



IMMUNE SYSTEM RESPONSE TO CHANGES IN DIFFERENT ANTIOXIDANT DIETARY INTERVENTION ON STAFF OF NASARAWA STATE UNIVERSITY, KEFFI

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ABSTRACT

Effect of antioxidant functional foods and nutraceuticals on malondialdehyde (MDA) level, cytotoxic clusters of differentiation antigen 4 (CD4) and Immunoglobulin M (IgM) in male and female individuals were studied for six months. The volunteers included a total of 150 healthy adults of 96 men and 54 women aged between 30 and 74 years. They were made to sign an informed consent form, questioner and an ethical approval was gotten. At the baseline, eligible candidates were randomized to either 1 capsule per day of antioxidant nutraceutical (Forever living product) (containing vitamin E 10 mg, vitamin C 60mg and β - carotene 2000 mcg of vitamin A, or cheap affordable indigenous antioxidant functional foods of equivalent vitamin composition of oranges, carrots, and soybean or bottled water, and the first dose was dispensed and followed up for six months. There was a positive decrease in MDA and IgM values and a positive increase in CD4 counts after antioxidant dietary intervention. However, a lower concentration of MDA, and higher level of IgM and CD4 in men than in female at some age ranges was observed. The study showed that the indigenous antioxidant functional foods could be used as an alternative to the expensive imported antioxidant nutraceuticals in boosting immunity and therefore suggest such for immune- compromised individuals especially in developing countries like Nigeria.

Keywords: Nutraceuticals, functional foods, antioxidant, malondialdehyde, immunoglobulin M, CD4

INTRODUCTION

Cellular immune functions and health generally can be compromised by severe nutritional deficiency (De La Fuenete, 2002). They also decline with age, and this decrease might be due to, at least in part, to alterations in nutritional status (Klasing & Leshchinsky, 2001). With increasing knowledge about micronutrients including minerals, vitamins and other compounds like carotenoids, flavonoids, anthocyanins on a molecular level together with results from epidemiological studies open a new and exciting field of nutritional science. Free radicals are incriminated in the pathogenesis of tissue injury in many diseases. They produce cellular injury by lipid peroxidation, enzyme activation, damage DNA and degradation of structural proteins. The body has evolve multiple defence mechanism through antioxidants against free radicals, these antioxidant include vitamin C, Vitamin E, vitamin A, carotenoids, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), however under normal circumstances, there is a balance between pro-oxidant and antioxidants (Khanna *et al.* 2012). The compound that have been studied most extremely are the antioxidants, many potential benefits have been attributed to antioxidant in the form of antioxidant supplements. These supplements have the potential to alleviate the health problems with ageing caused by excessive production of reactive oxygen species (ROS) (Packer & Werber, 2001). New concepts have appeared with this trend, as nutraceuticals, functional foods, nutritional therapy, phytonutrients, and phytotherapy (Bland, 1996; Berger & Shenkin, 2006; Bagchi, 2006).

The immune system is a system of biological structures and processes within an organism that protects against disease (Kahnamooi, Saber & Anzabi, 2013). The immune cell functions are strongly influenced by the antioxidant/oxidant balance and, therefore, the anti-oxidant levels in these cells play a pivotal role in maintaining immune cells in a reduced environment and in protecting them from oxidative stress and preserving their adequate function (Knight, 1998). More specifically, antioxidants maintain the integrity and function of membrane lipids, cellular proteins, nucleic acids and the control of signal transduction of gene expression in immune cells (Meydani *et al.*, 1995). For this reason the immune cells are particularly sensitive to changes in their antioxidant status. Moreover, since the immune system cells have a high percentage of polyunsaturated fatty acids (PUFA) in their plasma membrane, it is not surprising that these cells usually contain higher

concentrations of antioxidants than do other cells (Knight, 1998). The immune system is a two-edged sword: the extremely potent and toxic biological effector mechanisms of the immune system can destroy not only threatening microorganisms but also body tissues. Usually the tissue destruction and inflammation associated with the eradication of a microbiological threat are acceptable and functionally insignificant. However, in several human diseases, the immunologically associated tissue destruction and inflammation are harmful, for example, tuberculosis, fulminant hepatitis and meningitis, and, although this may be advantageous to the species as a whole, the effect on the individual may be devastating. It is because of their potential to destroy tissues that the effector mechanisms of the immune system are very tightly regulated. Failure of these regulatory mechanisms results in the full might of the immune system being inappropriately directed against body tissues and the development of diseases like rheumatoid arthritis and many other autoimmune diseases.

