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Anti-oxidation actions of Indian spice in simulated microgravity

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ABSTRACT

The effect of microgravity is more pronounced after long-duration space flights and can even last for several weeks after landing. The extensive research is going on the preventation of peroxidative damage due to microgravity. It has been evident that curcumin (diferuloylmethane), a yellow pigment in curry powder exhibits antioxidant, anti-inflammatory, and proapoptotic activities. To determine the preventive effects of curcumin on peroxidative damage due to two bed rest conditions, 20 healthy male volunteers were equally divided into two groups (10 with curcumin and 10 without curcumin). They were studied in condition before, during, and just on bed rest conditions at -6° head-down-tilt (HDT) bed rest for 10 days. We measured the salivary and serum oxidative markers such as Malonaldehyde, 8-hydroxydeoxyguanosine, vitamin C and E just before HDT , during HDT experiment, and in course time of recovery with curcumin and without curcumin groups. The values of serum and salivary Vitamin C & E showed statistically significant decrease in both bed rest conditions as compared to the condition before and during the recovery stage. However, levels were not significantly lowered in curcumin groups in contrast to the groups without curcumin (Table-1, $P<0.05$) . MDA and 8-OHdG levels showed significant increase in simulating microgravity condition as compared to the condition before and in the recovery stage. Hence, curcumin prevent peroxidative damage in simulated conditions. Further study is required on antioxidation actions of curcumin in space microgravity conditions.

Key Words: Curcumin, Serum, Saliva, oxidative stress, two bed rest position, space microgravity.

INTRODUCTION

Current projects on missions to Mars results in 2 years of microgravity conditions, demands the critical need for the development of optimal nutritional programs and physical counter-measures to prevent body mass and functional alterations. On long duration space flights such as mars mission, astronauts undergo many physiological changes such as loss of bone mass, muscle strength, and cardiovascular fitness as a result of reduced metabolic activities, lower cellular and reduced tissue oxygen demand 1-12. There exists a balance in the body between oxidant production and antioxidant defence, with the balance shifted slightly in favour of oxidants 1-3. Mainly products of this "leakage" are the two ROS: superoxide radical (O2_) and H2O2 2. Other ROS includes the free radicals such as nitric oxide and compounds such as ozone and HOCl. ROS can attack and damage cellular constituents such as DNA, proteins, and membrane lipids. Oxidative damage from free radicals to DNA and lipids has been implicated in the etiology of a wide variety of chronic diseases and acute pathologic states 2-8. The chronic diseases range from oral diseases such as periodontitis and oral cancer to cardiovascular diseases and neuro-

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degenerative diseases including Alzheimer and Parkinson diseases.9-13 It has been observed that there is an increased lipid peroxidation in human erythrocytic membranes and reductions in some blood anti-oxidants after long-duration space flights 13-15 It has also been observed that there was urinary excretion of 8-iso-prostaglandin F2- and 8-oxo-7,8 dihydro-2 deoxyguanosine (8-OHdG) in six subjects during and after long-duration space flights (90 to 180 d) 16-17. Isoprostane 8-isoprostaglandin F2- and 8-OHdG are the markers for oxidative damage to lipids and DNA respectively 16-17 . Most rodent studies showed increased production of lipid peroxidation products in postflights and decreased antioxidant enzyme activity in same post-flights 18. It has been found that space flights simultaneously down regulate anti-oxidant defence capacity and elicit an oxidative stress in the liver. There was an approximately 50% increase in the liver malondialdehyde concentration with space flights19. Vitamin E is the primary chain-breaking anti-oxidant in the cell membranes 9,20,21. The protective role of vitamin C seems to lie in its ability to reduce the oxidized form of vitamin E, thereby making it reusable by the cell 9,23.

Curcumin (diferuloylmethane), a dietary pigment responsible for the yellow color of turmeric is used as a traditional medicine well documented in the Ayurveda for the treatment of numerous inflammatory conditions. Extensive research within the past half-decade has confirmed that curcumin mediates anti-inflammatory effects through the downregulation of transcription factor, nuclear factor-?B (NF-?B) , tumor necrosis factor (TNF) , interleukin-6 , interleukin-8 , adhesion molecules , inducible nitric oxide synthase (iNOS) , matrix metalloproteinase-9 (MMP-9) , cyclooxygenase-2 (COX-2) , 5-lipoxygenase (5-LOX), and glutathione reverses the inhibition 24-34 . It has been reported that Curcumin act as anti-oxidant agent 30-34. Curcumin has shown that agent can be administered safely at a oral doses of up to 8 g/d .There was no dose-limiting toxicity; dosing was limited by the number of pills that patients could or would swallow daily 35-36. Hence, this study was planned to study the effect of curcumin on serum and salivary markers of oxidative stress in simulated microgravity.

