BLOOD HAEMATOLOGY RESPONSE TO CHANGES IN ANTIOXIDANTS: THE INFLUENCE OF SIX MONTHS NUTRACEUTICALS AND FUNCTIONAL FOODS INTERVENTION ON AGE AND SEX AS RISK FACTORS FOR IMMUNE-COMPROMISED INDIVIDUALS

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ABSTRACT

To assess the long-term effects of antioxidants in individuals according to age and sex distribution, we studied the effects of antioxidant functional foods and nutraceuticals on blood haematology such as White blood cells (WBC), Red blood cells (RBC), Haemoglobin (HGB) and Platellets (PLT). This study performed as a randomized, prospective, parallel group, comparative, open dose and single centre study. The studied subjects included a total of 150 healthy adults of 96 men and 54 women aged between 30 and 74 years. The included 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. At the baseline visit, eligible candidate were randomized to either 1 capsule per day of antioxidant nutraceutical (Forever living product) (containing vitamin E 10 mg, vitamin C 60 mg and β- carotene 2000 mcg of vitamin A, or antioxidant functional foods of equivalent vitamin composition oranges, carrots, and soybean or placebo, and the first dose was dispensed and followed up for six months. At the end of the six months of the dietary intervention, significant decline in HGB, RBC, and PLT with ageing, and more pronounced in male though males have higher HGB and PLT than the female. No significant difference in WBC and RBC count of male compare to female but WBC increases or decreases to the extreme range with age. In the present study, the authors have demonstrated that functional foods significantly improve blood haematology and its function.

Keywords: Antioxidant nutraceuticals, antioxidant functional foods, haematological parameters, age, sex.

INTRODUCTION

In cells and tissue, the redox state solely depends on the balance between oxidants and antioxidants at a given time rate. The oxidants are the radicals and reactive oxygen or nitrogen species produced in the body due to the regular intermediary metabolism and other oxidative processes such as the respiratory bursts seen during phagocytosis and regulate mediators in signalling processes (Anand *et al.*, 2010). High concentration of oxidants may damage some major cellular constituents and termed the process is termed oxidative stress (Valko *et al.*, 2007).

However, oxidative stress is implicated in the pathogenesis of various disorders such as cancer (Diplock, 1994; Gwarzo *et al.*, 2010), diabetes mellitus (Mates *et al.*, 1999), cardiovascular diseases (Kayode *et al.*, 2009), autoimmune and neurodegerative diseases (Gwarzo *et al.*, 2010; Vaghasiya & Chanda, 2010; Diplock, 1994). Potentially damaging oxidative stress is kept in check by endogenous antioxidant systems, which includes

antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) and nutrient derived antioxidants small molecules. A potent scavenger of radicals from plants caused by oxidative stress may serve as a possible preventive intervention for the diseases and disorders (Gyamfi *et al.*, 1999).

In recent years, the beneficial effects of dietary antioxidants have been considered, because it helps in the treatment of cardiovascular diseases (Nair *et al.*, 2007), cancer, diabetes and even common cold (Frei, 2003). Clinical studies carried out by researchers, provided information on some of the functions of antioxidants in the treatment of Alzheimer's disease (Nair *et al.*, 2007; Sano *et al.*, 1997) and managing arteriosclerosis (Saloneu, 2003). There is a growing interest in the use of natural antioxidants as bioactive component in food, and such food has been termed "functional foods" (Hertog *et al.*, 1993).

In the past two decades, the concept of functional food has become popular and known to have potential benefits in promoting health and preventing the risk of certain diseases beyond its basic nutritional function (Ballali & Lanciai, 2012; Gregori & Gafare, 2012). However, new healthy paradigm on diets has been evolving which places more emphasis on the positive aspects of diet. The new lifestyle adopted by people of 21st century has changed the basic food habits. Consumption of the junk foods has increased leading to a number of diseases caused due to improper nutrition (Das *et al.*, 2012). This problem leads to the seeking of complementary alternative beneficial products that could reduce the effect caused by improper meals (Das *et al.*, 2012). In addition to functional foods, nutraceuticals may holds an exciting opportunities for the food industry to create novel food products in future. Functional foods and nutraceuticals provide an opportunity to improve human health by reducing the cost of living and support the lives of indigenous people in rural communities. However, little or no clinical benefits of these amazing foods has been reported especially in Nigeria. The present study was designed to investigate the long-term dietary intervention of antioxidant functional foods and nutraceuticals on blood haematology in individuals according to age and sex distribution.