A food can be regarded as functional if it satisfactorily demonstrated to affect beneficially one or more target functions in the body exceeding just a adequate nutritional effect, in a way which is necessary to either the well being and health or reduction of the risk of infections and diseases (Nagpal *et al.* 2014). Whole foods represent the simplest example of functional foods (carrots, oranges, soybean, avocado pears, and tomatoes) because of their high contents of physiologically active components (β -carotene, vitamin C, vitamin E, and lycopene respectively).

“Nutraceutical” is a term coined in 1989 by Stephen DeFelice as food or parts of food that provide medical or health benefits (such as diseases prevention and treatment) (Alissa & Ferns, 2012). Nutraceutical is any nontoxic food extract supplement that has scientifically proven health benefit for both the treatment and prevention of disease (De Felice, 2002).

Free radicals damage contribute to the aetiology of many chronic health problems associated with ageing such as cardiovascular diseases, inflammatory diseases, cataract, cancer, and diabetes (Fang, 2002). Antioxidants prevent free radical induced tissue damage by preventing the formation of radicals and scavenging them (Young & Woodside, 2001). Synthetic antioxidants are recently reported to be dangerous to human health (Balsano & Alisi, 2009). Thus, the search for effective, nontoxic, natural products with antioxidant activity has been intensified in recent years. In addition to endogenous

antioxidant defence systems, consumption of dietary and plant-derived, widely spread, affordable, antioxidants appears to be a suitable alternative. The Nigerian easily grown, widely spread, effective, nontoxic, cheap functional foods (carrots, tomatoes, oranges, soybean and groundnuts) are rich sources of natural antioxidants that should be studied.

There is little evidence of the efficacy of a long term intervention of antioxidant nutraceuticals and functional foods on immune functions in healthy individuals. However, limited or no data is available in support of comparative effects of antioxidant nutraceuticals and functional foods on Malondialdehyde (MDA) level, Cytotoxic cluster of differentiation antigen 4 (CD4) and Immunoglobulin M (IgM) in these subjects according to age and sex distribution. Hence, the present study was undertaken to evaluate the effect of antioxidant nutraceuticals and functional foods on MDA, CD4 and (IgM) levels in healthy subjects according to age and sex distribution.

MATERIALS AND METHODS

The study was conducted in Nasarawa State, Nigeria. Nasarawa State is located in the north central geopolitical zone of Nigeria. It lies between latitude 80035'N and longitude 08036'E. It is bounded to the North-west by Federal capital territory (FCT), Abuja, and to the North-east by plateau state, to the South-east by Taraba state and to the North by Kaduna state, to the South by Benue state and to the South-west by Kogi state (Figure I). It has a land mass of 21,117 square kilometre with a population of 2, 100, 000 making it the 10th largest state in Nigeria (Nigerian 2006 census).

The volunteers were randomly assigned to three groups with 3 sub-groups based on three intervals of baseline, six months and twelve months. Group 1; No antioxidant treatment was given for the twelve months but a 75 cl bottled water- placebo group (Grp 1 (BT)_{0m}: placebo basaline group, Grp 1 (AT-P)_{6m}: placebo six months group, GRP 1 (AT-P)_{12m}: placebo twelve months group). Group 2; the group that later received antioxidant nutraceuticals Forever living product 1 capsule per day of (vitamin E (10mg), vitamin C (60mg) and β - carotene(2,000mcg of vit A)-nutraceutical treatment group (Grp 2 (AT-N)_{0m}: baseline group that were later given antioxidant nutraceuticals, Grp 2 (AT.N)_{6m}: nutraceutical treatment group that received antioxidant for six months, Grp 2 (AT-N)_{12m}: nutraceutical treatment group that reived antioxidant for twelve months). Group 3; the group that later received antioxidant

