

# Effect of Vitamin C and E Co-Administration on Lipid Profile and Hematological Parameters in Healthy Wister Albino Rats

<sup>1</sup>C.C. Nweze, <sup>2</sup>A.E. Uzoukwu, <sup>3</sup>M.H. Abdullahi and <sup>1</sup>N.O. Rasaq

<sup>1</sup>Department of Biochemistry and Molecular Biology, Nasarawa State University, Keffi, Nigeria <sup>2</sup>Department of Food Science and Technology, Federal University of Technology, Owerri, Nigeria <sup>3</sup>Department of Biochemistry, Bayero University, Kano, Nigeria

## Abstract

This study investigated the significance of varying combination of vitamin C and E on lipid profile and hematological parameters in healthy Wister albino rats. To achieve this, fifteen (15) healthy female adult albino rats weighing between 225-230g were procured and grouped into three (3) experimental groups of five (5) animals each. Group A animals received (100IU/g) of vitamin E with 60mg/kg of vitamin C, Group B received (100IU/g) of vitamin E with 30mg/kg of vitamin C while group C which served as the control received normal rat chow respectively for six (6) weeks. The result obtained showed a significant ( $p < 0.05$ ) increase in HDL in the test groups A and B compared to the control which was dose dependent, this was more prominent in group A. Although, there were also non significant decrease ( $p > 0.05$ ) in serum cholesterol (CHO), triglyceride (TG) and LDL- levels. Also, from the result, it was observed that antioxidant supplementation brought about a non significant decrease ( $p > 0.05$ ) in the levels of HGB, RBC, PCV and WBC of the test groups A and B compared to the control and it was also dose dependent with group A showing more prominence. In conclusion, the synergistic potential of antioxidant vitamins combination appears beneficial in the management of cardiovascular disease and blood-related infections.

**Keywords:** antioxidants, nutritional supplementation, lipid profile. haematology

## Introduction

It is a known fact that adequate Vitamin C intake helps in the management and treatment of cardiovascular diseases, cancer and diabetes mellitus (Frei, 2003). Food sources with varying vitamin C content include fruit juice, orange juice, tomato, sweet red pepper and broccoli. Vitamin C refers to a number of vitamers that have vitamin C activity in animals, including ascorbic acid and its salt and some oxidized form of the molecule like dehydroascorbic acid. Vitamin E either as alpha or gamma tocopherol has been suggested as a potent antioxidant. Clinical studies carried out by researchers have implied the function of vitamin E in

the treatment of Alzheimer's disease (Sano, *et al.*, 1997). It also helps stop atherosclerosis (Saloneu, 2003).

Reports had shown that vitamin C and vitamin E co-administration result in a synergistic interaction. Vitamin C was observed to be useful in regenerating vitamin E (Saloneu, 2003). Vitamin C and vitamin E co-administration have been reported to ameliorate the cellular damage caused by free radical formation and hence oxidative damage. Alfalfa sprouts, avocado, bee pollen, carrot, lemon and garlic. Antioxidant vitamins prevent lipid peroxidation both *in vivo* and *in vitro* (Gey *et al.*, 1993; Reaven, *et al.*, 1993). Numerous epidemiological evidences support the beneficial role of the dietary antioxidant vitamins (Donaldson, 1982; Hodis, *et al.*, 1995; Stephens, *et al.*, 1996). However, some studies have questioned the beneficial role of antioxidant vitamins (Gazano, *et al.*, 1995; Zhang, *et al.*, 1997). Vitamins are ideal antioxidants to increase tissue protection from oxidative stress due to their easy, effective and safe dietary administration in a large range of concentration without harmful side effect (Cadenas and Packer, 2002). Multivitamins and minerals have been found to be associated with lower body weight, body mass index and fat mass (Stephens, *et al.*, 1996).

### **The paper is aimed**

The aim of this paper was to establish the effect of vitamin C and E co-administration on lipid profile and hematological parameters of healthy Wistar albino rats

## **Material and Methods**

### **Experimental Animals**

A total of 15 female adult albino rats weighing (225--230)g were obtained from National Veterinary Research Institute (NVRI), Vom near Jos, Plateau state of Nigeria. The animals were randomly assigned into three study groups of 10 animals each. Each study group was individually housed in a stainless steel cage with plastic bottom grid and a wire screen top.