MATERIAL AND METHODS

Study Area

The study was conducted in Nasarawa State, Nigeria. Nasarawa State is located in the north central geopolitical zone of Nigeria. It lies between latitude 80^{0}35'N and longitude 08^{0}36'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. 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 according to Nigerian 2006 population census.

Nutraceutical and Functional Foods Intervention

The volunteers were randomly assigned to three groups. Group 1 (control group) received oral antioxidant nutraceutical (Forever living product) 1 capsule per day (containing vitamin E 10mg, vitamin C 60mg and β- carotene 2,000mcg of vitamin A). Group 2 (treatment group) received antioxidant functional foods of equivalent vitamin composition oranges, carrots, and soybean).

Study Population

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.

Study Design, Inclusion and Exclusion Criteria

A randomized, prospective, parallel group, comparative, open dose and single centre study was undertaken by the150 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.

Ethical Review and Independent Monitoring

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 questioneer. The protocol was reviewed and approved by the Chairman Ethical committee, Federal Ministry of Health Abuja through the Chief Medical Director, Hospital Management Board Lafia Nasarawa State and the Medical Director, Nasarawa State University Medical Clinic, Keffi. The study was independently monitored by an Ethical committee desk officer from Federal Ministry of Health, Abuja according to Quality Assurance programme such that Good clinical practice were followed throughout the one year study.

Specimen Collection and Laboratory Analysis

Three hundred (300) volunteers' veinous blood samples were taken by local physicians from the university staff clinic in 3rd week of June, 2012 and clinical records were taken. 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 ≤3.33mmol/L), extremely low/high blood pressure (< 100/60 mmHg and >140/95mmHg), 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.

Six Months Dietary Intervention and Samples Collection and Evaluation

Baseline samples were collected six months ago and the blood parameters analysed as reported in these six months antioxidants dietary intervention. Six months blood samples were drawn from the volunteers who are assumed healthy according to their groups, after a 12-14 hour fast, in a 0.1% EDTA tubes, Lithium hyperinized bottles, and sterile bottles for biochemical analysis. Volunteers were six months ago randomly assigned to groups of three. Control group volunteers were given antioxidant nutraceuticals ((Forever living product) 1 capsule per day of vitamin E (10mg), vitamin C (60mg) and β- carotene (2,000mcg of vitamin A). The treatment group volunteers were given antioxidant functional foods of equivalent vitamin composition (oranges (100g), carrots (100g), and soybean drink (75cl) and 1 heaped table spoon of soybean powder (35g/day)). The placebo group volunteers were giving clean drinking water (ordinary Swan table water (75cl) with no antioxidants).

Haematological Parameters

White blood cells (WBC), Red blood cells (RBC), Haemoglobin (HGB), Platellets (PLT) blood collected in 0.1% EDTA bottles were used to obtain above parameters using a fully automated Abacus Junior hematologic analyzer that uses Coulter-method for counting cells passing through a small aperture, and measures the haemoglobin content of the red cells.

RESULTS

The results of blood haematology after six months dietary intervention are presented in Table 1. Results in figure 1 to 4 showed that antioxidant dietary intervention has a significant improvement on haematology. These figures showed that antioxidant functional foods affected the haematology better than the antioxidant nutraceuticals.

There was no significant difference in age of White blood cell (WBC) count of male compare to female with LSD-Gender (p-value) 4.01 (0.0800). Though in raw result the WBC count of most of the elderly are beyond upper/lower normal range of white blood cells in both male and female. Also in the raw result, WBC count of the male was higher/lower at the extreme of normal ranges (5-10) $\times 10^3$ cells/µL than the female. There was no significant difference among the males with LSD-Male (p-value) 3.94 (0.2378), and a significance difference among the female with LSD-Female (p-value) 1.86 (0.0229). Though in raw result the WBC count of most of the elderly are beyond reference/normal range of WBC in both male and female.

