EFFECT OF ZOBO DRINK (HIBISCUS SABDARIFFA WATER EXTRACT) ON BIO-CHEMICAL AND HEMATOLOGICAL PARAMETERS IN MALE CYCLISTS

***[1]Akara E. and [2]Osemwenkhae O. P.**

[1]Department of Physical and Health Education, Faculty of Education, Delta State University, Abraka, Nigeria.

[2]Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin-City, Nigeria.

*Corresponding Author.

ABSTRACT

The aim of the present study was to evaluate, in a group of male cyclists the effects of short-term consumption of aqueous extract of the dried calyx of Hibiscus sabdariffa (popularly referred to as zobo in Nigeria) on some hematological and biochemical indices. Mmale clyclists who consumed the placebo (test control group) had significantly reduced hemoglobin (Hb) concentration, packed cell volume (PCV) and red blood cell (RBC) count, while the white blood cell (WBC) was significantly increased relative to inactive control subjects after one and two months of study. These parameters were restored to a level comparable to controls in the group of cylists who consumed zobo drink (test group) for one and two months. The levels of erythrocyte superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation (LPO) were significantly elevated in cyclists in the test control group relative sedentary controls after both durations of the study. These parameters were restored toward control values after two months of ingestion of zobo drink. The lactate dehydrogenase activity in the erythrocyte of the cyclist was significantly high with or without zobo ingestion relative to control after both durations of the study. In conclusion, the results obtained indicate that consumption of zobo drink (which is rich in antioxidants and other components) may be beneficial to athletes as it restored the exercise induced alterations of some hematological and biochemical parameters in cyclists under regular training.

INTRODUCTION

Strenous exercise has been reported to cause a dramatic increase in oxygen uptake in various organs, particularly in the skeletal muscle (Alessio, 1993). Physical activity increases the generation of free radicals in several ways, including increased cellular oxidative phosphorylation, catecholamine release, prostanoid metabolism, metmyoglobin release from damaged muscle, and radical release from macrophages recruited to repair damaged tissue (Cooper et al., 2002; Jackson, 1999). Exercise can produce an imbalance between reactive oxygen species (ROS) and antioxidants, which is referred to as oxidative stress. Oxidative stress has been linked to lipid peroxidation of polyunsaturated fatty acids in biological membranes and blood, disturbing cell function (Duthie et al., 1990). It has also been reported to cause damage or destruction of tissue and cell macromolecules, such as lipids, proteins, and nucleic acids (Packer, 1997). Thus, oxidative stress has been associated with decreased physical performance, muscular fatigue, muscle damage, and anaemia. Amelioration of oxidative stress in the body involves a large number of enzymatic and nonenzymatic antioxidants that either prevent ROS formation or scavenge radical species. It has been reported that strenuous physical exercise produces a decrease in antioxidants levels and an increase in the markers of lipid peroxidation (LPO) in target tissues and blood (Powers and Jackson, 2008; Davies et al., 1982). For these reasons, supplementation of antioxidants may be desirable for athletes to reduce oxidative stress and provide protection against its possible consequences (Zoppi et al., 2006; Ji, 1999; Schroder et al., 2000). It is therefore not surprising that antioxidant supplements are now highly marketed for and used by athletes as a means to counteract the oxidative stress of exercise. In addition, a number of synthetic compounds of diverse structure and presumed mechanism of action have displayed significant protection against

exercise-induced oxidative stress (Kerksick and Willoughby, 2005; Williams, 2005). However clinical applications of these compounds are very few owing to their high toxicity at optimum dose level. However, the use of plants and natural products may be beneficial in protecting against free radical induced-damage, as they are less toxic or practically non-toxic compared to the synthetic compounds at their optimum protective dose levels. Flavonoids and hydroxycinnamic acids are plant-derived extracts, which have been shown to possess several biologic properties, many of which may be related, partially at least, to their free radical–scavenging, metal-chelating, and enzyme-inhibiting ability (Cao et al., 1997; Rice-Evans et al., 1996). In Particular, cyanidin and its glycosides are considered dietary compounds with a potential beneficial role for human health (Galvano et al., 2004) and are excellent free radical scavengers and metal chelators (Köhkönen and Heeinononen, 2003). The need to counteract the oxidative stress of exercise with the use of natural products which are less toxic underscores the importance of the present study.

