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Effect of ACE Plus Selenium on Total Antioxidant/Oxidant Capacity and Nitric Oxide Levels in Rabbits

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Abstract: Pills including vitamins and minerals have been used as part of a sedentary lifestyle, malnutrition, aging, and age-related illnesses in developed countries. This study specifically focuses on the effect of ACE Plus Selenium given rabbits through intraperitoneal (i.p) injection on their total oxidant, antioxidant capacity (TOC, TAC), and nitric oxide (NO) levels. In this study, 0,5 ml/kg of normal saline was injected into rabbits in the control group and 0,5 ml/kg of ACE Plus Selenium was conducted to the treatment group twice (every other day) via i.p. route. Following the injection, plasmas of blood samples obtained in the second and fourth days were separated, and stored at –20°C until the analysis. Plasma TAC, TOC and NO levels were determined spectrophotometrically. The TOC, TAC and NO levels of the rabbits were compared to the control group statistically. While TOC levels were observed to decrease ($p<0,05$) on the fourth day, the NO levels increased ($p<0,01$) on the second day and became normal on the fourth day. Further no statistical alteration was observed in the TAC levels. As a result, it can be concluded that ACE Plus Selenium had no effect on TAC level, it may decrease TOC levels in parallel to the decline in oxidative stress; further, it can increase NO levels acutely as a result of α-tocopherol and ascorbate ingredients.

Keywords: Multivitamin, Oxidative stress, Antioxidant, Nitric oxide

1. INTRODUCTION

There is increasing evidence that oxidative stress plays a causal role in cancer, cardiovascular and neurological diseases, and aging-related disorders when free radicals are over-produced and/or insufficiently eliminated (Phaniendra et al., 2015; Deveci 2017; Deveci 2018). Free radicals formed as a consequence of the normal metabolism of the cell or by various external factors (food, ionizing radiation, etc.) are neutralized by enzymatic antioxidants such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) or by non-enzymatic antioxidants such as A, C, E vitamins, alpha-lipoic acid, ubiquinone and flavonoids (Mercan, 2004; Urso and Clarkson, 2003; Deveci 2019). Oxidants and antioxidants

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are stable in living organisms and any shift in this stability in favor of oxidants causes oxidative stress which is believed to play a causal role in aging and tissue damage associated with various diseases such as cancer, rheumatoid arthritis, Alzheimer's and Parkinson's (Phaniendra et al., 2015; Nur 2017; Deveci 2018). Although NO synthesized from L-arginine by catalysis of nitric oxide synthase (NOS) enzyme is not very reactive, it forms intermediates with damaging effects on biomolecules. Aside from its function as a neurotransmitter and an antioxidant and its role in the regulation of blood pressure, NO also plays a role in ischemia-reperfusion injury, chronic inflammatory bone disease and neurodegenerative diseases when overproduced (Ozcan and Ogun, 2015). The use of antioxidant vitamins such as ascorbic acid, α-tocopherol and β-carotene has become an attractive strategy for reducing the risk of oxidative damage-induced illnesses (Wei et al., 2006) and numerous natural or synthetic antioxidants referred to as exogens are believed to have positive effects on health and disease prevention (Sivoňová et al., 2006).

According to various epidemiological studies, it is suggested that a diet rich in antioxidants may be a strategy for preventing oxidative stress-related diseases (Tabart, 2009). The amount of vitamins and minerals received from foods alters as a result of changes in nutrition patterns of people due to climatic and ecological differences, and cultural and socio-economic factors (Report of a Joint FAO/WHO expert consultation, 1998). Today, especially in the developed countries, people have appealed to supplements that contain both vitamins and minerals owing to their concerns about sedentary lifestyle, malnutrition, aging and aging-related diseases (Fletcher et al., 2002). It is reported that in the United States about seven out of ten Americans spend $ 4 billion every year on products containing 3500 different vitamins and minerals (Erden and Tanyeri, 2004) and that the food supplement market in Europe is expected to grow 9.5% accounting for 7.9 Billion euros by 2020 (Ergen and Bozkurt, 2016).

Given that oxidative stress, which is the balance between pro-oxidative and antioxidative processes, is the cause or consequence of many diseases, oxidant/antioxidant capacity estimation may be important for the health of individuals. Despite the availability in the literature of studies on multivitamins and mineral supplements concerning diseases, there is no study examining the effect of multivitamins and mineral supplements on TAC, TOC and NO levels. This study was conducted to evaluate the effects of ACE Plus Selenium on TOC, TAC and NO levels in rabbits.

2. MATERIAL AND METHOD

The study was confirmed by the Ethics Committee of the Animal Experiments of Kafkas University (Decision no. KAU-CAE / 2012-87). The study was conducted on 18 New Zealand rabbits (*Oryctolagus cuniculus*) with an average live weight of 3.45± 0.4 kg and 14 to 20 months of age. The rabbits were divided into two groups: Control group (n=9) and ACE Plus Selenium Experiment group (n=9). The control group was applied 0.5 ml kg^{-1} normal saline while the treatment group was applied 0.5 ml kg^{-1} ACE Plus Selenium (Provitamin A 20 mg, Vitamin C 200 mg, vitamin E 200 mg, 50 µg selenium) i.p twice every other day. Blood samples were taken from the rabbits on days 2 and 4 following the injection. Their plasma was separated and stored at -20 °C until analysis. TAC and TOC levels in the samples were measured with Rel Assay Diagnostics Assay (Gaziantep-Turkey, Catalog No. RL0017, RL0024) commercial kits developed by Erel (2004; 2005) while NO levels were measured using a spectrophotometer depending on the method suggested by Miranda et al. (2001).