There was no significant difference noted in different ages in the red blood cell (RBC) count of male compare to female with LSD-Gender (p-value) 0.82 (0.4582), also in the raw result the RBC count of the male was higher/lower compare to upper/lower normal range (4.5-5.5) $\times 10^6$ cells/µL of RBC count of the female. Also, there was no significance difference among the male RBC count with LSD-Male (p-value) 0.76 (0.1719), and the female with LSD-Female (p-value) 0.58 (0.5038).

The blood Haemoglobin (HGB) concentration showed a significant difference in the ages of male compare to female with LSD-Gender (p-value) 2.59 (0.0053) the raw result showed that both male and female has normal HGB level with regards to upper/lower normal range (9.00-16.00) g/dL but with male HGB higher than that of female, though the lowest value was from male. There was a significant difference on the HGB concentration among the male with LSD-Male (p-value) 1.48 (0.0009). There was no significant difference among the female with LSD-Female (p-value) 0.90 (0.0130). HGB concentration raw result showed that most elderly has low result.

There was a significant difference in blood platelet (PLT) count in ages of male compare to female with LSD-Gender (p-value) 132.73 (0.0228) the raw result showed that the male has higher levels of PLT with regard s to normal range (150-400)$\times 10^3$ cells/µL compare to female of the same age. It was noted that the PLT result decrease with increase in age. There is a significance difference in PLT count among the male with LSD-Male (p-value) 31.51 (< 0.0001). There is also a significance difference in PLT count among the female with LSD-Female (p-value) 65.76 (0.0029).

Table 1: Effect of antioxidant nutraceuticals and functional foods on Haematology after six months treatment

		WBC ($\times 10^3$cells/L)	RBC ($\times 10^6$cells/L)	HGB (g/dL)	PLT ($\times 10^3$cells/L)
Group	**Age(years)**	**Male**	**Male**	**Male**	**Male**
PLACEBO	**30-39(n=08)**	08.70 ± 0.03	5.50 ± 0.03	14.50 ±0.06	360.00±0.03
(n=31)	**40-49(n=09)**	09.50 ±0.05	5.50 ± 0.04	13.30±0.05	310.00±0.07
	50-59(n=06)	11.00 ±0.04	3.50 ±0.02	10.50±0.03	300.00±0.05
	60-69(n=05)	12.00 ±0.02	4.90 ± 0.01	10.00±0.02	260.00±0.03
	70-79(n=03)	04.80 ±0.01	4.50 ± 0.03	09.50±0.02	150.00±0.01
CONTROL (	**30-39(n=10)**	07.00 ±0.02	5.00 ± 0.03	13.50±0.07	350.00±0.04
n=32)	**40-49(n=10)**	07.50 ± 0.06	5.00 ±0. 40	12.50±0.06	310.00±0.06
	50-59(n=06)	09.50 ± 0.04	4.70 ±0.03	10.50±0.05	300.00±0.03
	60-69(n=05)	12.00 ± 0.02	5.00 ±0.01	10.00±0.03	290.00±0.02
	70-79(n=01)	04.50 ±0.00	4.90 ± 0.00	09.50 ±0.00	155.00±0.00
TREATMENT	**30-39(n=09)**	08.00 ±0.03	5.10 ± 0.03	12.00±0.05	340.00±0.04
(n=31)	**40-49(n=10)**	09.00 ±0.05	5.00 ± 0.05	12.50±0.04	310.00±0.06
	50-59(n=07)	10.00 ±0.03	5.05 ± 0.04	12.50±0.03	305.00±0.05
	60-69(n=03)	09.00 ±0.02	4.90 ± 0.02	10.50±0.02	240.00±0.03
	70-79(n=02)	12.00 ±0.01	4.50 ± 0.01	10.00±0.01	200.00±0.02
		Female	**Female**	**Female**	**Female**
PLACEBO	**30-39(n=07)**	05.50 ±0.02	4.60±0.03	10.50±0.03	300.00±0.04
(n=19)	**40-49(n=06)**	05.50 ±0.04	4.65±0.05	10.00±0.05	200.00±0.04
	50-59(n=03)	07.00 ±0.03	4.55±0.04	09.50±0.04	260.00±0.03
	60-69(n=02)	10.50 ±0.02	4.60 ±0.03	09.00±0.02	150.00±0.02
	70-79(n=01)	10.50 ±0.00	4.00 ±0.00	08.50±0.00	135.00±0.00
CONTROL	**30-39(n=05)**	07.00 ±0.03	5.00 ±0.05	11.00±0.02	310.00±0.03
(n=18)	**40-49(n=05)**	07.00 ±0.03	5.00±0.06	10.50±0.03	290.00±0.05
	50-59(n=04)	07.50 ±0.03	4.80±0.03	10.00±0.03	170.00±0.04
	60-69(n=02)	08.00 ±0.02	5.10±0.02	10.00±0.02	225.00±0.02
	70-79(n=02)	09.00 ±0.01	5.00±0.01	10.00±0.01	160.00±0.01
TREATMENT	**30-39(n=06)**	07.00 ± 0.04	5.10±0.06	11.50±0.04	300.00±0.02
(n=19)	**40-49(n=06)**	07.00 ± 0.04	4.50±0.06	10.50±0.05	280.00±0.08
	50-59(n=04)	07.50± 0.03	4.90±0.05	10.00±0.03	230.00±0.07
	60-69(n=02)	08.00 ±0.02	5.10±0.04	10.00±0.02	155.00±0.02
	70-79(n=01)	08.00 ±0.00	4.50±0.00	09.50±0.00	160.00±0.00
LSD- Gender (p-value)		4.01 (0.0800*)	0.82 (0.4582*)	2.59 (0.0053)	132.73 (0.0228)
LSD-Male (p-value)		3.94 (0.2378*)	0.76 (0.1719*)	1.48 (0.0009)	31.51 (< 0.0001)
LSD-Female (p-value)		1.86(0.0229)	0.58 (0.5038*)	0.90 (0.0130)	65.76 (0.0029)

