

Role of Vitamin E & Vitamin C as Antioxidants in Parkinson's disease: A Community Based Study In Urban Areas of Karachi

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ABSTRACT

Objective: Pharmacological studies of vitamin E and vitamin C as antioxidants in patients with Parkinson's disease.

Design of study: Randomized, comparative and categorical study.

Place and Duration of Study: This study was conducted in the Department of Pharmacology, Faculty of Pharmacy, University of Karachi in collaboration with Jinnah Post Graduate Medical College Hospital and Mamji Hospital from April 11, 2010 to August, 2010.

Materials and Methods: On enrollment each patient received complete physical examination and laboratory tests were performed. All registered patients were advised to attend the respective outpatient department (OPDs) every week and at the end of 3rd month at a special counter allocated for the purpose of this study. Evaluation of the subjects was done on Patients Health Questionnaire and Unified Parkinson's disease Rating Scale (UPDRS).

Results: Patients responded to this combination in a very energizing way making them more active, less depressed and motivated. In some patients who were younger around age of 43 (as compared to others ≤ 65 years) diseases free period duration increased to two folds. Some male patients found this combination very aphrodisiac Statistical analysis confirms the promising future of Antioxidants in patients with Parkinson's disease (PD).

Conclusion: Antioxidants in combination must be given to Parkinson's patients in order to improve the quality of life. Especially at the early stages of Parkinson's disease worsening of symptoms can be prevented or prolonged.

Key Words: Oxidative stress, Parkinson's diseases, tremors, vitamin E, vitamin C.

INTRODUCTION

It is an established fact that aging brings many changes at neuronal level which are further progresses in the presence of neurodegenerative diseases like Parkinson's disease, Alzheimer's diseases and lateral sclerosis. These changes may be seen in the form of alteration in calcium homeostasis, ¹ sensitivity of adrenergic, ² and dopamine ³⁻⁶ and opium ⁷⁻⁸ receptors ³. All these changes are further seen in disturbances in motor ⁹⁻¹⁰ and cognitive functions ¹¹. A great deal of research has suggested that these decline result due to the absence of effective antioxidants defense system which increases oxidative stress and it is particularly evident in diseases like Parkinson's ¹² and Alzheimer's disease ⁶⁻¹³.

Parkinson's disease: Parkinson's disease is a movement disorder, though it bears cognitive difficulties¹⁴. It is the second most prevalent neurodegenerative disorder after Alzheimer's disease. In industrialized countries its occurrence is 0.3% in the whole population and increases to 1% in those over 60 years of age and to 4% among individuals 80 years and above¹⁵. The mean age of onset is around 60 years, although 5–10% of cases, classified as young onset, begin between the ages of 20 and 50 years¹⁴. Some studies have proposed that it is more common in men than women, but others have failed to detect any such differences between the two sexes. The basal ganglia which are innervated by the dopaminergic system are the most seriously affected brain areas in PD ¹⁶.

The symptoms of PD arise after the death of cells in pars compacta region of the substantia nigra that produces dopamine¹⁶. The most characteristic pathological finding in PD is a progressive accumulation of Lewy bodies in the substantia nigra and several other brain regions. A diagnosis of Parkinson's disease is usually made based on the medical history and neurological examination. The physician conducts an interview, looking for cardinal motor symptoms, while attending to other possible symptoms that would exclude a diagnosis of PD ¹⁷. Neurofibrillary tangles are present in affected areas of brain while dementia occurs at advanced stage ¹⁸. Parkinson's disease includes motor and non-motor symptoms. Non-motor symptoms include autonomic dysfunction, difficulties in sleep, sensory disturbance, cognitive and behavioral problems (neuropsychiatric). Tremor, rigidity, slowness of movement and postural instability are considered cardinal in PD while tremors are most noticeable. Cognitive symptoms usually appear as the disease progresses but may appear in many cases years before diagnosis of the disease ¹⁷. There is no cure for Parkinson's disease, but medications, surgery and multidisciplinary management can provide relief from the symptoms.

