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SHORT COMMUNICATION

Serum Micronutrient (α-tocopherol, retinol, copper, zinc) Status of Immunosuppressed Rats

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ABSTRACT

Micronutrients are immuno-active and some of the nutraceuticals have been reported to modify immune responses. An attempt was made to investigate the immuno-suppression effects by a nutraceutical- volatile oil of *Nigella sativa* (NSVO). To induce immuno-suppression, animals were challenged with a specific antigen, and then treated with NSVO intramuscularly for 30 days. Serum micronutrients (alpha-tocopherol, retinol, copper and zinc) of immune-suppressed rats were determined. The HPLC was employed to determine the serum concentrations of vitamin E and A, and atomic absorption flame spectrophotometry was used to estimate the concentrations of serum zinc and copper. The SPSS software package (version 10.0) was used to analyse the data. It was found that immuno-suppressed rats showed significant deceases in serum concentrations of retinol (P=0.005) and zinc (P=0.003), when compared with control rats. Serum concentrations of alpha-tocopherol and copper also increased but not significantly. This study revealed that immune suppression might bring about changes in the concentrations of certain serum micronutrients.

INTRODUCTION

Immune suppression induces down-regulation of humoral and cellular immune functions. Immuno-modulating properties of some nutraceuticals have been reported to decrease serum antibody titre, splenocytes, neutrophils etc (Islam *et al.,* 2004, Chisty *et al.,* 1996). It is well reported that micronutrients play a key role in immuno-physiological functions, and their deficiencies may suppress immune responses, while their overload is immunotoxic (Chandra, 1997; 2001; Scrimshaw and San Giovanni, 1997; Shankar and Prasad, 1998). Alpha-tocopherol is an essential constituent of cell membrane including the nuclear and mitochondrial membranes. It has been reported that deficiency of alpha-tocopherol impairs generation of immunoreactive molecules (cytokines, postaglandin), production and secretion of immunoglobulins, expression of surface markers, and causes damage to cell membranes like lymphocytes etc (Bendich, 1988). Vitamin A deficiency results in suppression of phagocytosis, specific antibody response, delayed type of hypersensitivity etc (Watson & Rybski, 1988). Copper and zinc

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are also involved in some key immune functions. Deficiencies of these minerals have been reported to impair production and secretion of immune-regulating constituents (Lukasewycz & Prohaska, 1990; Malave, 1990; Driessen, 1994).

In addition to the immune functions, it is further documented that most of the micronutrients act as the first line of defence against free radical attack and lipid peroxidation (Czernichow & Hercberg, 2001). Alpha-tocopherol, ascorbic acid and b-carotene (pro-vitamin A) are known to be powerful antioxidants. Alpha-tocopherol is the major lipid-soluble antioxidant that protects cells against lipid peroxidation. Ascorbic acid and beta-carotene are quenchers of free radicals including singlet oxygen (Czernichow & Hercberg, 2001). Ascorbic acid also regenerates the vitamin E. Some of the trace elements such as copper, zinc, manganese, selenium also act as antioxidants through their incorporation into specific enzymes. The present study investigated the serum immuno-active micronutrients like α-tocopherol, retinol, copper and zinc in immune-suppressed rats.

MATERIALS AND METHODS

The study was conducted in immune-suppressed rats. Immune-suppression was indicated by down-regulation of serum antibody titre, splenocytes, neutrophils (Islam *et al.,* 2004). The animals were immunised with specific typhoid TH antigen followed by treatment with volatile oil of *Nigella sativa* (NSVO) as reported elsewhere. On completion of immunisation and treatment, the animals were sacrificed to collect the blood specimen. Blood sample was kept undisturbed for at least 60min and was then centrifuged at 3000rmp for 10min. Serum extracted was stored at –20^{O}C for analysis of immune parameters, and micronutrient contents.

Determination of micronutrient concentrations

Reversed phase HPLC (LC-10AD, SHIMADZU, HPLC 1991, Model-7125, Japan) was used for simultaneous determination of retinol and α-tocopherol in the serum as described by Islam *et al.* (2001). The retinol and α-tocopherol were isolated from the serum by liquid-liquid extraction using n-hexane (Merck, Germany), concentrated by evaporation of n-hexane under nitrogen stream and reconstituted with HPLC grade ethanol (Merck, Germany). The reconstituted retinol and α-tocopherol (50μl) was injected into chromatography on a C_{18} shim pack column with methanol:water (95:5) mobile phase. Retinol and α-tocopherol were detected spectrophotometrically at 291nm. To verify the assay accuracy, standard analytes retinol and α-tocopherol (Sigma Chemical Co, St. Louis, USA) were injected for every 10 test samples.