* Not Significant

Normal/Reference range **5.00-10.00** **4.50-5.50** **9.00-16.00** **150-400**

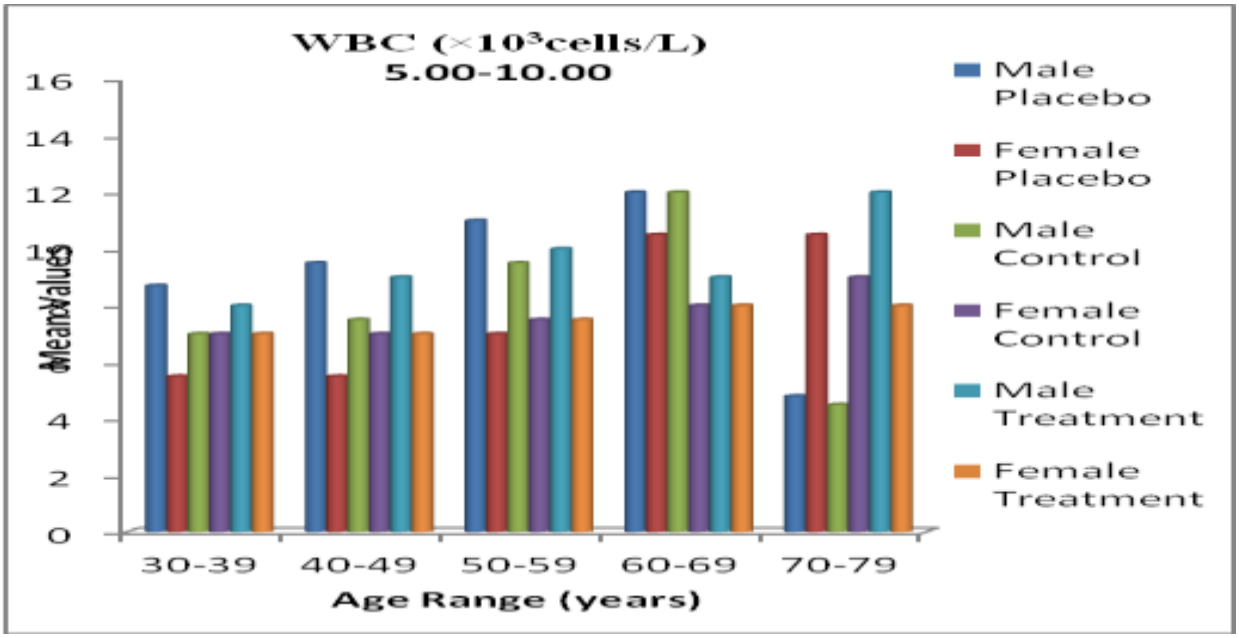

Figure 1: Effect of antioxidants on WBC after treatment

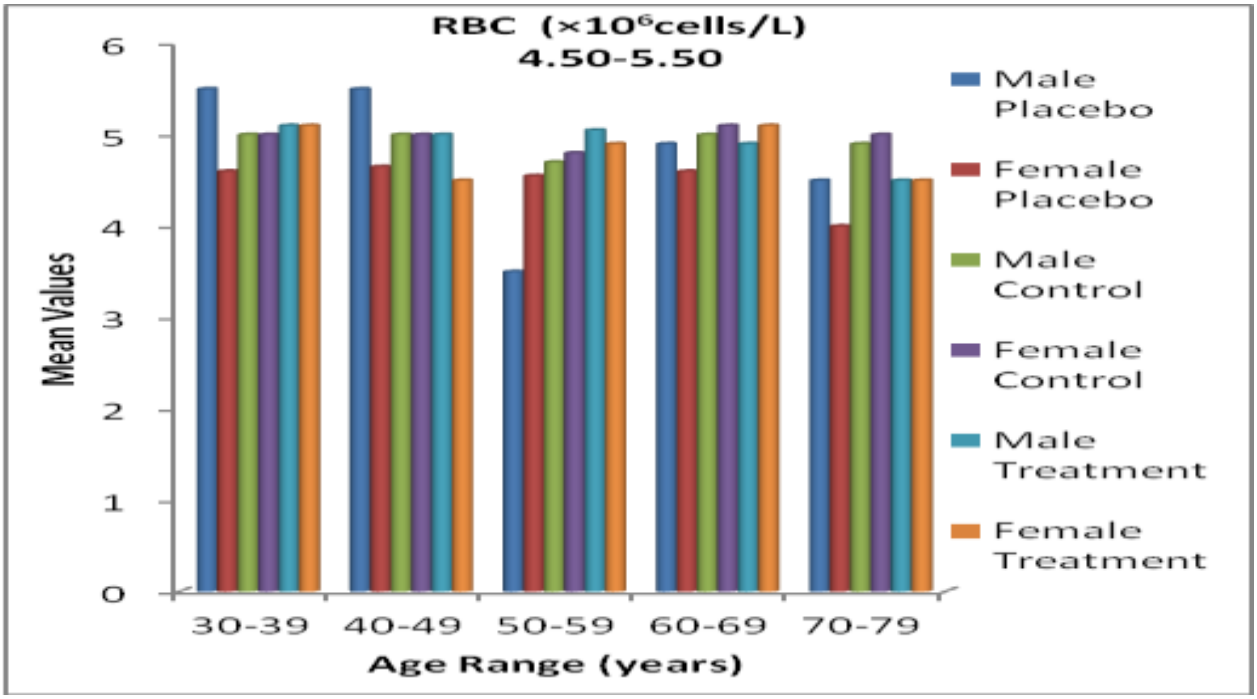

Figure 2: Effect of antioxidants on RBC after treatment

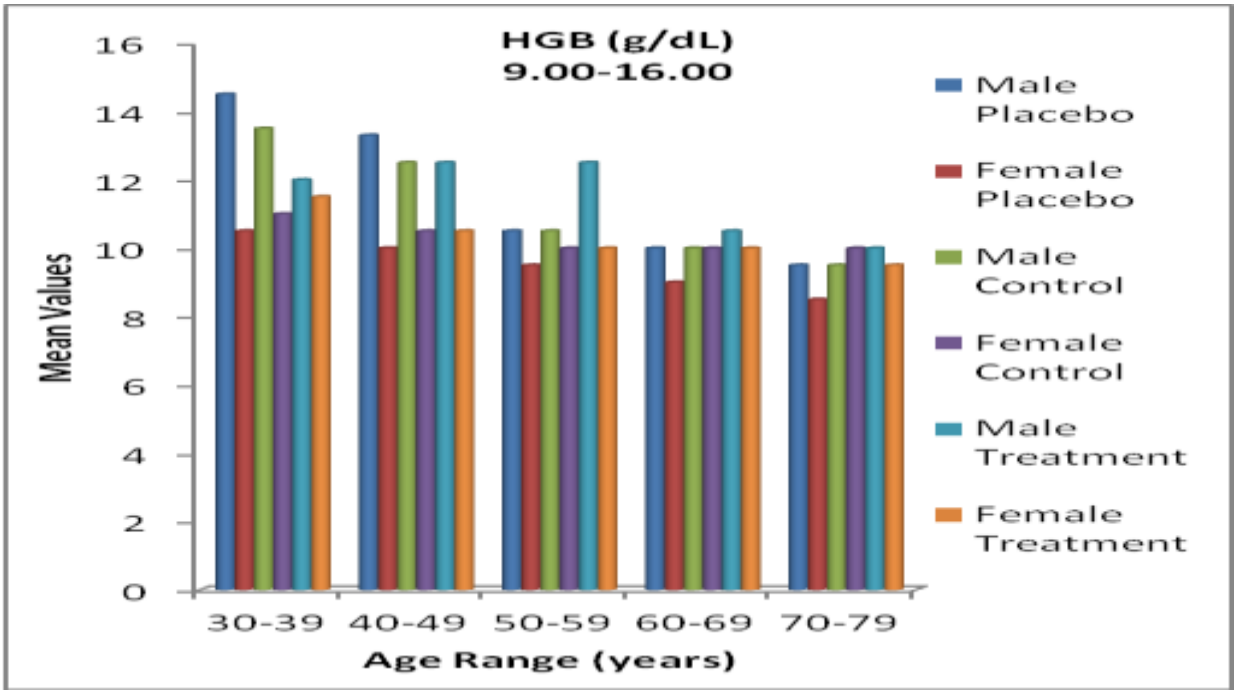

Figure 3: Effect of antioxidants on HGB after treatment

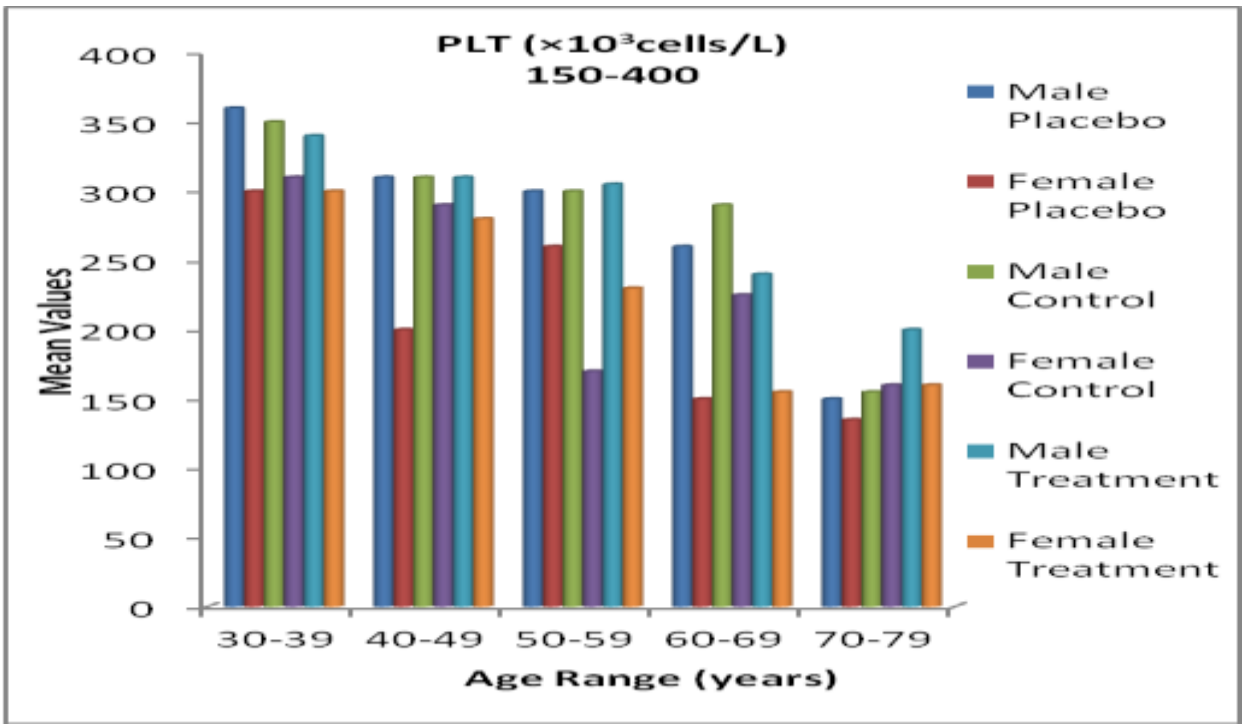

Figure 4: Effect of antioxidants on Platelet count after treatment

DISCUSSION

Blood cells (haematocytes) are cells produced by haemotoporiesis in mammals. There are red cells (erythrocytes), white blood cells (leucocytes) and platelets (thrombocytes). These cells make about 45% while plasma is about 55% (Maton *et al.*, 1993). The majority of the cells that are suspended in the plasma are erythrocytes which has high concentration of

haemoglobin the oxygen carrier pigment that impart red colour, the leucocytes are few in number they play important defence mechanism, and the platelets are small cells which are concerned with blood clothing process.