MATERIALS AND METHODS

The subjects of this investigation were 10 male volunteers aged (18-22 years, mean weight of 72.5 +_ 3.2 kg and mean height of 174.9+_ 3.4 cm) participated in an 8-hour 6° HDT bed-rest exposure (18-21 years, mean weight of 71.8 +_ 2.3 kg and mean height of 174.8+_ 3.3 cm) and bed rest position (18-24 years, mean weight of 73.6 +_ 3.4 kg and mean height of 175.1+_ 4.1 cm), who had not participated in systemic endurance training for 10 days prior to study and each subject was given a detailed explanation of the experimental protocol and were provided written and verbal consent. Each subject completed a medical and dental history questionnaire to determine the status of systemic diseases, smoking, alcoholic and drugs history as well as clinical examination for systemic diseases, chronic diseases and oral & dental diseases. Patients were excluded from study who had systemic diseases, chronic diseases, oral & dental disease, smoking, alcoholic and drugs history. Five volunteers of each HDT was selected and given a curcumin once a day and others five volunteers of each HDT was not given anything.

Curcumin- 1 g caplet form Curcumin (900 mg curcumin, 80 mg desmethoxycurcumin, and 20 mg bisdesmethoxycurcumin) from Sabinsa was obtained .

Blood and saliva samples were taken just before HDT, throughout the time course of the HDT experiment, and during recovery period. Subjects were asked to awake at 6 A.M on the day of the study and to remain seated or in standing position until arrival at the research centre. Baseline control measurements were obtained during the hour before HDT. At -9 A.M. the subjects were transferred supine to a gurney and tilted to 6' HDT, where they remained for the next 8 h. At -5 P.M. till 10 days, after 10 days the subjects returned to a

chair and stayed in seated position for the 4-h recovery period. Blood and saliva samples were prepared at the same time.

Whole unstimulated saliva was collected over a five-min period from subjects with directions to allow saliva to pool at the bottom of the mouth and drain into a collection tube, when necessary. Unstimulated whole saliva produced in a 5-min period (about 3 mL) was collected, allowed to drain into a plastic container, and centrifuged at 3,000 ×g in 4°C for 5 min to remove bacterial and cellular debris. Saliva samples were stored at -80°C until analysis. Blood samples were collected into Vacutainer tubes. The blood was centrifuged at 1,700 g for 10 minutes and the plasma was separated. Plasma was stored at -80°C until analysis. Serum and salivary levels were assessed for MDA using thiobarbituric acid (TBA) method of Buege and Aust 37 . Concentrations of both vitamins were measured using liquid chromatography 38 .Quantitative measurements of the oxidative DNA adduct 8-OHdG was performed according to the method described by Toyokuni et al.39 Briefly, the saliva samples were centrifuged at 10,000g for 10 minutes and the supernatant was used to determine 8-OHdG levels with a competitive ELISA kit (Japan Institute for the Control of Aging, Shizuoka, Japan). The determination range was 0.5-200 ng/mL. Serum 8-OHdG levels were measured in duplicate by a competitive ELISA kit (OXIS, Portland, OR, USA) according to the manufacturer's instructions. The sensitivity of the method was 1 ng/ml. All data were statistically analyzed using SPSS statistical package (SPSS, version13, Chicago,IL,USA). Data were expressed as mean ± standard deviation. Differences between pre, during and after microgravity simulation were analyzed for significant values using one-way ANOVA test. Correlation assessment was performed using the Spearman correlation analysis. Statistical significance was defined as $p< 0.05$.

RESULTS

The values of serum and salivary Vitamin C & E showed statistically significant decrease in simulating microgravity as compared to the period before and during the recovery stages, with and without Curcumin groups. It was also observed lower in recovery stage as compared to the period before when examined in microgravity conditions (Table-1&2, $P<0.05$). However decrease in curcumin groups was lower as compared to that examined in without curcumin groups. MDA and 8-OH dG levels showed statistically significant increase in both conditions as compared to period before and in recovery stages, also observed relatively higher in without curcumin groups as compared with curcumin groups (Table-1&2, $P<0.05$).