functional foods of equivalent vitamin composition oranges (100g), carrots (100g), 75cl soybean drink and a heaped tablespoon of powdered soybean (35g) daily-functional group treatment group (Grp 3 (AT-F)_{0m}: baseline group that was later given antioxidant, Grp 3 (AT-F)_{6m}: functional food treatment group that received antioxidant for six months, Grp 3 (AT-F)_{12m}: functional food treatment group that received antioxidant for twelve months).

The study subjects included a total of 150 healthy adults of 96 men and 54 women aged between 30 and 74 years. All volunteers are staff of Nasarawa State University, Keffi.

A Single-centred open dose study was undertaken by the 150 healthy subjects (96 men and 54 women). The inclusion and exclusion criteria were that the subjects had no history of gastrointestinal surgery, or other significant pathology, were non-smokers, had no history of alcohol or drug abuse, were non-diabetic, were not on a calorie-reduced or vegetable diet nor were taking antioxidant/vitamin supplement, female were not pregnant or lactating. No concomitant medication was allowed throughout the study except contraceptive pill.

The scope, nature, aim and objectives of this study were thoroughly explained to voluntary participants for their consent, and all of them were made to sign an informed consent letter and a questionnaire. Three hundred (300) volunteers' venous blood samples were taken by local physicians. Volunteers with desirable health status were chosen. Most of the people not chosen have either HIV+ve, hepatitis B or C, very high/low blood sugar (≥ 7.77 and ≤ 3.33 mmol/L), extremely low/high blood pressure ($< 100/60$ mmHg and $> 140/95$ mmHg), those that are not sure of their date of birth. Some due to personal reasons refuse to participate. At the end we had 180 assumed healthy participants. Volunteers who were assumed healthy were randomly assigned to three groups as in dietary intervention above. At six months of dietary intervention blood samples were collected from all groups for biochemical analysis just as at baseline.

Blood biochemical analysis/assays were carried out at Innovative Biotechnology Research Laboratory, Keffi, and Nasarawa state with the exception of the Micronutrients that were carried out at Nigerian Institute of Pharmaceutical Research and Development, Idu Industrial District, Abuja. Lipid peroxidation (Malondialdehyde, MDA) level in serum were estimated spectrophotometrically using Thiobarbituric acid- reactive substances (TBARS) method as described by Ohkawa, Ohishi & Yagi, 1979).

Cytotoxic cluster of differentiation antigen 4 (CD4) in blood serum was determined using BD FACScount machines using the method described by Giorgi, Cheng & Margolic (1990). Immunoglobulin M (IgM antibody) Titers of antibodies IgM in sera were quantified by evaluating serum antibodies to *Helicobacter Pylori* (Isotypes of immunoglobulin M(IgM)) were measured by enzyme immunoassay (EIA) using goat-antihuman IgM with alkaline phosphatase (EC 3.1.3.1) (Wernette *et al.*, 2003).

The experimental results were expressed as the mean \pm SD. The parameters were analysed statistically using one-way analysis of variance (ANOVA) for comparing the mean using Smith's Statistical package (SSP).