Vitamin C: Vitamin C is a potent antioxidant and works as an electron donor, it is this property which accounts for its most beneficial characteristic. Human

diseases such as atherosclerosis and cancer might occur in part from oxidant damage to tissues.¹⁹

Vitamin C is essential for a healthy diet as it lowers oxidative stress and work as potent antioxidant. It serves as enzyme cofactor and electron donor for important enzymes.²⁰ Ascorbic acid performs numerous physiological functions in the human body. These functions include synthesis of collagen, carnitine and neurotransmitters; the synthesis and catabolism of tyrosine; and the metabolism of microsome.²¹ During biosynthesis, ascorbate acts as a reducing agent by donating electrons and preventing oxidation so as to keep iron and copper atoms in their reduced states. Vitamin C acts as an electron donor for eight different enzymes.²⁰ Vitamin C functions as an antioxidant and is necessary for the treatment and prevention of scurvy, though in nearly all cases dietary intake is adequate to prevent deficiency and supplementation is not necessary.²¹⁻²²

Vitamin E: The specific function of α -tocopherol is to protect long chain polyunsaturated fatty acids and thus maintain their concentrations for important signaling events. Vitamin E is the collective name for a group of fat-soluble compounds with distinctive antioxidant activities. Unshared electrons are highly energetic and react rapidly with oxygen to form reactive oxygen species (ROS). The body forms reactive oxygen species endogenously when it converts food to energy and antioxidants might protect cells from the damaging effects of reactive oxygen species. The body is also exposed to free radicals from environmental exposures, such as cigarette smoke, air pollution and ultraviolet radiation from the sun. In addition to its activities as an antioxidant, vitamin E is involved in immune function, cell signaling, regulation of gene expression and other metabolic processes.²³⁻²⁴ Alpha-tocopherol inhibits the activity of protein kinase C, an enzyme involved in cell proliferation and differentiation of smooth muscle cells, platelets and monocytes. Vitamin-E-replete endothelial cells lining the interior surface of blood vessels and are better able to resist blood-cell components that adheres to this surface. Keeping in view the importance of antioxidants, the study is planned to observe the effects of antioxidants in combinations to patients with Parkinson's diseases.

MATERIALS AND METHODS

The study was conducted in Out Patient Department of Jinnah Post Graduate Medical College Hospital and Maamji Hospital on 25 out of 100 Patients diagnosed with Parkinson's disease. The proposed study was spread over a period of 12 weeks. All the patients fulfilling the following inclusion and exclusion criteria were selected after taking the written consent.

Inclusion Criteria: Male and female outpatients aged between 50-75 years.

The patients must be receiving anti-Parkinson's disease medications confirming earlier diagnosis of PD.

No History of other Neurodegenerative/Motor disease other than PD.

Drugs and vitamins bio availability must be reproducible.

Patient's family members were available for feedback.

History of patient was available in Hospital.

Dietary assessment was possible.

Exclusion Criteria: History of substance abuse/disorder within last 6 months which affects neuronal/motor functions.

Presence of any other neurodegenerative disease other than Parkinson's disease.

Patient who displayed any medical illness that would compromise their safety or interfere with implementations of the protocol or interpretation of study results.

Patients not reachable or out of city residents.

Unreliable answers from patients and family members regarding dietary assessment.

Subject Recruitment: The criteria for diagnoses of PD are the core signs and symptoms, movement-related, including shaking, rigidity, slowness of movement, and difficulty with walking and gait. Cognitive and behavioral problems with dementia are also considered. Other symptoms include sensory, sleep and emotional problems.

The subjects were recruited in OPD of JPMC and Maamji Hospital Karachi with the permission of Head of the departments and Ethical committee. 25 patients were enrolled in this study. All received vitamin C 500 mg once daily and Vitamin E 400 IU capsule once daily which is the standard adult dose.

Table 1: Effects of Vitamin E & Vitamin C on UPDRS Subscale 1.

Sub scores	Possible Range of Scores	Baseline N=29	Three Months after dosage (N=29)	P Value Three Months after dosage Vs. Baseline
Motivation Behavior and Mood: 0-16				
Intellectual Impairment	0-4	1.0±0.0	0.862±0.350	0.0437
Thought disorder	0-4	0.276±0.702	0.138±0.351	0.349
Depression	0-4	1.517±0.509	1.138±0.351	0.002**
Motivation/Initiation	0-4	1.06±0.651	1.138±0.351	0.50

Values are mean + S.D (n=29). Significant difference by Newman-Keuls test.

*p<0.05, **p<0.01, ***p<0.001 compared to baseline, following Student's t-test.

