changes resulting from the deposition of energy, generating free radicals, reactive oxygen species their accumulation and the subsequent cellular alterations [1]. Agents which can prevent the formation of free radicals during radiation or destroy free radicals by reacting with them, thereby inhibiting their reaction with biomolecules, might be considered as radioprotectors [2]. The use of dietary antioxidants to prevent antitumor agent-induced chromosomal damage in normal cells is also of considerable interest. The ability of dietary antioxidants to protect cellular DNA from radiation induced and chemical induced damages has been the focus of active research [3] and the present work

concerns monitoring of the ability of dietary supplements, such as curcumin and ascorbic acid, to protect the cellular genome from radiation hazard by single cell electrophoresis or comet assay. The Comet assay has been widely used to detect DNA damage such as strand breaks, alkali-labile sites, DNA cross-linking, and incomplete excision repair sites [4, 5]. This technique is a very sensitive and useful tool to detect genotoxic damage at the individual cell level particularly in humans. Exposure to gamma -radiation will induce strand breaks in cellular DNA and the extent of this damage can be observed as increased % DNA in tail, tail length, tail moment and olive tail moment in comets formed after electrophoresis of irradiated cells [6].

MATERIALS AND METHODS

Chemicals

Low melting point agarose was from Sigma Chemical Company Inc., St Louis, MO, USA. All other chemicals were of analytical grade procured from reputed Indian manufacturers. The nutraceuticals curcumin and ascorbic acid used in the present study were commercially available capsules. Curcumin was obtained from LivLong Nutraceuticals Limited; Alwaye which is available in the market as a dietary supplement under the brand name 'Vivomeric' and ascorbic acid was a gift from Prof. V.T Kagia, Health Research foundation, Kyoto, Japan.

Blood collection

Nine non-smoking male volunteers, of age group 23-25 and not suffering from any known serious acute or chronic illness, with an average weight of 60 kg were selected and divided into 3 groups. 2 ml of whole blood was collected into heparinized vacutainers.

In vitro radioprotection of cellular DNA

Human peripheral blood was collected in heparinized tubes and exposed to 2 doses of gamma radiation (0 - 6 Gy) as detailed below:

Group 1 - Blood + 0 Gy
Group 2 - Blood + 3 Gy
Group 3 - Blood + 6 Gy
Group 4 - Blood + Curcumin (100 µg/ml) + 0 Gy
Group 5 - Blood + Curcumin (100 µg/ml) + 3 Gy
Group 6 - Blood + Curcumin (100 µg/ml) + 6 Gy
Group 7 - Blood + Ascorbic acid (100 µg/ml) + 0 Gy
Group 8 - Blood + Ascorbic acid (100 µg/ml) + 3 Gy
Group 9 - Blood + Ascorbic acid (100 µg/ml) + 6 Gy

The experiment was done in duplicates and was repeated twice.

In vivo radioprotection of cellular DNA

9 non-smoking male volunteers, of age group 23-25 and not suffering from any known serious acute or chronic illness, with an average weight of 60 kg were selected and divided into 3 groups. Group 1 was kept as the control while group 2 and 3 were taking commercially available capsules of curcumin (daily dose of 8.33 mg/kg) and tablets of ascorbic acid (daily dose of 8.33 mg/kg) as dietary supplement for health reasons and willing to donate blood. Peripheral blood was collected from these volunteers before taking the supplements and after consuming the capsules of curcumin or the tablets of ascorbic acid for five consecutive days. The blood samples were exposed to different doses of gamma-radiation (0-6 Gy) and alkaline single cell gel electrophoresis or comet assay was carried out to examine the extent of DNA damage in the blood leukocytes.

Alkaline single-cell gel electrophoresis (comet assay)

Alkaline single-cell gel electrophoresis was performed using the method given by Singh (2000), with minor modifications [7]. Microscope slides were coated with normal melting point agarose (1% in PBS) and kept at 4°C till the agarose was solidified. On these slides, 200 µl of 0.8% low melting point agarose containing 50 µl of treated cells were added. After solidification of the low melting agarose, the slides were immersed in pre-chilled lysing solution containing 2.5 M NaCl, 100 mM EDTA, 10 mM Tris-HCl, pH 10, 1% DMSO, 1% Triton X and kept for 1 hour at 4°C for lysis of the cells. After lysis, the