Hibiscus sabdariffa (Roselle) is an annual herb native to tropical Africa reaching up to 2 meters. Extracts from this plant have previously been shown to possess antioxidant properties (Asagba et al., 2007). The dried calyces contain the flavonoids gossypetins, hibiscetine and sabdaretine and 0.004-0.005 % ascorbic acid (Christie, 1984; Perry, 1980). Its major pigment formerly reported as hibiscin, has been identified as daphniphylline. Small amounts of delphinidin 3-monoglucoside, cyanidine 3-monoglucoside (chrysanthenin) and delphinidin are also present (Bernd and Franz, 1990). Juice made by cooking a quantity of calyces with water is used as a cold drink in the West Indies, tropical America, Jamaica, Mexico and Egypt. In Nigeria, the wine coloured drink known as "zobo" is now a favored drink on dinning tables in many homes and for entertaining guests.

With the purpose of studying the effect of ingestion of a drink of zobo in sportsmen, a pilot trial with male cyclists under regular training was carried out. The aim of the study was to evaluate, the effects of short-term consumption of the extracts by male cyclists on a number of biochemical and hematological parameters.

MATERIALS AND METHODS

Preparation of zobo

The dried calyx of H. sabdariffa (180 g) was extracted with 6 liters of boiling water for 1 hour. The extract was then filtered into another clean bowl and made up to 6 liters. The extract was sweetened with sugar (as locally prepared) and refrigerated until the time of use.

Study design

A group of 30 sportsmen (males, age 18-25 years) was selected for the present study. The sportsmen were cyclists under the employment of Edo State Sport Council, Benin City, Nigeria. These cyclists have been engaged in a controlled physical training programme consisting of 3h of training per day for one year during which they were under strict lifestyle particularly with regard to diet. A sex- and age -matched control group of 15 sedentary, healthy individuals who were students of the University of Benin, Benin City, Nigeria were used as controls. The study was conducted in accordance with the Declaration of Helsinki; written informed consent was obtained from each subject participating in the study, which was approved by the ethics committee of the University of Benin Teaching Hospital, Benin City, Nigeria. All subjects were subjected to a routine clinical checkup, including hematology and liver and kidney function tests; they were taking no medication (including vitamin and antioxidant supplements) and were required not to modify their dietary habits during the course of the study. Exclusion criteria were obesity, active smoking, occupational exposure to toxic agents, the use of antioxidant dietary supplementation, and vegetarian dietary habits. The cyclists used for the study were divided into two subgroups with 15 sportsmen each. While each member of one subgroup was recommended to take 300 ml of a drink of zobo at breakfast and bedtime, members of the other subgroup served as a test control. Individuals in this subgroup were also recommended to take same volume of a

drink (placebo) whose composition was basically water with sugar and natural colourants. Individuals in the control group were made to take similar volumes of the same drink offered to the test controls. Both subgroups of cyclists and the controls were biochemically assessed after 1 and 2 months.

Preparation of samples

After one and two months blood samples were collected by venous puncture from the antecubital vein of the volunteers after an overnight fast by means of a sterile needle and syringe. The blood samples were then transferred to heparinized tubes. The plasma was separated by centrifugation at 3000 g for 10 min. The erythrocytes remaining after the removal of the plasma were washed three times with 310 mM isotonic Tris HCl buffer (pH 7.4). Hemolysis was performed by pipetting out the washed erythrocyte suspension into polypropylene centrifuge tubes, which contained 20 mM hypotonic Tris HCl buffer (pH 7.2). The erythrocytes membranes were sedimented in a high speed centrifuge at 20 000 g for 40 min. The supanatant was decanted and subsequently used for biochemical analysis. Some portion of the blood was also collected in EDTA containers and used for hematological analysis.

Biochemical analysis

Erythrocyte superoxide dismutase (SOD) activity was determined essentially by the method described by Misra and Fridovich, (1972) which is an indirect method based on the inhibition by SOD of the auto-oxidation of adrenaline to adrenochrome. The activity of the enzyme was expressed as SOD units, where one unit represents the amount of enzyme that causes 50% inhibition of the autooxidation in 1 min as monitored by colorimetry. The activity of erythrocyte catalase (CAT) was determined by the method of Kaplan and Groves (1972) and was based on residual H_2O_2 after incubation with the samples; each CAT unit specifies the relative logarithmic disappearance of hydrogen peroxide per min and is expressed as K min^{-1} where K is the rate constant for a first order reaction kinetics. Erythrocyte lactate dehydrogenase (LDH) activity was assayed using kit from Quimica Clinica Applicada (Spain). The assay was carried out by incubating the sample with nicotinamide adenine dinucleotide (3mg mL^{-1}) and DL lactic acid (0.45 M) with sodium pyrophosphate buffer, pH 8.8. The activity of LDH is expressed in Units / L. The level of lipid peroxidation (LPO) in the erythrocytes of the cyclists was assayed by the method of Gutteridge and Wilkins (1982). The procedure involved the determination of thiobarbituric acid-reactive substances (TBARS), which are indicators of LPO. Values for TBARS are reported as malondialdehyde (MDA), quantified using a molar extinction coefficient of 1.5×10^5 M^{-1} cm^{-1} and expressed as micromole MDA per ml of erythrocytes.