Table 2: Effects of Vitamin E and Vitamin C on UPDRS Sub scale 3

Sub scores	Possible Range of Scores	Baseline N=11	Three Months after dosage N=11	P Value Three Months after dosage Vs. Baseline
Motor Examination:	0-56			
Speech	0-4	1.448±0.506	1.310±0.471	0.287
Facial Expression	0-4	1.552±0.506	2.00±0.535	0.00***
Tremors at Rest	0-4	2.621±0.494	0.276±0.455	0.00***
Action or postural Tremors	0-4	2.172±0.384	1.517±0.738	0.00***
Rigidity	0-4	2.276±0.702	1.828±0.658	0.015***
Finger Taps	0-4	1.832±0.351	1.379±0.494	0.00***
Hand movements	0-4	2.0±0.0	1.379±0.493	0.00***
Rapid Alternative Movement	0-4	1.724±0.455	1.241±0.435	0.00***
Leg agility	0-4	1.862±0.351	1.379±0.494	0.00***
Arising from chair	0-4	1.138±0.351	0.966±0.499	0.134
Posture	0-4	1.586±0.501	1.483±0.509	0.439
Gait	0-4	1.690±0.471	1.552±0.506	0.287
Postural stability	0-4	1.172±0.658	1.138±0.639	0.840
Body Bradykinesia	0-4	1.690±0.471	1.552±0.506	0.287

Values are mean + S.D (n=29). Significant difference by Newman-Keuls test. *p<0.05, **p<0.01, ***p<0.001 compared to baseline, following Student's t-test.

All subjects gave written consent before induction in this study. All subjects were elderly patients above 50 years and conversant in Urdu and willing to be available for participation in this study.

On enrollment each patient received complete physical examination and lab tests were performed.

All registered patients were advised to attend the respective OPDs every week and at the end of 3rd month at a special counter allocated for the purpose of this study.

Evaluation of Subjects: Evaluation of the subjects was done on the basis of

- Patients Health Questionnaire with complete family history and medications.
- Unified Parkinson's disease Rating Scale (UPDRS).

Dietary Assessment: Mini nutritional assessment (MNA) was used to evaluate the risk of malnutrition in the elderly. Evaluation includes questions regarding previous medical illnesses, drug history, musculoskeletal disorders, dementia, and dermatological history, number of family members at home, psychiatric assessment and anthropometric measurement. None of the patient was found to be malnourished or had any signs of vitamin C and vitamin E deficiency.

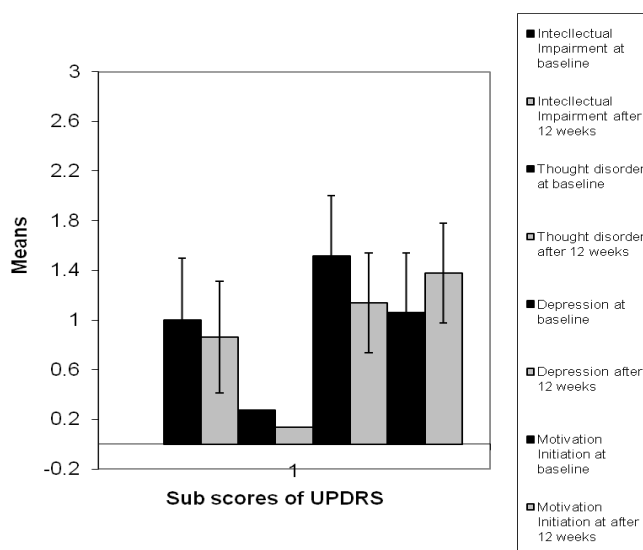
Statistical Analysis: We examined the patient before and after giving antioxidants on UPDRS. Sample t test was applied and charts were drawn using MINITAB

software. Each Subject was compared with his own baseline value and *p-value* was calculated statistically.

RESULT

Effects of Vitamin E& Vitamin C after 2 Months on UPDRS Sub scores

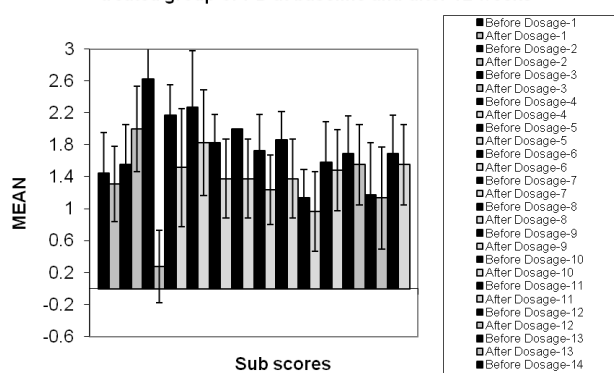
Comparison of Menation ,Behavior and Mood in Vitamin E and Vitamin C treated group of PD at baseline and after 12 weeks



Values are mean + S.D (n=29). Significant difference by Newman-Keuls test. *p<0.05, **p<0.01, ***p<0.001 compared to baseline ,following Student's t-test.