### **Animal treatment and study**

The animal rooms were adequately ventilated and kept at room temperature with a 12-hour natural light-dark cycle. Good hygiene was maintained by constant cleaning and removal of faeces and spilled feed from cages daily. All the animals in the group were fed with Rat chows for first two weeks for acclimatization before commencing the antioxidant vitamins supplement to various groups i.e. group (A and group B, while group C serve as control) for six (6) weeks

**Group A** animals were fed with (1001u/g) of vitamin E and 60mg/kg of vitamin C administered to the rats through water and feed respectively. **Group B** animals were fed with (1001u/g) of vitamin E and 30mg/kg of vitamin. This lasted for a period of 40 days and the experiments were conducted between the hours of 09.00 am and 10.00am daily. **Group C** animals which served as the control were fed with only with normal rat chow and water.

### **Preparation of Blood Serum and Assays**

Twenty four hours after the administration of the last dose of dietary supplements on test groups and control respectively, the animals were sacrificed by inhalation of an over dose of chloroform. Blood samples were collected by cardiac puncture into sterilized sample test -tubes, serum samples were then collected into plain container after centrifugation at 3000 rpm for 5 minutes and used for serum lipid analysis.

**Assay of Lipid Profile:** Component lipids were estimated using enzymatic colorimetric diagnostic kits obtained from Randox Laboratories, Antrim, United Kingdom BT 294 QY.

### **Total cholesterol estimation**

The estimation of serum cholesterol was done by end point method of (Allain *et al*, 1974).

$$\text{Conc. of cholesterol in sample} = \frac{\Delta A \text{ sample} \times \text{conc. of standard}}{\Delta A \text{ standard}}$$

Where  $\Delta$  = absorbance

### **Triacylglycerol Estimation (TG)**

The estimation of triglycerides was done by end point method of McGowan *et al.*, (1983).

$$\text{Triglyceride concentration} = \frac{\text{Absorbance of Sample} \times 200}{\text{Absorbance of Standard}}$$

### **High density lipoprotein Estimation (HDL)**

The estimation of serum HDL was done by precipitation method of Lopez-Virella *et al.* (1977).

$$\text{HDL-Cholesterol in mg/dl} = \frac{\text{Absorbance of Sample} \times 200}{\text{Absorbance of Standard}}$$

Factor of 200 was used instead of 50 for calculation due to serum dilution during precipitating step.

### **Low density lipoprotein-cholesterol Estimation (LDL-CHOL)**

The estimation of LDL-cholesterol was determined by using formula of Friedwald *et al.* (1972-).

$$\text{LDL-cholesterol} = \text{Total cholesterol} - (\text{TG}/5 + \text{HDL})$$

### **Assay of hematological parameters:**

Red blood count, total white blood cell count, platelet count, total hemoglobin and packed cell volume (PCV) were determined using fully automated hematology analyzer (Pentra-XL 80, Horbia ABX, USA).

### **Statistical analysis**

Data collected were expressed as mean  $\pm$  standard deviation (SD) and the Student's T test was used for analysis. Values of  $P < 0.05$  were regarded as significant.

### Result and Discussion

**Table 1:** Showing the results of the effect of oral administration of vitamin C and E on lipid profile

Study Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-CHOL (mg/dl)	LDL-CHOL (mg/dl)
A	208.8±7.33	168.6±4.22	43.6±2.07	131.48±6.58
B	225.2±6.48	180.6±4.22	38.0±1.58	151.08±5.08
C	250.4±7.83	242.6±7.27	30.0±1.58	171.88±6.58

Values are expressed as mean±SD. values of (p<0.05) are considered significant.