DISCUSSION

In the present study, serum and salivary Vitamin C & E values were significantly lowered in condition and in both groups (Table-1, $P<0.05$) which support the previous studies 40-42. Decreased anti-oxidant defence may be one of the reasons for increased levels of ROS and subsequent tissue damage in two bed rest conditions. MDA levels in both rest conditions environment were significantly elevated in both groups in contrast to the period before and in the recovery stages. This indicates that increased lipid peroxidation due to 'free radical'-mediated injury occurs in the both rest conditions. Increased lipid peroxidation can occur if the rate of production of reactive oxygen species is higher or the antioxidant level is low which concur with the previous studies 40-44. The 8-OHdG levels were increased in both conditions as observed in the previous studies 28, 30-33. Different aspects of oxidative stress are measured by 8-OHG namely DNA damage and cell membrane damage respectively 44-48. The increased 8-OHG, MDA levels and decreased Vitamin C and E levels were low in curcumin groups as compared to the values observed in without curcumin groups in accordance with the previous studies 34 . Several reports suggest that curcumin can induce ROS 47,48 . There are also reports which suggest that curcumin quenches ROS production and thus acts as an

Table-1 Salivary and serum MDA, Vitamin C& E and 8 dihydro-2 deoxyguanosine (8-OH dG) concentrations in the plasma and saliva of 20 Normal healthy subjects in period before HDT without Curcumin (A), throughout the time course of the HDT experiment (B), during recovery (C) and before HDT with Curcumin (AA), throughout the time course of the HDT experiment (BB), during recovery (CC)

Markers	Serum and saliva	A	AA €	B	BB £,€	C	CC £
MDA	Salivary (µmol/L)	0.24±0.06[a]	0.22±0.13	0.34±0.12[*a]	0.25±0.14	0.25±0.13[*]	0.24±0.23
	Serum (µmol/L)	1.14 ±0.37[a]	1.06 ±0.89	1.36 ±0.36[*a]	1.01 ±0.68	1.18 ±0.24[*]	1.01 ±0.75
Vitamin C	Salivary (µg/L)	1.01±0.32[a]	1.56±0.66	0.82±0.21[*a]	1.23±0.67	0.97±0.24[*]	1.29±0.68
	Serum (µg/L)	8.23±1.23[a]	8.96±2.46	7.56±1.89[*a]	8.82±2.33	8.05±1.95[*]	8.88±2.86
Vitamin E	Salivary (µg/L)	0.43±0.12[a]	0.56±0.46	0.31±0.14[*a]	0.48±0.45	0.41±0.16[*]	0.54±0.29
	Serum (µg/L)	8.01±1.12[a]	8.46±2.32	7.32±1.21[*a]	8.23±3.34	7.90±1.12[*]	8.94±3.32
8-OH dG	Salivary (ng/ml)	0.32±0.04[a]	0.22±0.13	0.45±0.07[*a]	0.24±0.11	0.38±0.08[*]	0.22±0.12
	Serum (ng/ml)	2.12 ± 1.24[a]	1.45 ± 1.11	2.79 ± 1.23[*a]	1.89 ± 1.36	2.32 ± 1.26[*]	1.77 ± 1.12

*p < 0.05, as compared to after condition (C)
ap < 0.05, as compared to Before condition (A).
£p < 0.05, as compared to after condition (CC)
•p < 0.05, as compared to Before condition (AA).

antioxidant 49. Other reports suggest that curcumin quenches ROS production at low concentrations and induces ROS production at high concentrations 50. It might be like the vitamin C which acts as both a pro-oxidant and an antioxidant. Whereas the pro-oxidant mechanism mediates apoptotic effects, the antioxidant mechanism mediates NF-?B-suppressive effects.

Hence, Microgravity condition had not only systemic alterations but it also lowered the oral antioxidant levels. Antioxidant defence (vitamin E and C) was compromised and oxidative stress was higher in both rest condition. Hence, better formulations of curcumin might provide more antioxidant effects. Further study is required on the effect of curcumin as an anti-oxidant agent in microgravity & zero gravity conditions.

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