Graph 1: Effects of Vitamin E and Vitamin C on UPDRS Sub scale 1 in PD patients at baseline and after 12 weeks

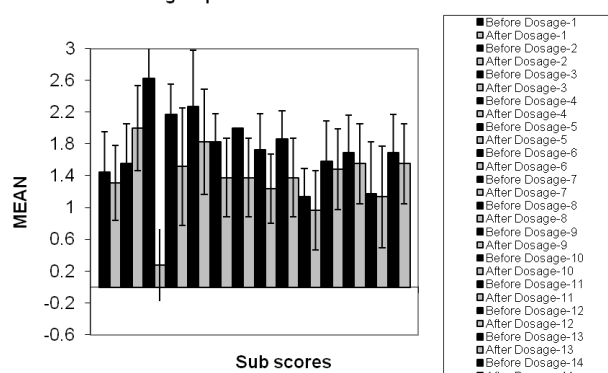
Comparison of Motor functions in Vitamin E and Vitamin C treated group of PD at baseline and after 12 weeks



Values are mean + S.D (n=29). Significant difference by Newman-Keuls test * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to baseline, following Student's t-test.

Graph 2: Effects of Vitamin E and Vitamin C on UPDRS Sub scale 3 in PD patients at baseline and after 12 weeks.

Comparison of Motor functions in Vitamin E and Vitamin C treated group of PD at baseline and after 12 weeks



Values are mean + S.D (n=29). Significant difference by Newman-Keuls test.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to baseline, following Student's t-test.

Graph 3: Effects of Vitamin E and Vitamin C on UPDRS Sub scale 3 in PD patients at baseline and after 12 weeks

Table 3: Effects of Vitamin E and Vitamin C on UPDRS Sub scale 3

Sub scores	Possible Range of Scores	Baseline N=11	Three Months after dosage N=11	P Value Three Months after dosage Vs. Baseline
Motor Examination: 0-56				
Speech	0-4	1.448±0.506	1.310±0.471	0.287
Facial Expression	0-4	1.552±0.506	2.00±0.535	0.00***
Tremors at Rest	0-4	2.621±0.494	0.276±0.455	0.00***
Action or postural Tremors	0-4	2.172±0.384	1.517±0.738	0.00***
Rigidity	0-4	2.276±0.702	1.828±0.658	0.015*
Finger Taps	0-4	1.832±0.351	1.379±0.494	0.00***
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Gait	0-4	1.690±0.471	1.552±0.506	0.287
Postural stability	0-4	1.172±0.658	1.138±0.639	0.840
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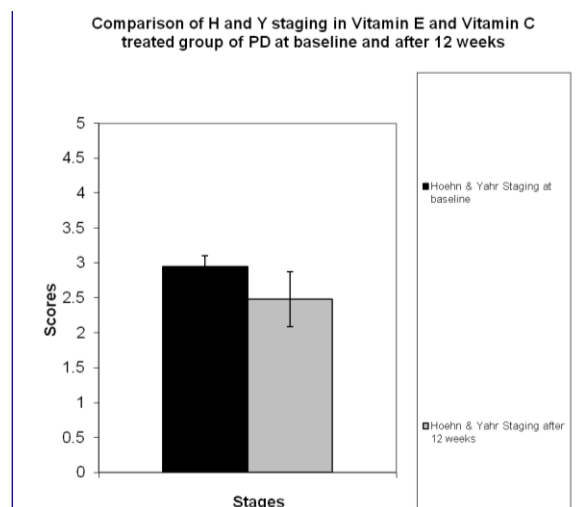
Values are mean + S.D (n=29). Significant difference by Newman-Keuls test * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to baseline, following Student's t-test.

