Biochemical Effect of Antioxidant Nutraceuticals and Functional Foods on Hematology and Serum Lipid of Healthy Wister Rats

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Abstract
Scientific Research supports biological activity of antioxidant Nutraceuticals and Functional foods. The study compared effect of these two antioxidants in healthy albino rats of the Wistar strain. Eighteen (18) albino rats of opposite sexes weighing between 150-300g were randomly assigned to three groups of six animals each. The control group- Group C were fed with rat chow (vital feed) and clean drinking water, Group A were fed with nutraceutical supplement consisting of caplets of vitamin C - 60mg/day and B-carotene mixed with vitamin E - vitamin A as β-carotene-12,500IU, vitamin E- 200IU and clean drinking water was also given. Group B were fed with functional foods (Oranges, carrot and soybean) and clean drinking water. Hematological parameters were positively influenced by the vitamin supplementation ($p>0.0\ 5$) and greatest effect was achieved in group B. The result also showed a significant ($P>0.05$) decrease in TC, TG and LDL-C in the antioxidants treated groups compared to the control and a non-significant ($P<0.05$) increase in HDL-C.

Keywords: Antioxidants, Nutraceuticals, Functional Foods, Hematology, Lipid Profile

Introduction

Nutrition science during recent decades has been focused on the detection and understanding of deficiencies. With increasing knowledge of the existence and action of vitamins, specific recommendations were given with the aim of avoiding classical deficiency diseases (Knight, 2000). However, the increasing knowledge about micronutrient including minerals and further compounds like carotenoids, flavonoids, anthocyanin on a molecular level together with result from epidemiological studies open a new and exciting field of nutrition science. Free radicals are known to have effect in the pathogenesis of tissue injury in many diseases which is detrimental to the cell (Devasagayam *et al,* 2003). They produce cellular injury by lipid peroxidation, enzyme inhibition, damage DNA and degradation of structural proteins (Devasagayam *et al,* 2003). The body has multiple defence mechanism against free radicals, these include vitamin A, vitamin E, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), under normal circumstances there is a critical balance between pro-oxidant and antioxidants (Vertuani *et al,* 2004) . The compound that have been studied most extremely are the antioxidants, many potential benefits have been attributed to antioxidant in the form of dietary intake or supplementation. Antioxidant in general may be useful in the prevention of cancer and cerebrovascular diseases, supplementation with vitamin C may be beneficial in the management of asthma and high dietary intake of vitamin E may prevent Parkinson diseases (Ogden *et al,* 2000, Wood-Kaczmar, 2006, Di Matteo and Esposito, 2003). An important field of research today is the control of 'redox' status with the properties of food and food components. However, natural antioxidants present in the diet increase the resistance toward oxidative damages and they may have a substantial impact on human health (Boskou, 2006). The concepts of antioxidants, free radicals, and singlet O_2 species are terms that have been topics of research for decades (Azizan, 2006). Antioxidant compounds play an important role in our body due to their favourable effects on human health. Consumption of foods containing phytochemical with potential antioxidant properties can reduce the risk of human disease (Temple, 2000).Oxidation Chain breaking antioxidants are highly reactive with free radicals and form stable compounds that do not contribute to the oxidation chain reaction (El Diwani *et al*, 2009). In the past five years, the world has witnessed the explosive growth of a multi-billion dollar industry known as nutraceuticals. The term "nutraceutical" combines the word "nutrient" (a nourishing food or food component) with "pharmaceutical" (a medical drug). The word "nutraceutical" has been used to describe a broad list of products sold under the premise of being dietary supplements (i.e. a food) and most times in predispose form with the intention of treatment or prevention of diseases, but for the expressed intent of treatment or prevention of disease (Dzanis,1998).Canada defines functional foods as "ordinary food that has components or ingredients added to give it a specific medical or physiological benefit, other than a purely nutritional effect" In Japan, all functional foods must meet three established requirements: foods should be (1) present in their naturally-occurring form, rather than a capsule, tablet, or powder; (2) consumed in the diet as often as daily; and (3) should regulate a biological process in hopes of preventing or controlling disease (Hardy,2000). These foods contain biologically active substances such as antioxidants that may lower the risks of certain diseases associated with aging (Shibamato *et al*, 2008). Examples of functional foods include fruits and vegetables, whole grains, soy milk, enhanced foods and beverages and some dietary supplements.