RESULTS AND DISCUSSION

Immune status results after six months intervention of antioxidants nutraceuticals and antioxidant functional foods are shown in Tables 1-3. The result in Table 1 showed that males have higher MDA concentration than females even after antioxidants treatment. The result showed that the MDA of antioxidant treated groups were positively influenced than the placebo group at six (Grp 1 (AT-P), and the indigenous antioxidant functional foods (Grp 3 (AT-F)_{6m} were affected more positively with decrease in MDA than the imported nutraceutical antioxidant (Grp 2 (AT-N)_{6m}). The result showed that male MDA levels were mostly higher than the female of same age range. Result showed that the MDA levels of males and females decrease with increase in age. Statistically, the Serum MDA level of Grp 1 (AT-P)_{6m} showed statistically difference with negative increase in MDA except for the males of age range of 30-39 years and males of age range 40-49 compare to Grp 1 (BT). Grp 2 (AT-N)_{6m} showed a significant difference in all ages of male and female except for males in 40-49 age range compared to Grp 2 (BT)_{0m}. Grp 3 (AT-F)_{6m} showed a significant different in positive decrease of all age groups for male and females compared to Grp 3 (BT)_{0m}. Table 2 showed that the serum CD4 count of antioxidant treated groups were positively influenced than the placebo group (Grp1 (AT-P)_{6m}). Grp 3 (AT-F)_{6m} were affected more positively than the antioxidant Grp 2 (AT-N)_{6m}. There was a statistical difference in all the treatment groups at six. There is a difference in serum CD₄ level of different ages of male and female after treatment. Result showed that the serum CD4 levels of males and females decrease with increase in age. The result showed that most of the female has good increase reference values of CD4 than the male before and after treatment. The

result showed that most of the placebo groups did not show any improvement on any range that was not within the reference range like in ages 60-69 of the placebo group the values did not improve within the six months rather it negatively reduced (Grp 1 (BT)_{0m}=55, Grp 1 (AT-P)_{6m}=50). The older adult from 60-79 have serum IgM concentration that is either above or below the reference range though some of the antioxidant group were slightly improved at that age. The IgM values are more positive in the younger adults than older adults. Generally, from the result more of the males have better serum IgM concentration than the females. Statistically, there was a significant difference between Grp 2 (AT-N)_{6m} and Grp 2 (BT)_{0m} except for the females in age groups 70-79. Also there is a significant difference between Grp 3 (AT-F)_{6m} and Grp 3 (BT)_{0m} though most of them are within normal reference ranges.

This randomized, prospective, parallel group, comparative, open dose and single centre study was conducted to determine the effects of antioxidant nutraceuticals and functional foods intervention on Malondialdehyde (MDA), Cytotoxic cluster of differentiation antigen 4 (CD4) and Immunoglobulin M (IgM) in relation to age and sex distribution as risk factors for immuno-compromised individuals. Immune cells are influenced by antioxidant/oxidant balance; the antioxidant levels play a vital role in maintaining immune cells in a reduced environment and in protecting them from oxidative stress (Knight, 1998). Because the immune system is critically dependent on accurate cell-cell communication in order to mount a response, immune cell integrity is essential. The effects of antioxidants are very beneficial during period of oxidative stress like the periods of infections, and in elderly. In the study the blood parameters evaluated and assayed relates to their levels in the blood with ageing and most times sex. The antioxidant intervention positively influenced the blood parameters with the antioxidant functional foods showing a better influence than the antioxidant nutraceuticals.

Persistent low-grade systemic inflammation has been increasingly recognized as a common tissue destruction process, and an important contributing factor to cardiovascular diseases and its risk factor-metabolic syndrome. MDA, CD4+-cell counts, and IgM is one of central important parameter in monitoring of immune function (Tumwebaze, 2012). During ageing, the balance between the generation of reactive oxygen species (ROS) and ROS clearance can be disturbed resulting in oxidative damage to macromolecules such as membrane phospholipids.

The study has confirmed that the immune status level evaluated and assayed shows that MDA increases with age, while CD4 count and IgM reduces with age. The result showed that the younger males have lower malondialdehyde than younger females; the males have good increase reference values of CD4 and Immunoglobulin M than the female. Figures 1 to 3 showed a significant improvement on the immune status level with antioxidant dietary intervention with the antioxidant functional foods given better results than the antioxidant nutraceuticals. Many different types of immune defects in elderly have been identified. The previous focus on defects in cell-mediated immune responses provided a possible explanation for the increased risk of cancer, viral infections, and infections with intracellular bacterial pathogens, such as *Helicobacter pylori*, *Mycobacterium tuberculosis*, also pronounced susceptibility to extracellular bacterial infections, such as *Streptococcus pneumoniae* and T-cell deficit infections, all these infections increase with age (Miller, 1996).