**Table 2:** Showing the results of the effect of oral administration of vitamin C and E on some hematological parameters

Study groups	PCV (%)	WBC (10 <sup>9</sup> /L)	HGB (g/l)	RBC (10 <sup>12</sup> /L)
A	53.4±4.63	2.35±0.22	17.4±3.30	6.17±0.30
B	51.2±4.20	2.45±1.18	16.28±0.97	5.92±0.21
C	23.1±8.01	2.68±0.16	16.01±6.00	5.46±0.89

Values are expressed as mean± SD. values of (p<0.05) are considered significant

From the result, vitamin A and E treated groups showed a non-significant (p>0.05) decrease in the level of cholesterol when compared to the control as revealed by the mean values for group A, B and C (208.8± 7.33, 225.2± 6.42 and 250.4 ±7.83) and the decrease was dose dependent with group A showing more prominence. Also, the triglyceride (TG) and the low density lipoprotein (LDL-CHOL) levels of the groups A and B were decreased, though not significantly (p>0.05) as revealed by their mean values when compared to the control. (168.6±4.22, 180.6±4.22, and 242.6±7.27) and (131.6±6.58, 151±5.01, and 171.9±6.6) respectively. This is mainly due to the antioxidative potentials of the vitamins and the synergistic effect of their co-

administration. This is also in line with Esterbaue*et.al.*, (1987) who reported that lipid peroxidation starts only after the depletion of natural antioxidant, in the last 2 decades, many basic, clinical and epidemiological research data have suggested a potential protective effect of antioxidants nutrients such as betacarotene, vitamin C and vitamin E on the risk of cancer and cardiovascular diseases (Sies*et al.*, 1992, Diplock, 1991, Weisburger 1991; Byers and Pery 1992). Many research data have confirmed the relationship between intake of antioxidant vitamins and trace elements and the risk of pathologies (Block *et al.*, 1992; Stampfer and Rimm, 1993, 1995). Thus, it is plausible to be considered advantageous a combination of these vitamins since individually they produce beneficial effects. Multivitamins and mineral supplementations have been shown to have beneficial effects on lipid profile (Li,*et al.*, 2010). Also from the result, there is a significant increase ( $p < 0.05$ ) in HDL-C in the test groups (A and B) when compared to the control and it was dose dependent with group A 60mg/kg of vitamin C showing more prominence.

The result, there was a non significant ( $p > 0.05$ ) increase in the levels of hemoglobin (HGB), packed cell volume (PCV) and red blood cells (RBC), however, the co-administration of vitamin E and A brought about a non significant ( $p > 0.05$ ) decrease in the levels of WBC which is an indication of the level of immune activation of the experimental animals and the results were also dose dependent. This is not surprising, giving that vitamin C has antioxidative potentials and vitamin E acts mainly has a free radical chain – breaking antioxidants in liposome and cellular membrane (Liu, 1995) antioxidants generally are type of molecules that neutralize harmful free radical, produced through a chain of reaction (Joseph *et al.*, 2009) that damage living cells, spoils food, degrade materials, such as rubber, gasoline, lubricating oil. Antioxidant terminates these chain reactions through the removal of free radical intermediates and inhibition of other oxidation reactions (Sies, 1997). This is why plants and animals maintain complex systems of multiple antioxidants such as glutathione, vitamin C and vitamin E along with some enzymes like catalase, superoxide dismutase and various peroxidases.

### Conclusion

Vitamin combination appears beneficial in the management and treatment of hyperlipidemia and the attendant of cardiovascular diseases and blood-related infection as demonstrated in this study. The potency of vitamin E and vitamin C combination influence HDL-C, LDL-C levels and WBC; however the toxicity of the vitamin E and A combination should be studied since the benefit increases with increasing dose.