slides were drained properly and placed in a horizontal electrophoretic apparatus filled with freshly prepared electrophoresis buffer containing 300 mM NaOH, 1 mM EDTA, 0.2% DMSO, pH ≥ 13. The slides were equilibrated in buffer for 20 minutes and electrophoresis was carried out for 30 minutes at 20 V. After electrophoresis the slides were washed gently with 0.4 mM Tris-HCl buffer, pH 7.4, to remove alkali. The slides were again washed with distilled water, kept at 37°C for 2 hours, to dry the gel and silver staining was carried out. The comets were visualized under a binocular microscope and the images captured were analyzed using the software 'CASP' to find out the extent of DNA damage measured in terms of different comet parameters such as % DNA in tail, tail length, Tail Moment (TM) and Olive Tail Moment (OTM) [8]. The parameter tail moment is the product of tail length and % DNA in tail, and olive tail moment is the product of the distance between the centre of gravity of the head and the centre of gravity of the tail and % DNA in tail. Results are presented as mean ± standard deviation.

Statistical analysis

The results are presented as mean ± SD of the studied group. Statistical analyses of the results were performed using ANOVA with Tukey–Kramer multiple comparisons test.

RESULTS

In vitro radioprotection of cellular DNA

There was an increase in all the comet parameters of the leukocytes upon exposure to ionizing radiation. The increase in these parameters was dependent on the dose of irradiation. Comet parameters of blood leukocytes exposed to 3 Gy gamma radiation were brought down to 6.29±1.51, 8.28±2.66, 0.51±0.25, 2.47±0.65 in curcumin treated group and 6.06±0.97, 9.94±2.07, 0.62±0.17, 2.57±0.36 in ascorbic acid treated group from 11.09±2.29, 14.79±3.8, 1.62±0.67, 3.96±0.78. While the comet parameters were brought down from 20.05±4.96, 32.59±6.33, 6.27±2.39, 8.75±1.9 in the 6 Gy irradiated group to 10.36±2.05, 13.95±3.57, 1.42±0.57, 4.44±1.1 in curcumin treated group and 12.19±2.67, 15.98±4.97, 1.92±0.87, 5.16±1.18 in ascorbic acid treated group. The increased comet parameters of the leukocytes (due to radiation exposure) were found significantly reduced in the presence of curcumin and ascorbic acid as evident from figure 1.

The effect of intake of curcumin or ascorbic acid

Comet assay was performed to analyze the extent of cellular DNA damage in peripheral blood leucocytes of individuals consuming curcumin or Ascorbic acid. The blood cells obtained from the volunteers before taking the supplements and after consuming capsules of curcumin (daily dose of 8.33 mg/kg) or tablets of ascorbic acid (daily dose of 8.33 mg/kg) for five consecutive days were exposed to 6 Gy gamma radiation. Blood from the individuals when exposed to gamma radiation showed tail formation after comet assay due to radiation induced DNA damage, while unirradiated blood cells of normal and individuals who had consumed the curcumin or ascorbic acid appeared as homologous disc. The blood cells obtained from individuals after 5 days of curcumin or ascorbic intake when exposed to 6 Gy radiation showed reduction in the extent of radiation induced DNA damage as revealed from figure 2.

Protection of cellular DNA

Percentage protection of cellular DNA is calculated using the formula given below:

$$\% \text{ Protection} = \frac{(P_i - P_u) - (P_t - P_u)}{(P_i - P_u)} \times 100$$

Here 'P_i' represents the value of a parameter of the comet for irradiated control, 'P_u' represents the value of the parameter of the comet for unirradiated control and 'P_t' stands for the value of the parameter of the comet for irradiated-drug treated group.