Hematological analysis

Red blood cell (RBC) counts and white blood cell (WBC) counts were determined in blood using a cell coulter T540 (Coulter Electronic limited) based on the method described by Baker and Silverton (1978). The packed cell volume (PCV) was also determined by the method of Baker and Silverton (1978). This involved the transfer of 0.8 ml of blood into plain capillary tubes. The samples in the capillary tubes were centrifuged in the microhematocrit centrifuge for 5 mins at 5000 g after which the percentage of blood (PCV) was calculated. The hemoglobin (Hb) concentration of the blood samples was determined by the method of Fairbanks and George (1986).

Statistical Analysis: Data are expressed as means ± SEM. Differences between the experimental groups were evaluated using the non-parametric Mann-Whitney U-test. A $p < 0.05$ was considered significant. All statistical calculations were done using STATISTICA version 5.0 computer program.

RESULTS

Table 1 presents the effect of zobo drink on the hematological parameters of male cyclists. The male cyclists in the test contol group who were not offered zobo drink had a significantly reduced hemoglobin concentration, packed cell volume and RBC count after one and two months of study as compared to sedentary control subjects. Conversely the WBC count was significantly elevated after both

durations in this group of male cyclists relative to the same controls. However these hematological parameters were restored to a level comparable with the control in cyclists who ingested zobo drink for one and two months. The study indicates that hematological parameters of male cyclists are responsive to zobo drink after one and two months of consumption.

Table 1. Effect of zobo drink on some hematological parameters in male cyclists

	Experimental groups		
Parameters	CO	TC	TE
One month			
Hemoglobin (g/l)	152.7±8.3[a]	140.8±12.4[b]	155.0±10.5[a]
Packed cell volume (%)	0.48±0.03[a]	0.40±0.04[b]	0.46±0.03[a]
Red cell count (x 10^{12}/l)	5.6±0.4[a]	4.5±0.4[b]	5.8±0.5[a]
White cell count (x 10^{9}/l)	5.5±0.6[a]	10.5±1.8[b]	6.7±3.4[c]
Two months			
Hemoglobin (g/l)	158.0±5.6[a]	145.0±10.5[b]	159±12.0[a]
Packed cell volume (%)	0.47±0.02[a]	0.39±0.04[b]	0.48±0.04[a]
Red cell count (x 10^{12}/l)	5.7±0.3[a]	4.8±0.5[b]	5.9±0.5[a]
White cell count (x 10^{9}/l)	5.3±0.5[a]	10.7±2.0[b]	5.8±0.5[a]

CON= control subjects who are sedentary individuals, TE= test control subjects, who are cyclists not under zobo supplementation, TE = test subjects, who are cyclists under zobo supplementation. Results are presented as Means±SEM. Values with different letters as superscripts are significantly different ($p<0.05$).

The effect of zobo drink consumption on some biochemical parameters in the RBCs of male cyclists under regular training is shown in Table 2. The levels of erythrocyte SOD, CAT and LPO were significantly elevated in cyclists in the test control group who consumed the placebo relative to the sedentary subjects used as controls after both durations of the study. These parameters remained significantly elevated in cyclists in the test group who ingested zobo drink for one month, but were restored toward control values after two months. The erythrocyte LDH activity was significantly high in cyclists with or without zobo ingestion after both durations of the study relative to controls. Thus the results of the study indicate that the levels of SOD, CAT and LPO in the erythrocytes of cyclists were responsive to zobo drink after two months of consumpyion.