Malondialdehyde (MDA) is a secondary product of lipid peroxidation and is used as an indicator of tissue damage. MDA results agreed with a previous work that plasma thiobarbituric acid reacting substances increases with age, indicating increases lipid peroxidation (Coudray *et al.*, 1997). Previous studies showed that patients with immune diseases have higher increase in MDA with increase in ageing than healthy ageing adult group (Prasher, Pandev & Gupta, 1993; Cohen, Olin & Feur, 1994). The raw result also showed that males have higher MDA concentration than females. The plasma level of MDA is a reliable and common biomarker of the overall lipid peroxidation. Nielsen, Mikkelsen & Nielsen (1997) showed that increased plasma MDA levels in ageing is not only consistent with the role of oxidative stress in ageing but also supports the idea that plasma MDA levels may be used as a marker of oxidative stress on immunological studies.

Cytotoxic cluster of differentiation antigen 4 (CD4) are types of white blood cells that fight infection it is also called T-helper cells they are made in the lymphoid organs (spleen, lymph nodes and thymus gland) which are path of the lymph or infection fighting system. Its measure in the blood shows how strong the immune system is. CD4 results showed that there is a significant decrease in CD4 count with age. The results showed that males have good increase reference value of CD4 count than the females. A study support the result of these study, age and sex is significantly related to CD4 count, men have lower CD4 count than women, and CD4 count

decreases with each decade increase in age, exclusion of pregnant women in a HIV +ve study (Aina *et al.*, 2005). A study in Ethiopia disagrees with this result that women have higher CD4 count than men (Lee *et al.* 1996) but studies among Indians (Uppal, Verma & Dhot, 2003) and also in Uganda (Tugume *et al.*, 1995) agrees with this result. It is not clear whether there are true variations across countries in the relationship between gender and CD4 cell counts or these results are due to confounding factors. Our result showed that there is significant increase in CD4 cell counts on age of the elderly. This is consistent with previously reported findings in Central African republic (Menard *et al.*, 2003) it reported significant increase in CD4 cell count by decades in age.

After an initial antigen encounter, Immunoglobulin M (IgM) is said to be the first antibody to be produced during an immune response and is the predominant isotype secreted in T-cell independent of immune response (Ehrenstein & Notley, 2010). IgM concentration is reactive to wide variety of autoantigen, and its levels are found markedly elevated in a series of autoimmune diseases (Duarte-Rey *et al.* 2012). It is therefore believed to be an important component of autoimmunity (Duarte *et al.*, 2012; Marchalonies *et al.*, 1993). IgM are related to elevated trig, chol, LDL, CD4, MDA, Uric acid level and white blood cell count. Titers of antibody to *Helicobacter pylori* (Isotype of Immunoglobulin M (IgM)) were evaluated, results are shown in table 1. It showed that female has higher antibodies-IgM antibodies to *H. pylori* than male. Our result is consistent with previous studies, that females have higher IgM levels than males (Gonzalez-Quintelan *et al.*, 2008) although the precise mechanism is yet unclear, the stimulatory action of estrogens' on B-lymphocytes could be the cause (Bouman, Heineman & Faas, 2005). A large number of studies have established that most autoimmune diseases occurs significantly more frequently in females than males (Gleicher & Barad, 2007). The results showed that there is a significant decrease on IgM antibody to *H.pylori* with increase in age. According to the biochemical indicators, the elderly has lower antibodies when compared to the younger age. Thus, the elderly are more prone to infections and immune diseases than the young. The reference range for IgM is between (55-575 mg/Dl) higher levels of IgM above the upper limit can mean mononucleosis, macroglobulinemia, rheumatoid arthritis, early viral hepatitis kidney damage (nephritic syndrome, or a parasite infection; lower level of IgM below the lower limit occur in multiple myeloma, some types of

leukaemia, and in some inherited types of immune diseases (Ehrenstein & Notley, 2010).