The result of haematological parameters are in table 1 it showed that conclusion can be drawn from the data that ranges of haematological indices are age and sex-specific, at present this pertains to RBC, HGB, and PLT. In the study it is noted that there are significant decline in HGB, RBC, and PLT with ageing, and more pronounced in male though males have higher HGB and PLT than the female. The raw result is consistent with a study of 215 men and 275 women aged 62-90 years found that men had significant higher total leukocytes counts than women (Milne & Williamson, 1972). The raw result is in contrast with study of healthy Caucasian hospital staff found that the total leukocytes counts were significantly higher in women than in men (Bain & England, 1975). But normal laboratory reference ranges for leukocytes counts often do not differ between the different cells in healthy cells. There is no significant difference in WBC and RBC count of male compare to female but WBC increases or decreases to the extreme range with age.

In the study, the RBC count showed no significant difference in ages of male compare to female. The raw result showed that male RBC is lower than that of female. This is supported by a study that erythrocyte count and haemoglobin concentration start to decline in men around 40 years of age; age associated changes in women are less (Kelly & Munan, 1977).
In the study males have higher HGB concentration than females this agrees with a World Health Organisation record that women has lower HGB than men (WHO, 2001). This could be concluded that women of menstruating age are likely to be iron-deficient and therefore lower HGB concentration. Though some studies that looked at ferritin levels do not support this (Waalen *et al.,* 2002), this leading to the suggestion that the difference may be due to hormonal influences on red cell production (Shahidi, 1973). Among the sexes haemoglobin level decline with increase in age, and this is supported by a study (Kelly & Munan, 1977), and the increase is pronounced in men than women (Nilsson-Ehle *et al.,* 2000).

PLT count showed that there females have higher PLT count than the males. This is consistent with previous studies, that platelet counts have been found to be significantly higher in women (Butkiewiez *et al.,* 2006; Kemona *et al.,* 1997), with possible explanation of compensation for menstrual blood loss or increased thrombopoietin in women being suggested.

CONCLUSION

We demonstrated the beneficial effects of antioxidant functional foods and nutraceuticals in improving the blood haematology. This study did not explore the underlying cellular and molecular mechanisms of action for the observed haematological changes. This however, has important benefits for the food and medical industry which may offer a degree of protection from cardiovascular diseases and improving the immune function and further studies are needed to be establish the effects of these physiological changes.

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