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Anti-oxidation actions of curcumin in two forms of bed rest: oxidative stress serum and salivary markers

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ABSTRACT

Objective: To determine the preventive effects of curcumin on peroxidative damage under two bed rest conditions. **Methods:** 20 healthy male (10 with curcumin and 10 without curcumin) volunteers were selected. They were studied before, during, and just on bed rest conditions at -6° head-down-tilt (HDT) bed rest and bed rest position (BD) for 10 days. We measured the salivary and serum oxidative markers such as Malonaldehyde, 8-hydroxydeoxyguanosine, vitamin C and E just before HDT & BD, during HDT & BD experiment, and in course time of recovery with curcumin and without curcumin groups. **Results:** The values of serum and salivary vitamin C & E showed statistically significant decrease in both bed rest conditions as compared to those of the conditions before and during the recovery stage. However, these levels were not significantly lowered in curcumin groups in contrast to the groups without curcumin ($P>0.05$) . MDA and 8-OHdG levels showed significant increase in simulating microgravity and zero gravity conditions as compared to those before and in the recovery stage. However, these levels were lower in curcumin groups in contrast to the groups without curcumin($P<0.05$). Serum and salivary correlation analysis revealed a strong and highly significant correlation for MDA, vitamin C & E and 8 dihydro-2 deoxyguanosine (8-OHdG) in the conditions before, during and in the recovery periods in both bed rest conditions. Since saliva collection is easy and non-invasive, measurements of salivary marker levels may prove to be useful in the space research. **Conclusions:** Curcumin prevents peroxidative damage in both bed rest conditions. Further study is required on antioxidation actions of curcumin in space microgravity conditions.

1. Introduction

Current projects on Mission to Mars result in 2 years of microgravity condition, which 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 results of reduced metabolic activities, lower cellular and tissue oxygen demand[1-8]. There exists a balance in the body between oxidant production and antioxidant defence, with the balance shift slightly in favour of oxidants[1-3]. Main products of this "leakage" are two ROS: superoxide radical (O^-_2) and H_2O_2[2]. Other ROS includes the free radicals such as nitric oxide and compounds e.g. 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[2-6]. The chronic diseases range from oral diseases such as periodontitis and oral cancer to cardiovascular diseases and neuro-degenerative diseases including Alzheimer and Parkinson diseases[9]. 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[10]. 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

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long-duration space flights (90 to 180 d)[11]. Isoprostane 8-isoprostaglandin F2- and 8-OHdG are the markers for oxidative damage to lipids and DNA respectively[12]. Most rodent studies showed increased production of lipid peroxidation products and decreased antioxidant enzyme activity in postflights[10]. 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 malondialdehyde concentration of liver with space flights[1]. Vitamin E is the primary chain-breaking anti-oxidant in the cell membranes[9]. 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[13-18].

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 researches over the past half-decade have confirmed that curcumin mediates anti-inflammatory effects through the downregulation of transcription factor, nuclear factor-κB (NF-κB), 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[13]. It has been reported that curcumin act as anti-oxidant agent. Curcumin has been administered safely at a oral dose of up to 8g/d. There was no dose-limiting toxicity; dosing was limited by the number of pills that patients could or would swallow daily[14]. Hence, this study was planned to investigate the effects of curcumin on serum and salivary markers of oxidative stress in conditions of two forms of bed rest.

2. Materials and methods

The subjects of this investigation were 20 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 no systemic endurance training in 10 days prior to study. Each subject was given a detailed explanation of the experimental protocol and 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 diseases, smoking, alcoholic and drugs history. Five volunteers of each HDT and BD were selected and given a curcumin once a day and others five volunteers of each HDT and BD were 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 & BD experiment, and during recovery period. Subjects were asked to be 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 & BD. At 9 A.M. the subjects were transferred supine to a gurney, tilted to 6′ HDT & BD, and remained for 8 hrs. in following 10 consecutive days. After 10 days the subjects returned to a chair and stayed in seated position for 4 hrs recovery period. Blood and saliva samples were prepared at the same time.