In conclusion, data of the present study suggest that the dietary intervention of antioxidant nutraceuticals and functional foods intervention is likely to compromise immune functions. These findings also showed possible effects of age and gender on immune parameters of healthy individuals treated with antioxidant diets and supplements and this should pave a way for more research on antioxidants functional foods. Findings from this study showing lower levels of MDA and higher level of CD4 and IgM among men, point out to an advantage of males in relation to females. We recommend more intact of antioxidant functional food as an alternate to antioxidant nutraceuticals for both healthy and immuno-compromised individuals.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

Table 1: Effect of antioxidants on Serum Malondialdehyde level (µmol/L)

Groups	30-39		40-49		50-59		60-69		70-79	
	M	F	M	F	M	F	M	F	M	F
Baseline										
Grp 1 (BT) _{0m}	0.88±0.04	0.87±0.02	0.93±0.09	0.89±0.10	1.36±0.08	1.48±0.09	1.52±0.07	1.58±0.06	2.00±0.04	1.91±0.00
Grp 2 (BT) _{0m}	0.91±0.03	0.88±0.03	0.96±0.09	0.90±0.11	1.22±0.08	1.32±0.08	1.92±0.06	1.81±0.05	2.30±0.00	2.18±0.04
Grp 3 (BT) _{0m}	0.87±0.03	0.90±0.04	0.98±0.09	0.93±0.08	1.25±0.10	1.27±0.11	2.00±0.06	1.98±0.07	2.29±0.04	2.16±0.00
Antioxidant Treatment (6 months)										
Grp 1 (AT-P) _{6m}	0.89±0.04	0.85±0.03a	0.93±0.09	0.87±0.08a	1.38±0.07a	1.50±0.06a	1.60±0.06a	1.60±0.02a	2.20±0.02a	2.00±0.00a
Grp 2 (AT-N) _{6m}	0.95±0.02b	1.00±0.03b	0.95±0.05	1.10±0.04b	1.20±0.04b	1.20±0.06b	1.75±0.03b	1.50±0.03b	1.90±0.00b	1.88±0.01b
Grp 3 (AT-F) _{6m}	0.90±0.03c*	1.10±0.04c	0.10±0.05c*	1.12±0.05c*	1.20±0.07 c	1.15±0.06c*	1.70±0.03c*	1.30±0.02c*	1.85±0.01c*	1.70±0.00c*

Reference value: 0.88-1.61 (µmol/L); Grp 1 (BT): Group 1 before treatment; Grp 2 (BT): Group two before treatment; Grp 3 (BT): Group 3 before treatment; Grp 1 (AT-P)_{6m}: Group 1 after treatment- placebo; Grp 2 (AT-N)_{6m}: Group 2 after treatment- nutraceuticals; Grp 3 (AT-F)_{6m}: Group 3 after treatment- functional food; p<0.05 was considered statistically significant; n: 150; a: was considered statistically significant when compared with Grp 1 (BT); b: was considered statistically significant when compared with Grp 2 (BT); c: was considered statistically significant when compared with Grp 3 (BT); *: was considered statistically significant when compared with Grp 2 (AT-F)_{6m}

Table 2: Effect of antioxidants on Serum Cytotoxic clusters differentiation antigen 4 level (µmol/L)

Groups	30-39		40-49		50-59		60-69		70-79	
	M	F	M	F	M	F	M	F	M	F
Baseline										
Grp 1 (BT) _{0m}	944±0.03	981±0.01	935±0.07	1028±0.12	898±0.09	500±0.08	680±0.04	510±0.04	512±0.02	550±0.00
Grp 2 (BT) _{0m}	810±0.02	1004±0.03	813±0.10	780±0.09	463±0.11	773±0.11	705±0.05	801±0.05	432±0.00	569±0.04
Grp 3 (BT) _{0m}	999±0.02	992±0.03	960±0.12	817±0.11	708±0.09	482±0.07	501±0.05	760±0.05	476±0.01	522±0.00
Antioxidant Treatment (6 months)										
Grp 1 (AT-P) _{6m}	925±0.04a	985±0.02a	920±0.03a	1030±0.08a	900±0.07a	510±0.06a	600±0.04a	555±0.02a	500±0.02a	600±0.00a
Grp 2 (AT-N) _{6m}	840±0.03b	1010±0.03b	845±0.10b	800±0.09b	500±0.08b	850±0.08b	730±0.03b	900±0.03b	490±0.00b	600±0.01b
Grp 3 (AT-F) _{6m}	1010±0.02c*	1000±0.04c*	990±0.10c*	850±0.08c*	740±0.08c*	580±0.05c*	540±0.04c*	800±0.03c*	510±0.01c*	600±0.00c*