## Reference

- Block, G., B. Patterson and A. Subar, (199). Fruit, vegetables, and cancer prevention: A review of the epidemiological evidence. *Nutr. Cancer*, 18: 1-29.
- Byers, T. and G. Perry, (1992). Dietary carotenes, vitamin C, and vitamin E as protective antioxidants in human cancers. *Ann. Rev. Nutr.*, 12: 139-159.
- Cadenas, E. and L. Packer, (2002). *Handbook of Antioxidants*. 2nd Edn., Marcel Dekker Inc., New York.
- cardiovascular disease. *Am. J. Clin. Nutr.*, 62: 1365S-1369S.
- Diplock, A.T., (1991). Antioxidant nutrients and disease prevention: an overview. *Am. J. Clin. Nutr.*, 53: 189S-193S.
- Donaldson, W.E., (1982). Atherosclerosis in cholesterol fed Japanese quill: Evidence for amelioration by dietary vitamin E. *Poult. Sci.*, 61: 2097-2102.
- Esterbauer, H., G. Jargons, O. Quehenberger and E. Koller, (1987). Auto-oxidation of human low density lipoprotein: loss of polyunsaturated fatty acids and vitamin-E and generation of aldehydes. *J. Lip. Res.*, 28: 495-509.
- Frei, B., 2003. To C or not to C. That is the question! *Am Coll. Cardiol.*, 42: 253-255.
- Gaziano, J.M., A. Hatta, M. Flynn, E.J. Johnson, N.J. Krinsky, P.M. Ridker, C.H. Hennekens and B. Frei, (1995). Supplementation with beta-carotene in vivo and in vitro does not inhibit low-density lipoprotein oxidation. *Atherosclerosis*, 112: 187-195.
- Gey, K.F., U.K. Moaer, P. Jordan, H.B. Stahelin, M. Eichholzer and E. Ludin, (1993). Increased risk of cardiovascular disease at suboptimal plasma concentrations of essential antioxidants: An epidemiological update with special attention to carotene and vitamin-C. *Am. J. Clin. Nutr.*, 57: 787s-797s.
- Hodis, H.N., E.J. Mack, L. LaBree, L. Cashin-Hemphill, A. Sevanian, R. Johnson and S.P. Azen, (1995). Serial coronary angiographic evidence that antioxidant vitamin intake reduces progression of coronary artery atherosclerosis. *J. Am. Med. Assoc.*, 271: 1849-1854.
- Joseph, N.M., M. Sabharwal, A. Shashi, A. Mahor and S. Rawal, (2009). *Intl. J. Pharmaceutical Science and Research*, 1: 1.
- Liu, Z.L., (1995). Antioxidant activity of Vitamin E and C derivatives in membrane m In: *Bioradicals detected by ESR spectroscopy*. imeticsystems Ohyanishiguch, H. and Packer, L., Eds. Birkhauser V.B., Switzerland: 259-275.
- Reaven, P.D., A. Khouw, W.F. Beltz, S. Parathasarathy and J.L. Witztum, (1993). Effect of dietary antioxidant combinations in humans. Protection of LDL by vitamin-E but not by beta-carotene. *ArteriosclerThromb*, 13: 590-600.
- Saloneu, R.M., (2003). Six year effect of combined vitamin C and E supplementation on atherosclerotic progression. *Circulation*, 107: 947-953.
- Sano, M., C. Ernestoe, R.G. Thomas, M.R. Klauber, K. Schafer, M. Grundman, P. Woodbury, J. Growdon, C.V. Cotman, E. Pfeiffer, L. Schneider and L.J. Thal (1997). A controlled trial

- of selejihini alpha-tocopherol, or both as treatment for Alzheimers disease. *N. Engl. J. Med.*, 336: 1216-1222.
- Sies, H., (1997). Oxidative stress: oxidants and antioxidants *Exp Physiol.*, 82: 291.
- Stampfer, M.J. and B. Rimm, 1995. Epidemiologic evidence for vitamin E in prevention of Stampfer, M.J. and B. Rimm, (1993). A review of the epidemiology of dietary antioxidants and risk of coronary heart disease. *Can. J. Cardiol.*, 9: 14B-18B.
- Stephens, N.G., A. Parson, P.M. Shields, F. Kelly, K. Cheesman and M.J. Mitchinson, (1996). Randomized controlled trial of vitamin E in patients with coronary disease: Cambridge heart antioxidants study. *Lancets*, 347: 781-786.
- Weisburger, J.H., (1991). Nutritional approach to cancer prevention with emphasis on vitamins, antioxidants, and carotenoids. *Am. J. Clin. Nutr.*, 53: 226S-237S.
- Zhang, S.H., R.L. Reddick, E. Avdievich, L.K. Surles, R.G. Jones, J.B. Reynolds, S.H. Quarfordt and N. Maeda, (1997). Paradoxical enhancement of atherosclerosis by probucol treatment in apolipoprotein E deficient mice. *J. Clin. Invest.*, 99: 2858-2865.
- Li, Y., C. Wang, K. Zhu, R.N. Feng and C.H. Sun, (2010). Effects of multivitamin and mineral supplementation on adiposity, energy expenditure and lipid profiles in obese Chinese women. *Int. J. Obesity*, 34: 1070-1077.