Objectives of the study: The study compared effect of the antioxidants on hematology and serum lipid and lipoproteins of healthy wister rats**.**

Methodology

Animal study/treatment: Eighteen healthy adult albino rats of opposite sexes of weight 150-300g, were randomly grouped into three experimental groups of six rats in each group. Group A (control) nutraceutical (Forever living capsules: Vitamin C(60mg/day); β -carotene/ Vitamin E (2,000 mcg of Vitamin A and 10mg) respectively. Group B (Treatment) Functional foods (oranges, carrot, and soyabean). Group C (Placebo) no antioxidant group.

Study design: Animals in the three groups fed on rat chow and water *ad libitum*. Dietary intervention commenced after two weeks of acclimatization. Antioxidant supplementation was for ten weeks.

Preparation of blood samples: Blood samples were collected from jugular veins of sacrificed animals in sterile bottles and 0.1% EDTA bottles, serum was collected from sterile bottles after centrifugation at 3000 rpm for 5 minutes and used for plasma lipid and lipoproteins. While 0.1% EDTA bottle samples were used for hematological analysis.

Sample assays: Lipid profiles were analyzed using reflotron system (cholesterol and high density lipoprotein were analyzed by, Third report of National cholesterol education programme, 2001; Triglyceride was analyzed by GPO-PAD method of Tiez; Low density lipoprotein was Calculated with Chawla, 1999). Hematology (Hemoglobin, White blood cells, Red blood cells, and Platelet counts) were analyzed using fully automated Abaccus Junior hematology analyzer based on Coulter method for counting cells that pass through an aperture.

Statistical calculation

Data collected were expressed as mean $\pm$standard deviation (SD) and the Students T-test were used for analysis. Values of $P<0.05$ were regarded as significant.
Results and Discussion

The result of effect of nutraceuticals and functional food antioxidants on some hematological parameters and serum lipid of healthy Wister rats has been presented in tables 1-2. Statistical analyses of the results showed that the dietary supplements significantly increased ($p<0.05$) the levels of WBC, RBC, PCV and HC Compared with the control. Also there is a significant decrease ($P<0.05$) in the levels of LDL, TC and LDL. However, there is a non significant increase ($p>0.05$) in the level of HDL when compared to the control.

Among most aficans and other part of the world. The use of food supplement is steadily gaining acceptance as a mean of preventing diet related diseases. In this present study, the effects of dietary supplements (nutraceuticals and functional food antioxidants) is observed on lipid profile and some hematological parameters of healthy the treatment brought about a significant increase($p<0.05$) in the levels of WBC,PCV,RBC and HC. A significant increased in catalytic activities of erythrocytes

were found in the treatment groups (A&B) when compared with the control group(C) ($p<0.05$) (Chakra borty *et al.,* 2001). The increase is probably due to an increase in the proportion of red blood cells, and the compensatory mechanism after increased oxidant stress (Filiz *et al.,* 2005). Erythrocytes are protected from oxidative stress by intracellular enzymes such as superoxide dismutase (preventive antioxidant) and several other constituents such as vitamin E, A and C. The highest white blood cell count was recorded in group B (7.44±0.17) and lowest in group C (4.46±0.34).

This therefore suggests that after feeding with the supplement, the leukocytes levels of the test group increases which helps to defend the body against infectious diseases and foreign material (Alberts, 2005). An important part of this defence mechanism is the production of active oxygen and its reactive derivatives (e.g., hydrogen peroxide, hydroxyl group, and singlet oxygen) by NADPH oxidase, an activated specific enzyme system (Babior, 1978; Robinson and Badway, 1995; Kobayashi et al., 1998). Also there is a significant increase ($p<0.05$) in haemoglobin levels of the test groups compared to the control. the increase in the haemoglobin concentration increases it function in the blood by carrying oxygen from the respiratory organs (lungs or gills) to the rest of the body where it releases the oxygen to burn nutrients to provide energy to power the functions of the organism and collect the resultant carbon dioxide to bring it back to the respiratory organs to be dispensed from the organism.