Table 4: Effects of Vitamin E and Vitamin C on UPDRS Sub scale 4 (Hoehn and Yahr staging and Sub scale 5 (Schwab and England Activities of daily living ADL)

Scale	Possible Range	Baseline N=29	Three Months after dosage (N=29)	P Value Three Months after dosage Vs. Baseline
Hoehn&Yahr Staging	0-5	2.948±0.155	2.483±0.389	0.00***
Schwab & England activities of daily Living scale	0%-100 %	52.8±11.6	57.9±10.8	0.085

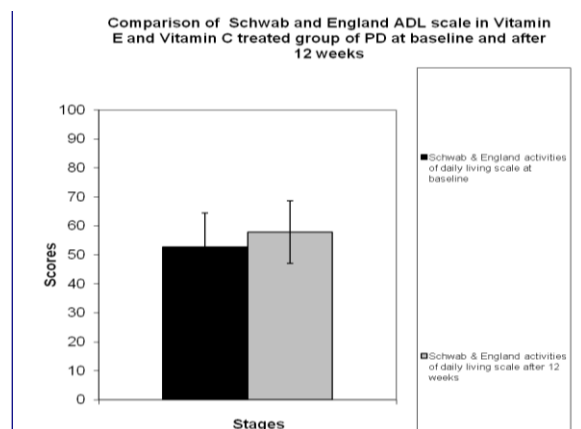
Values are mean + S.D (n=29). Significant difference by Newman-Keuls test.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to baseline, following Student's t-test



Values are mean + S.D (n=29). Significant difference by Newman-Keuls test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to baseline, following Student's t-test

Graph 4.1: Effects of Vitamin E and Vitamin C on UPDRS Sub scale 4 (Hoehn and Yahr staging) in PD patients at baseline and after 12 weeks



Values are mean + S.D (n=29). Significant difference by Newman-Keuls test * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to baseline, following Student's t-test

Graph 4.2: Effects of Vitamin E and Vitamin C on UPDRS Sub scale 5 (Schwab and England Activities of daily living) in PD patients at baseline and after 12 weeks.

DISCUSSION

It has been proved that an oxidative stress brings apoptosis in almost all cells of the body²⁵ due to high levels of free radicals which causes damage to lipid membrane especially long chain fatty acids.²⁶ We have used two different vitamins yet most potent antioxidants having synergistic effects on each other.²⁷ Both antioxidants have been used in combination in

patients suffering from Parkinson's disease. Up till now no study has ever been conducted in urban areas of Karachi which has used this combination in patients suffering from Parkinson's disease in their different stages.

It was found that patients responded to this combination in a very energizing way making them more active, less depressed and motivated. In some patients who were younger i.e. around age of 43 years as compared to others who were around 65 years, their disease free period duration increased by two folds. Some male patients found this combination very aphrodisiac thus resulting in increased libido.

In a study by Etminan et al, it was observed that there was a decrease in the level of vitamin E in PD patients as compared to control individuals which is compatible with the results of a meta-analysis study, suggesting a neuroprotective effect of dietary vitamin E in attenuating the risk of PD²⁸ and same can be applied to other antioxidant vitamins.

As all of the patients were living in urban areas of Karachi, thus inhaling polluted environment on regular basis which can result in increased oxidative stress²⁹⁻³⁰ as pollution is a powerful source of oxidants and causes aging or death of brain cells even in normal healthy individuals. If the patients have PD and they are living in such conditions where there is high exposure to oxidants from air then one can imagine the degree of worsening of symptoms by two folds and above all worsening of symptoms in patients with neurodegenerative diseases. The statistical analysis confirms the promising future of antioxidants in patients with PD.

CONCLUSION

Antioxidants must be given in combination to Parkinson's patients especially in the early stages of their disease in order to improve their quality of life and to prevent worsening of their disease.

REFERENCES

1. Landfield PW, Elbridge JC. The Glucocorticoid hypothesis of age related hippocampus neurodegeneration: Role of dysregulated intraneuronal Ca²⁺. Ann NY Acad Sci 1994; 746: 308-321.
2. Golub TJ, Bickford P. Age related deficits in the cerebellar beta adrenergic signal transduction cascade in Fischer 344 rats. J Pharmacol Exp Ther 1997; 281:965-971.
3. Joseph JA, Kowatch MA, Muki T, Roth GS. Selective cross activation /inhibition of second messenger system and the reduction of age related deficits in the muscarinic control of dopamine release from the perfused rat striata. Brain Res 1990; 537:40-48.