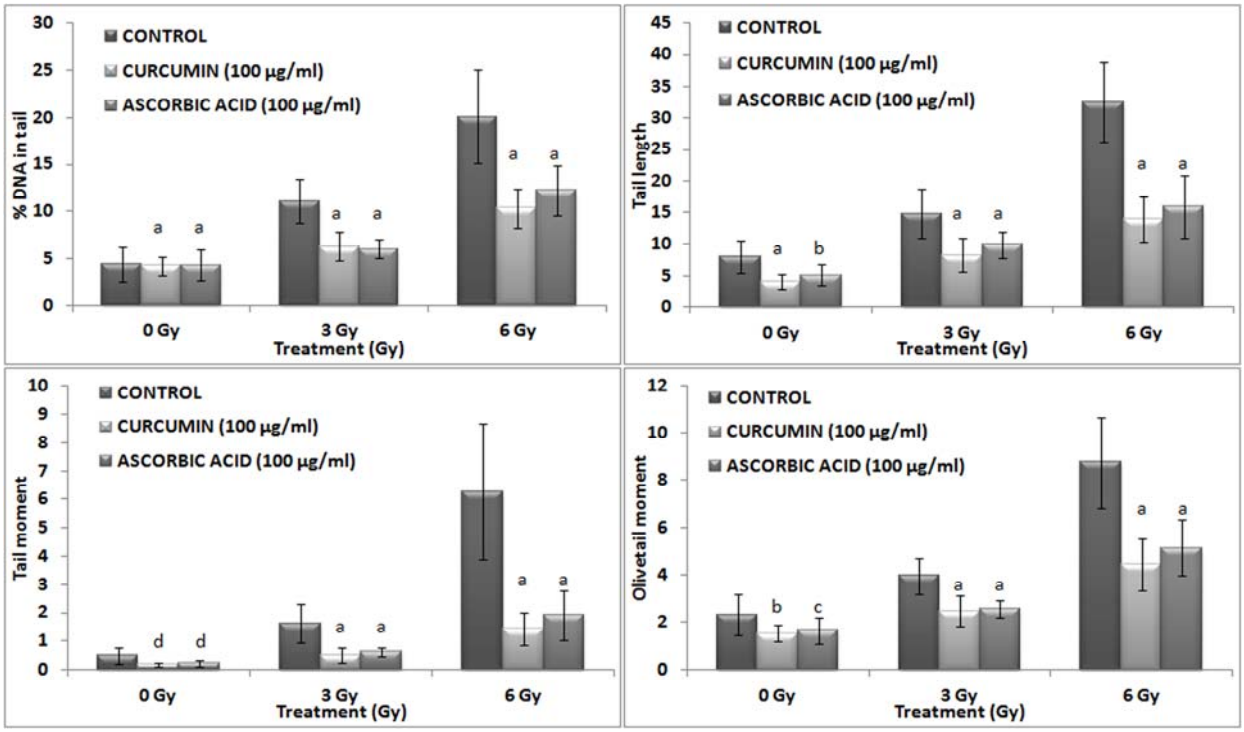

Figure 1. DNA damage of human peripheral blood leukocytes exposed to 2 doses of gamma radiation (0–6 Gy) in the presence and absence of curcumin (100 µg/ml) and ascorbic acid (100 µg/ml) expressed in terms of various comet factors. Values are expressed as mean ± S.D. 'a' indicated $p \leq 0.001$; 'b' indicates $p \leq 0.01$; 'c' indicated $p \leq 0.05$; 'd' indicated not significant.

Table 1. Percentage protection of cellular DNA exposed to 6 Gy gamma radiation under ex vivo and in vitro conditions expressed in terms of various comet parameters.

Percentage protection in the Comet Parameter under ex vivo condition				
Treatment	% DNA in Tail	Tail length	Tail moment	Olive tail moment
Curcumin (8.33 mg/kg) + 6 Gy	25.17	65.61	61.01	41.6
Ascorbic Acid (8.33 mg/kg) + 6 Gy	12.79	54.19	43.11	28.16
Percentage protection in the Comet Parameter under ex vivo condition				
Treatment	% DNA in Tail	Tail length	Tail moment	Olive tail moment
Curcumin (100 µg/ml)	62.07	76.01	83.91	67.02
Ascorbic Acid (100 µg/ml)	50.35	67.74	75.25	55.83

Comparison of the % protection of comet parameters indicated that curcumin (8.33 mg/kg) offered higher protection than ascorbic acid (8.33 mg/kg) to leukocytes exposed to 6 Gy gamma radiation (table 1). This would suggest that intake of dietary antioxidants such as curcumin and ascorbic acid offer protection to cellular DNA in blood leukocytes against genotoxic damage by ionizing radiation.