Statistical analysis of the data of the study was evaluated using the SPSS.16 (SPSS 16, USA) package program. Means between the groups were determined by one-way analysis of variance (ANOVA) and differences between the groups were detected by the Tukey test. The results were presented as; mean (±) and standard error (x ± Sx).

3. RESULTS

There was a statistically significant decrease ($p<0.05$) in TOC levels of the experiment group on day 4, and a statistically significant increase in NO levels on day 2, and a decrease back to the normal NO levels on day 4. There was, however, no statistically significant difference in TAC levels between the two groups (Table 1).

Table 1. Plasma TAC, TOC and NO values of ACE Plus Selenium-given rabbits

Parameter	Control	ACE Experimental		P-value
		Day 2	Day 4	
TAC (mmol Trolox Equiv. L^{-1})	0.59 ± 0.04^{a}	0.71 ± 0.06^{a}	0.69 ± 0.05^{a}	ns
TOC (µmol H_2O_2 Equiv.L^{-1})	9.17 ± 1.09^{a}	7.49 ± 0.61^{ab}	6.14 ± 0.58^{b}	*
NO (µmol L^{-1})	17.88 ± 2.28^{b}	27.34 ± 1.73^{a}	21.13 ± 1.75^{b}	**

***:** Differences in the same line are statistically significant ($p<0.05$), **: Differences in the same line are statistically significant ($p<0.01$), ns: Differences in the same line are statistically insignificant

4. DISCUSSION AND CONCLUSION

In vivo, the position of each compound constitutes a substantial factor in preventing the cell from oxidative damage. Being more lipophilic, β- carotene is located in the interior of the membranes and lipoproteins; α- tocopherol is located in the vicinity of the membrane and water interface despite its lipophilic nature while ascorbic acid is located in the extracellular matrix and hydrophilic parts of the cells. Thus, ascorbic acid constitutes the first line of defense (Zhang and Omaye, 2001).

Antioxidant vitamins are thought to be more effective when used together than when used individually. For example, a study using a membrane model suggested that the combination of β-carotene and α-tocopherol inhibited radical-induced lipid peroxidation and that this inhibition was more pronounced when the two compounds were used together than when used individually (Palozza and Krinsky, 1992) while another study suggested that ascorbic acid when added to the test environment, protected low-density lipoprotein (LDL) from oxidation synergistically together with β-carotene (Packer, 1993).

There is no precise information regarding the contributions of multivitamin and mineral supplements to prevent diseases and protect health. In some studies, β-carotene has been reported to significantly increase the incidence of lung cancer (ATBC Study Group, 1994), and it has been claimed that E, C and partially high-dose supplementation of vitamin A increases the risk of mortality and therefore does not have any benefit (Bjelakovic et al., 2015). Yet, there are some objections to these arguments that they cannot lead to the same effects in healthy individuals due to disease conditions (inflammation, etc.). For example, it has been suggested that iron is released from ferrite during inflammation and sepsis (Biemond et al., 1984) while vitamin C reacts easily with Fe^{+3} and forms ascorbyl radical and Fe^{+2} which in turn reacts with H_2O_2 and generates the $HO^{\cdot}$ radical which is extremely harmful to biomolecules (Childs et al., 2001).

Studies in volunteers suggested that multivitamins and mineral supplements decreased aging-related oxidative DNA damage (Ribeiro et al., 2007) and when used together with fish oil, reduced F_2- isoprostane level as a parameter of oxidative stress (Pipingas et al., 2015). A recent study reported that the combined use of vitamin E and selenium nanoparticles increased cock sperm quality and significantly reduced the amount of lipid peroxidation after freeze-thawing (Safa et al., 2016). Henning et al. (2000) observed that 3 weeks of multivitamin and mineral supplement use did not change the plasma antioxidant capacity in young volunteers.

This study shows that ACE plus Selenium does not affect TAC levels while leads to a statistically significant decrease in TOC levels on day 4. It has been concluded that the reduction

in TOC levels may be because of the synergistic effect of antioxidants in the content of multivitamins and mineral supplements used in the study (Palozza and Krinsky, 1992; Packer, 1993).

It is stated that NO formation also depends on tetrahydrobiopterin (BH_4) which is one of the intracellular cofactors and a reduction in BH_4 leads to short-term endothelial dysfunctions, however, BH_4 support can increase cellular NO formation and restore endothelium-dependent relaxation (Ignarro, 2010). It is showed that BH_4 deficiency may be related to oxidative stress in the vascular system; and ascorbic acid may protect BH_4 from oxidative stress (dUscio et al., 2003), and increase NO synthesis by regressing the neutral trihydrobiopterin generated by the radical reactions back to BH_4 (Patel et al., 2002).

Other studies showing the interaction between NO and antioxidants report that NO protected α-tocopherol from oxidation and inhibited the lipid peroxidation process in liposomes together with α-tocopherol (Rubbo et al., 2000); and recovered UV-A induced cell damage in fibroblast cells together with ascorbic acid (Oplander et al., 2007).

The statistically significant increase in the amount of NO on day 2 and then the decrease back to the normal level observed in this study may be due to α-tocopherol and ascorbic acid (Patel et al., 2002; dUscio et al., 2003; Heller et al., 2004).

It has been concluded that ACE Plus Selenium does not have any effect on TAC levels, and may result in a decrease in TOC levels due to reduced oxidative stress and may acutely increase NO levels owing to its α-tocopherol and ascorbate content.

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