The Packed cell volume level, increased significantly ($p<0.05$) in group B (feed with nutraceuticals) when compared to group A (feed with functional food) and the control group (group C). This implies that, there is an increase in the levels of antioxidants in the test groups when compared to the control after treatment

The result also showed a significant decrease ($p<0.0.5$) in the levels of Total cholesterol (TC), low-density lipoprotein (LDL) and a non significant increase ($p>0.05$) in high density lipoprotein (HDL).This may be due to ability of the vitamins to inhibit the oxidation of HDL even in humans (Hillstrom, 2003). A plausible explanation for the observed effect on serum lipids may be due to the activation of the enzyme 7 α-hydroxylase by vitamin C which enhances the conversion of plasma cholesterol into bile acid hence resulting in a decrease in serum levels of cholesterol. In fact (Mayes, 1996) observed that deficiency of vitamin C and E inhibits 7α-hydroxylase leading to the blockage in bile acid synthesis and accumulation of cholesterol in serum with subsequent atherosclerosis in scorbutic Guinea pigs. The present result agrees with previous reports as documented by (Rezaian *et al*, 2002) who reported that the antioxidant vitamins C and E alone or in combination decreased the serum cholesterol and low-density lipoprotein cholesterol (LDL-C) levels and raised the serum levels of high-density lipoprotein cholesterol (HDL-C).

Also (Chatterjea and Shinde 2002) observed a reduction in serum cholesterol levels in experimental animals administered with vitamin C. It also directly meditates through a rate limiting hydroxylation of side chains, the conversion of cholesterol into steroid hormones as documented by (White *et al*, 1978). The reduction in LDL-cholesterol points to the fact that adequate vitamin C intake can reduce the incidence of atherosclerosis. Anderson *et al,* 1999; Bsoul and Terezhalmy, (2004) noted that animal fed on vitamin C and E had reduced risk of coronary Heart disease. The observed decrease in total cholesterol, and most significantly the ability to lower the levels of the atherogenic predisposing factor (serum – LDL cholesterol) yet

desirably increasing the level of HDL implies that dietary vitamin C on account of its effect on lipid profile may have a protective effect against atherosclerosis.

Conclusion

In conclusion, the treatment of adult albino rats with the vitamin A, vitamin C, and vitamin E together improves the antioxidant status of these rats and protects their organs from the damage resulting from iron overload as compare to the untreated group. Also, treatment with antioxidant vitamins is known to improve the liver functions and reduced the percentage haemolysis of erythrocytes therefore improving the total haemoglobin concentration, white blood cell, packed cell volume and platelet. Also, since the dietary supplements brought about a decrease in the levels of low density lipoprotein and total cholesterol and an increase in high density lipoprotein levels, therefore the dietary supplements may therefore protect against arteriosclerosis and attendant coronary heart disease (CHD) and does not also predispose to hypertension.

Table 1: *Effects of dietary supplement on lipid profile of Albino Wistar Rats (mmol/L).*

Groups	Cholesterol	TG	HDL	LDL
Group C	2.92±0.08	2.10±0.07	0.95±0.25	0.94±0.16
Group A	2.00 ±0.20	0.75±0.39	1.52±0.08	0.36 ±0.05
Group B	2.11±0.13	0.80±0.38	1.50 ±0.29	0.40 0.27

Values are represented as mean±SD of three separate determinations from six rats. Values are not significantly different ($p>0.05$) from the control (group C)

Table 2: *Effect of dietary supplements on Haematological parameters of Albino Wistar Rats* (*mmol/L).*

Groups	WBC	RBC	PCV	HC
Group C	7.44± 0.34	2.28 ± 0.27	36.00 ± 2.92	13.12 ± 0.87
Group A	4.72 ± 0.19	4.48± 0.11	47.20 ± 1.30	18.8 ± 0.16
Group B	5.46 ± 0.17	5.28 ± 0.19	49.60 ± 1.14	18.50± 0.21

Values are represented as mean±SD of three separate determinations from six rats. Values are not significantly different ($p>0.05$) from the control (group C)

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