Table 2: Effect of zobo on some biochemical parameters in erythrocytes of male cyclists

	Experimental groups		
Parameters	**CO**	**TC**	**TE**
One month supplementation			
SOD (units / ml)	152.0±10.5[a]	180.8±12.4[b]	172.0±24.5[b]
CAT (units / ml)	16.5±1.4[a]	25.0±4.2[b]	24.8±5.0[b]
LDH activity (units / ml)	120±9.5[a]	145.6±10.0[b]	148.0±12.0[b]
LPO (nmoles / ml)	0.25±0.03[a]	0.48±0.04[b]	0.46±0.04[b]
Two months supplementation			
SOD (unit / ml)	148.0±5.6[a]	175.0±10.5[b]	146±12.0[a]
CAT (unit / ml)	15.0±0.8a	32.6±1.8[b]	20.4±2.0[c]
LDH activity (units / ml)	125.4±5.8[a]	140.0±9.2[b]	146.0±10.5[b]
LPO (nmoles / ml)	0.30±0.03[a]	0.46±0.06[b]	0.32±0.07[a]

CON= control subjects who are sedentary individuals, TE= test control subjects, who are cyclists bot under zobo supplementation, TE = test subjects, who are cyclists under zobo supplementation. Results are presented as Means±SEM. Values with different letters as superscripts are significantly different ($p<0.05$).

DISCUSSION

This work presents the findings from the study of the effect of zobo drink on some hematological and biochemical parameters in the blood of cyclists under regular training.

The significant decrease in Hb concentration, packed cell volume and RBC count in the blood of male cyclists in the test control group is in agreement with previous reports on sportsmen (Schumacher et al., 2002; Fallon et al., 1999). It has been demonstrated that these variables are highly sensitive to both acute and chronic effects of exercise. Nevertheless it is pertinent to note that except for the white blood cell count, the hematological status of the cyclist remained within the normal range. The exercise induced decrease in hematological parameters has been attributed to many factors amongst which are plasma volume (PV) expansion which sets in within a few days of prolonged training and increase turnover of RBCs as a result of accelerated destruction by exercise-induced oxidative stress (Schumacher et al., 2002). In this study zobo drink was able to produce useful effects by elevating the PVC, hemoglobin concentration and the RBC count of the male cyclists when they consume the drink in contrast to those who consumed a placebo (test control group).

The mechanism by which the herbal preparation produces the effect on these parameters is presently unclear. Nevertheless some possible mechanisms of action of the extract on these hematological parameters may be by stimulation of erythropoiesis or by increase absorption / utilisation of nutrients which in turn may have a stimulating effect on RBC synthesis.

White blood cells (WBC) occur in large numbers in the blood. Since they are actively involved in the destruction of bacteria, they are therefore part of the body's immune system. Thus the significant increase in WBC count demonstrated in cyclists enlisted for the present study is an indication that exercise may modulate immune function. This finding is corroborated by available experimental evidence which indicate that high intensity exercise has a negative influence on the immune system, affecting the subject health and performance (Romero et al., 2010). In the present study, it was shown that, short term ingestion of zobo drink restored the WBC count of male cyclists under regular training for one year to a level comparable to sedentary control subjects. Thus it is concievable that supplementation with zobo drink may be an excellent strategy for improving the immune system of athletes under high intensity exercise.

The high LDH activity observed in the RBCs of cyclists with or without zobo consumption infers a rise in energy generation since RBCs derive their energy entirely via glycolysis releasing lactate into the plasma for liver to generate glucose for its constant fuel supply. LDH is thus elaborated in mature RBCs and its activity would be directly related to the level of energy production. Low energy availability to RBCs will make such cells to become easily turgid and lyse. Besides, it will also increase the propensity for their destruction by ROS. It was not surprising therefore that the significant increase in erythrocyte LPO of the cyclists was associated with a significant increase in LDH.