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 at 4 ℃ for 5 min to remove bacterial and cellular debris. Saliva samples were stored at −80 ℃ 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 and stored at −80 ℃ until analysis. Serum and salivary levels were assessed for MDA using thiobarbituric acid (TBA) method[12]. Concentrations of both vitamins were measured using liquid chromatography[13]. Quantitative measurement of the oxidative DNA adduct 8-OHdG was performed according to the method described[14]. 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 among before, 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 $P<0.05$.

3. Results

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

P<0.05). Serum and salivary correlation analysis revealed strong and highly significant correlation among MDA, vitamin C & E and 8 dihydro-2 deoxyguanosine (8-OHdG) in both bed rest conditions and in both the groups (r=0.86, r=0.67, r=0.76, P<0.001 & r=0.67, r=0.66, r=0.64, P<0.001 respectively).

Table 1

Salivary and serum MDA, vitamin C & E and 8 dihydro-2 deoxyguanosine (8-OHdG) of 20 normal healthy subjects in period before, during and recovery phase in HDT with and without taking curcumin($\bar{x}\pm s$).

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

A: before HDT without curcumin; B: throughout the time course of the HDT experiment; C: during recovery; AA: before HDT with curcumin; BB: throughout the time course of the HDT experiment; CC: during recovery;*P<0.05, as compared to after condition (C); $^{\#}P$<0.05, as compared to Before condition (A).

Table 2

Salivary and serum MDA, vitamin C & E and 8 dihydro-2 deoxyguanosine (8-OHdG) of 20 normal healthy subjects in period before, during and recovery phase in BD with and without taking curcumin($\bar{x}\pm s$).

Markers		A	AA	B	BB	C	CC
MDA (μ mol/L)	Salivary	0.25±0.08$^{\#}$	0.11±0.03	0.37±0.14$^{*,\#}$	0.15±0.13	0.28±0.19*	0.12±0.16
	Serum	1.25±0.45$^{\#}$	0.78±0.65	1.35±0.41$^{*,\#}$	0.76±0.65	1.23±0.78*	0.78±0.67
Vitamin C (μ g/L)	Salivary	1.02±0.45$^{\#}$	1.23±0.67	0.85±0.47$^{*,\#}$	1.08±0.76	0.94±0.38*	1.09±0.69
	Serum	7.48±1.54$^{\#}$	8.32±2.01	7.06±1.02$^{*,\#}$	8.00±2.01	7.41±1.84*	8.03±2.09
Vitamin E (μ g/L)	Salivary	0.48±0.14$^{\#}$	0.59±0.23	0.35±0.15$^{*,\#}$	0.51±0.23	0.47±0.19*	0.53±0.22
	Serum	8.11±1.08$^{\#}$	8.88±2.13	7.54±1.09$^{*,\#}$	8.23±3.19	8.09±1.01*	8.56±2.56
8-OH dG (mg/L)	Salivary	0.35±0.06$^{\#}$	0.23±0.08	0.41±0.05$^{*,\#}$	0.26±0.08	0.34±0.02*	0.22±0.05
	Serum	2.15±1.26$^{\#}$	1.89±1.43	2.89±1.25$^{*,\#}$	1.98±1.13	2.14±1.26*	1.78±1.23

A: before BD without curcumin; B: throughout the time course of the BD experiment; C: during recovery; AA: before BD with curcumin; BB: throughout the time course of the BD experiment; CC: during recovery;*P<0.05, as compared to after condition (C); $^{\#}P$<0.05, as compared to before condition (A).

4. Discussion

In the present study, serum and salivary vitamin C & E values were significantly lowered in both conditions and both groups (P<0.05) which support the previous studies[14-19]. 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 lower than usual, which concur with the previous studies[10-14]. The 8-OHdG levels were increased in both conditions as observed in the previous studies[15]. Different aspects of oxidative stress are measured by 8-OHG namely DNA damage and cell membrane damage respectively[16]. The increased 8-OHG, MDA levels and decreased vitamin C and E levels were more significant in curcumin groups as compared with the values observed in non-curcumin groups in accordance with the previous studies. Several reports suggest that curcumin can induce ROS[17,18]. There are also reports which suggest that curcumin quenches ROS production and thus acts as an antioxidant. Other reports suggest that curcumin quenches ROS production at low concentrations and induces ROS production at high concentrations[18,19]. It might be like 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-κB-suppressive effects.

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

Conflict of interest statement

We declare that we have no conflict of interest.

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