4. Akayama F, Egashira T, Ymanaka Y. Effects of bifemelane on muscarinic receptors and choline acetyltransferase in the brains of aged rats following chronic cerebral hypo perfusion induced by permanent occlusion of bilateral carotid arteries. *Jpn J Pharmacol* 1996;72:57-65.
5. Yu Fu, Egashira T, Ymanaka Y. Age-related changes of cholinergic markers in the rat brain. *Jpn J Pharmacol* 1994;66:247-255.
6. Yu BP. Cellular defense against damage from reactive oxygen species. *Physiol Rev* 1994;76: 139-162.
7. Kornhuber J, Schoppmeyer K, Bendig C, Riederer P. Characterization of [3H] pentazocine binding sites in post-mortem human frontal cortex. *J Neural Transm* 1996;103:45-53.
8. Nagahara AH, Gill T M, Nicolle M, Gallagher M. Alteration in opiate receptors binding in the hippocampus aged Long evans rats. *Brains Res* 1996;707:22-30.
9. Joseph JA, Bartus RT, Clody DE, Morgan D, Finch C, Beer B, et al. Psychomotor performance in the senescent rodent: reduction of deficits via striatal dopamine receptors up-regulation. *Neurobiol Aging* 1983;4:313-319.
10. Kluger A, Giamutsos JG, Golomb J, Ferris SH, George AE, Frannssen E, et al. Patterns of motor impairment in normal aging mild cognitive decline and early. *Alzheimer's Disease Gerontol* 1997; 52:28-39.
11. Bartus RT. Drugs to treat age related neurodegenerative problems. The final frontier of medical science? *J Am Geriat Soc* 1990;38: 680-695.
12. Jenner P. Oxidative Stress in Parkinson's disease and other neurodegenerative disorders. *Pathol Boil* 1996;44:57-64.
13. Finch CE, Cohen DM. Aging metabolism and Alzheimer's disease: Review and hypothesis. *Exp Neurol* 1997;143:82-102.
14. Sami A, Nutt JG, Ransom BR. Parkinson's disease. *Lancet* 2004;363 (9423):1783-93.
15. De Lau L M, Breteler MM. Epidemiology of Parkinson's disease. *Lancet Neurol* 2006;5(6): 525-35.
16. Obeso JA, Rodríguez- Oroz MC, Benitez-Temino B, Blesa FJ, Guridi J, Marin C, et al. Functional organization of the basal ganglia: therapeutic implications for Parkinson's disease. *Mov Disord* 2008;23(3): S548-59.
17. Jankovic J. Parkinson's disease: clinical features and disease. *J Neurol Neurosurg Psychiatr* 2008; 79(4):368-76.
18. Galpern WR, Lang AE. Interface between tauopathies and synucleinopathies: a tale of two proteins. *Ann Neurol* 2006;59(3):449-58.
19. Sebastian JP, Katz A, Wang Y, Eck P, Kwon O, Je-Hyuk Lee, et al. Vitamin C as an Antioxidant: Evaluation of Its Role in Disease Prevention. *J Am Coll Nutr* 2003; 22(1):18-35.
20. Levine M, Rumsey SC, Wang Y, Park JB, Daruwala R. Vitamin C. In: Stipanuk MH, editor. *Biochemical and physiological aspects of human nutrition*. Philadelphia: WB Saunders; 2000.p. 541-67.
21. Shenkin A. The key role of micronutrients. *Clin Nutr* 2006;25(1):1-13.
22. Woodside J, McCall D, McGartland C, Young I. Micronutrients: dietary intake v. supplement use. *Proc Nutr Soc* 2005;64 (4):543-53.
23. Traber MG. Vitamin E. In: Shils ME, Shike M, Ross AC, Caballero B, Cousins R, editors. *Modern Nutrition in Health and Disease*. 10th ed. Baltimore, MD: Lippincott Williams & Wilkins; 2006.p.396-411.
24. Vitamin E. Dietary Reference Intakes: Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington, DC: The National Academies Press; 2000;p.195-196.
25. Ventura C, Maioli M. Protein kinase C control of gene expression. *Crit Rev Eukaryot Gene Expr* 2001;11(1-3):243-267.
26. Traber MG, Atikson J. Vitamin E, oxidation and nothing more. *Free Radic Biol Med* 2007;43(1): 4-15.
27. Eitenmiller R, Lee J. Vitamin E. In: Eitenmiller R, Lee J, editors. *Vitamin E: Food Chemistry, Composition, and Analysis*. New York: CRC Press; 2004.p.43-45.
28. Etminan M, Gill SS, Sami A. Intake of vitamin E, vitamin C, and carotenoids and the risk of Parkinson's disease: a meta-analysis, *Lancet Neurol* 2005;4:362-365.
29. MacNee W, Rahman I. Is oxidative stress central to the pathogenesis of chronic obstructive pulmonary disease? *Trends Mol. Med* 2001;7:55-62.
30. Bowler RP. Oxidative stress in the pathogenesis of asthma. *Curr Allergy Asthma Rep* 2004;4:116-122.

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