Copper and zinc levels in the sera were determined by an atomic absorption flame emission spectrophotometer (AA-6200 Series, Shimadzu Corporation, Kyoto, Japan) as described by Picciano and Guthrie (1976) using standard copper and zinc (Sigma Chemicals Co, St. Louis, USA). Absorbance of copper and zinc were read at 324.8nm and 213.9nm respectively. To verify the assay accuracy and quality, the standard mineral preparations were run for every 10 test samples. A software package (AA-6200, ver 1.1, Shimadzu Corporation, Kyoto,

Statistical analysis

SPSS software package (version 6.0) was used to analyse the data. Descriptive statistics were calculated for all variables. Values were expressed as percentage and mean±SD.

RESULTS

The immune-suppression induced by NSVO has been indicated by significant reduction in antibody titre, and counts of splenocytes, and neutrophils (Islam *et al.,* 2004; Chisty *et al.,* 1996). It was reported that antibody titre, splenocytes, and neutrophils of immune-suppressed rats were found to be 1280, $3.53x10^7$, and 33.88 $x10^7$ compared to 2560, $5.48x10^7$, and 59.84 $x10^7$ that in the control animals respectively (Islam *et al.,* 2004).

In the present study, serum micronutrients levels are presented in Table 1. Concentrations of serum retinol and α-tocopherol in experimental animals were estimated to be 25.33±14.76 µg/dl and 406.16± 182.89 µg/dl respectively, while these were 36.66±12.70 µg/dl and 297.97±201.50 µg/dl respectively in the controls. Copper and zinc concentrations were 119.64±17.63 µg/dl and 236.26±43.12 µg/dl respectively in experimental rats, and these were 109.73±15.28 µg/dl and 276.72±42.22 µg/dl respectively in the controls. The decreases in serum concentrations of retinol (P=0.005) and zinc (P=0.003) after immuno-suppression were significant. However, the increase in alpha-tocopherol concentration (P=0.052) and copper concentration were not significant (P=0.093).

DISCUSSION

Immune-suppression in the animals had been shown to lead to significant

Table 1. Serum levels of α-tocopherol, retinol, copper, zinc (case=50, control=25)

Micronutrient	Experimental			Control				
in µg/dl	No	%	Mean ± SD	No.	%	Mean ± SD	t value	P value
Retinol								
<20	15	30	25.33 ±14.76	4	16	36.66±12.70	3.11	0.005
20-50	27	54		18	72			
>50	8	16		3	12			
α-tocopherol								
<200	6	12	406.16±182.89	14	56	297.97±201.50	2.04	0.052
200-600	35	70		6	24			
>600	9	18		5	20			
Copper								
<100	03	06	119.64±17.63	8	32	109.73±15.28	1.75	0.093
100-150	40	80		16	64			
>150	07	14		1	04			
Zinc								
< 200	13	26	236.26±43.12	1	04	276.72±42.22	3.25	0.003
201 – 300	31	62		17	68			
>300	6	12		7	28			

Significance P<0.05
Values were expressed in mean ± sd.
Compare means: Independent-samples t-test

decreases in their antibody titre, splenocytes and neutrophils (Islam *et al.,* 2004; Chisty *et al.,* 1996). The effect of the NSVO on the serum immuno-nutrients in the immune-suppressed animals was investigated in this study.

Analysis of serum micronutrients showed that the NSVO treatment significantly ($p<0.05$) decreased serum concentrations of vitamin A and zinc. The decrease or deficiency of vitamin A and zinc has been previously reported to be associated with immuno-suppression (Watson and Rybski, 1988; Cunninghan-Rundles, 1988).

However, the elevation of serum vitamin E level in this study is not in agreement with immuno-suppression result of other workers. It has been documented that α-tocopherol supplementation to mice do not always affect immunosuppressive factors (Bendich, 1988), indicating that the rise of serum a-tocopherol may have no affect on the immune system. The increase in copper level may be associated with the deficiency of zinc and vitamin A (Baura & Ahmed, 1990). It may be further because of reciprocal interaction between serum zinc and copper levels (Turnlund, 1988). This study showed that immune suppression could bring about changes in serum micronutrient content.

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