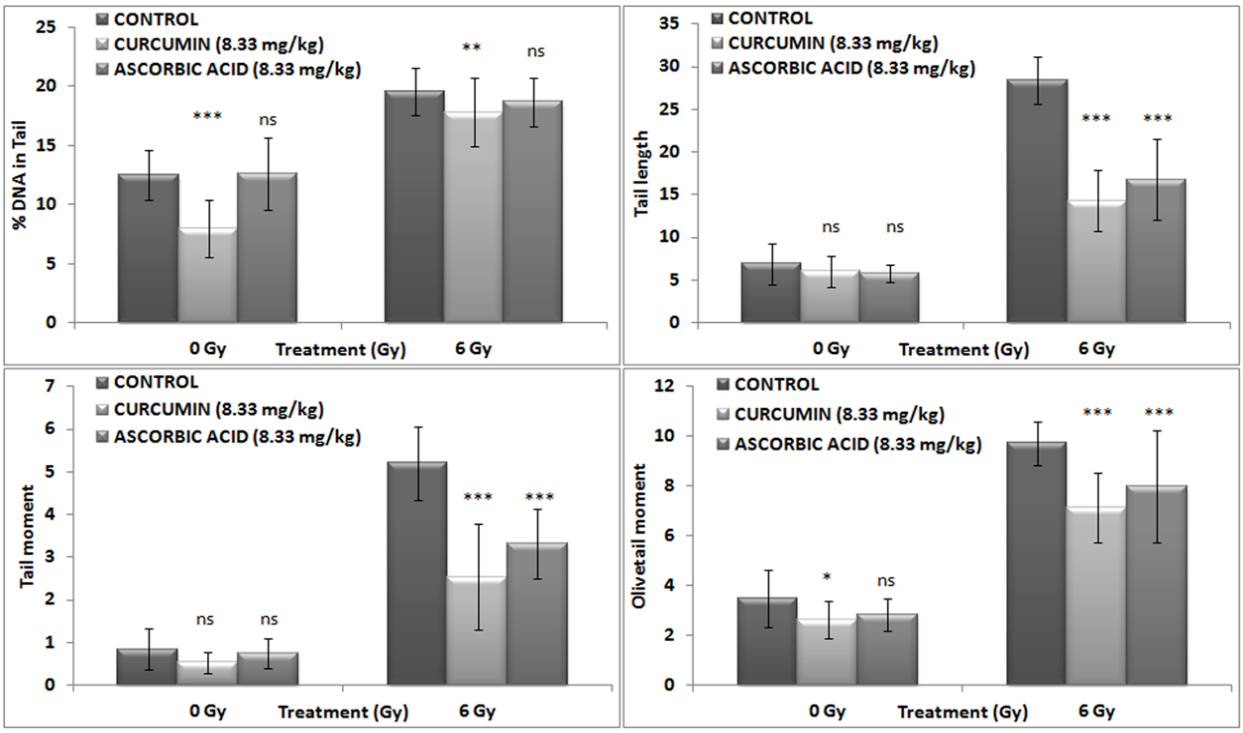

Figure 2. Effect of curcumin (8.33 mg/kg) and ascorbic acid (8.33 mg/kg) on radiation induced DNA damage in peripheral blood leukocytes exposed to 6 Gy gamma radiation. Values are expressed as mean ± S.D. *** indicated $p \leq 0.001$; * indicates $p \leq 0.05$.

DISCUSSION

Ionizing radiations are encountered in different spheres of human life. These radiations cause deleterious effects in animals and humans. Radioprotective agents offer a possible solution to counteract the radiation damage to living beings [2]. Radiation is used therapeutically for the treatment of various types of malignancies. It is well known that most of the damages induced by radiation to living cells are due to the generation of aqueous free radicals [9].

Cellular DNA is the principal target for radiation damages as free radicals generated during radiation exposure in the cellular milieu cause single strand breaks, DNA-DNA and DNA-protein cross links and damages to nucleotide bases. Alkaline comet assay is a sensitive technique to monitor strand breaks and alkali labile DNA lesions and is widely used to study genotoxicity, cellular DNA lesions such as single strand breaks or double strand breaks, apoptosis and DNA repair [10, 11].

Compounds with antioxidant properties have been shown to prevent the deleterious effects of ionizing radiations in living systems and bio-molecules due to their ability to scavenge free radicals [12]. Curcumin and ascorbic acid have been extensively reviewed for their antioxidant and free radical scavenging activity [13-16]. Presence of dietary supplements such as curcumin or ascorbic acid during radiation exposure protected cellular DNA from radiation induced strand breaks. As revealed by alkaline single cell gel electrophoresis on human peripheral blood, these two dietary supplements protect cellular DNA from radiation induced damages in a dose dependant manner.

Exposure to radiation changes the antioxidant levels in body. Antioxidant compounds with free radical scavenging property have been reported to prevent radiation induced lesions in cellular targets [2]. The observed effect can be attributed to the scavenging of free radicals generated during radiation exposure by these 2

compounds. Dietary supplements such as curcumin and ascorbic acid have high antioxidant activities and the present work reveals their application for radioprotection in human system. Consumption of these agents considerably reduced the radiation-induced damage to DNA in peripheral blood leukocytes. This indicated the ability of these dietary ingredients to protect against gamma-radiation induced cellular DNA damages.

Oral intake of curcumin and ascorbic acid, 5 days prior to radiation exposure significantly protected human cells against radiation induced cellular DNA damage. Curcumin had higher radioprotecting activity than ascorbic acid. The observed resistance to radiation injury could be due to radioprotection developed by the drugs in blood or indirectly through their metabolic activities by increasing the antioxidant levels in the blood cells. The present study does not distinguish these possibilities.

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