Reference value: 500-1000 (µmol/L); Grp 1 (BT): Group 1 before treatment; Grp 2 (BT): Group two before treatment; Grp 3 (BT): Group 3 before treatment; Grp 1 (AT-P)_{6m}: Group 1 after treatment- placebo; Grp 2 (AT-N)_{6m}: Group 2 after treatment- nutraceuticals; Grp 3 (AT-F)_{6m}: Group 3 after treatment- functional food; p<0.05 was considered statistically significant; n: 150; a: was considered statistically significant when compared with Grp 1 (BT); b: was considered statistically significant when compared with Grp 2 (BT); c: was considered statistically significant when compared with Grp 3 (BT); *: was considered statistically significant when compared with Grp 2 (AT-F)_{6m}

Table 3: Effect of antioxidants on Serum immunoglobulin level (mg/DL)

Groups	30-39		40-49		50-59		60-69		70-79	
	M	F	M	F	M	F	M	F	M	F
Baseline										
Grp 1 (BT) _{0m}	270.00±0.04	188.00±0.01	304.00±0.07	249.00±0.12	140.00±0.09	225.00±0.10	52.00±0.06	55.00±0.08	375.00±0.03	377.00±0.00
Grp 2 (BT) _{0m}	375.00±0.03	205.00±0.04	306.00±0.03	162.00±0.04	122.00±0.9	231.00±0.10	211.00±0.10	378.00±0.09	53.00±0.06	381.00±0.02
Grp 3 (BT) _{0m}	259.00±0.02	303.00±0.03	371.00±0.09	367.00±0.10	107.00±0.11	167.00±0.07	54.00±0.08	54.00±0.06	49.00±0.04	52.00±0.00
Antioxidant Treatment (6 months)										
Grp 1 (AT-P) _{6m}	270.00±0.04	180.00±0.02a	300.00±0.07a	240.00±0.09a	145.00±0.05a	225.00±0.06	50.00 ± 0.06a	50.00 ± 0.03a	378.00±0.03a	380.00±0.00a
Grp 2 (AT-N) _{6m}	316.00±0.03b	220.00±0.02b	132.00±0.07b	180.00±0.04b	220.00±0.10b	240.00±0.06b	64.00 ± 0.02b	370.00±0.02b	60.00 ± 0.00b	380.00±0.01
Grp 3 (AT-F) _{6m}	270.00±0.02c*	300.00±0.03c*	360.00±0.07c*	355.00±0.04c*	120.00±0.09c*	170.00±0.03c*	68.00 ± 0.06c*	60.00±0.02c*	59.00 ± 0.02c	54.00±0.00c*

Reference value: 55-375 (mg/DL); Grp 1 (BT): Group 1 before treatment; Grp 2 (BT): Group two before treatment; Grp 3 (BT): Group 3 before treatment; Grp 1 (AT-P) 6m: Group 1 after treatment- placebo; Grp 2 (AT-N)_{6m}: Group 2 after treatment- nutraceuticals; Grp 3 (AT-F)_{6m}: Group 3 after treatment- functional food; p<0.05 was considered statistically significant; n: 150; a: was considered statistically significant when compared with Grp 1 (BT); b: was considered statistically significant when compared with Grp 2 (BT); c: was considered statistically significant when compared with Grp 3 (BT); *: was considered statistically significant when compared with Grp 2 (AT-F)_{6m}

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