MDA is one of the major oxidation products of peroxidized polyunsaturated fatty acids of membranes and increased MDA content is an important indicator of cell membrane LPO. The significantly increased level of LPO observed in the erythrocytes of cyclists who consumed a placebo is an indication of oxidative stress. It therefore follows that oxidative stress/damage measured frequently in blood after exercise or any other experimental intervention derives, at least in part, from the erythrocytes in blood. Physical exercise can cause oxidative stress in erythrocytes because of increased generation of ROS, which may trigger antioxidant enzymes such as SOD and CAT to enhance their activities and reduce ROS to safe compounds (Lekhi et al., 2007; Powers and Jackson, 2008). Thus the significantly increased erythrocyte SOD activity of the cyclists under study may be due to increased level of MDA occasioned by increased generation of ROS. A similar increase in antioxidant enzymes in response to oxidative stress has been reported in the lymphocytes of recreationally active males after high intensive interval training (Fisher et al., 2010). It is noteworthy that an increase in erythrocyte SOD was followed by a corresponding increase in CAT in cyclists not under zobo supplementation. This is not surprising as SOD controls damage caused by free radicals, which reduces the superoxide anion to peroxide, which can be removed by CAT (Lukaski et al., 1990). Thus the similarities in the profiles of SOD and CAT activities observed in the erythrocytes of these cyclists (Table 2) are consistent with the observation that both enzymes which are involved in peroxide catabolism are not only functionally linked, but also occur in tandem (Bartkowiak and Bartkowiak, 1981; Halliwell, 1994). The decreased erythrocyte MDA level in cyclists under zobo supplementation for two months is an indication that the herbal drink contains antioxidant components and this may have also accounted for the return of erthrocyte SOD and CAT activities towards control values. An improvement in the antioxidant state and a decrease in the oxidative damage induced by exercise with the ingestion of soy beverage have been demonstrated by Rossi et al (2000). These investigators suggest that this improvement is attributable to the isoflavones (a flavonoid) of the soybean used in preparing the drink. The known antioxidant components in H. Sabdar-

iffa from which zobo drink is prepared include flavonoids and vitamins such as vitamin C and E (Christie, 1984). Because oxidative damage may occur with exercise, antioxidant administration has drawn much attention both in terms of preventing damage and in terms of affecting performance in athletes. It has been suggested that oxidative stress might play a role on the fatigue process, and antioxidant administration might reduce the fatigue, leading to an increase in performance (Chen et al., 2002).

In conclusion, although preliminary, our results clearly demonstrate that consumption of zobo drink (which is rich in antioxidants and other components) is able to decrease oxidative stress in erythrocytes, prevent anemia and improve the immune system of athletes such as cyclists under regular training exercise. However, further study is necessary to establish the optimal dose of zobo drink required to produce a beneficial effect not only on the antioxidant defense system but also on the hematological and immune systems.

REFERENCES

Alessio, H. (1993) Exercise-induced oxidative stress. *Medical Science & Sports Exercise*. **23**: 218-224.

Asagba, S.O., Adaikpoh, M.A., Kadiri, H. and Obi, F.O. (2007) Influence of aqueous extract of Hibiscus sabdariffa L Petal on cadmium toxicity in rats. *Biological Trace Element Research* **115**: 47 -57.

Baker, F.J. and Silverton, R.E. (1978) Introduction to medical laboratory technology, 5th edn, *The English language Book Society and Butterworths,* London.

Bartkowiak, A. and Bartkowiak, T. (1981) Superoxide dismutase and catalase activities in normal and cancerous tissues. *Comparative Biochemistry & Physiology* **70b**: 819-820.

Bernd, M. and Franz, M.J. (1990) Hibiscus flowers a mucillage drug? *Journal of Agriculture & Food Chemistry* **1**: 116D-118D.

Cao, G., Prior, R.C., Cutler, R.G. and Yu, B.D. (1997) Effect of dietary restriction on serum antioxidant capacity in rats. *Archive of Gerontology & Geriatrics* **25**: 245-253.

Chen, Y.C., Holtzman, G.I. and Bakhit, R.M. (2002) High- genistin isoflavone supplementation modulate erythrocyte antioxidant enzymes and increased running endurance in rats undergoing one session of exhausting exercise. A pilot study. *Pakistan Journal of Nutrition* **1 (1):** 1-7.

Christie, B.R. (1984) *The handbook of plant science in agriculture*, CRC, Boca Raton.

Cooper, C.E., Vollard, N.B., Choueri, T. And Wilson, M.T (2002) Exercise, free radicals and oxidative stress. *Biochemical Society Transactions* **30**: 280-283.

Davies, K.J.A., Quintanitha, A.T., Brooks, G.A. and Packer, L (1982) Free radicals and tissue damage produced by exercise. *Biochemistry & Biophysics Research Communications* **107** (4): 1198-1205.

Duthie, G.G., Robertson, J.D., Maughan, R.J. and Morrice, P.C. (1990) Blood antioxidant status and erythrocyte lipid peroxidation following distance running. *Archives of Biochemistry & Biophysics* **282** (1): 78-83.

Fallon, K.E., Sivyer, G., Sivyer, K. And Dare, A. (1999) Changes in hematological parameters and iron metabolism associated with 1600 kilometers ultra marathon. *British Journal of Sports Medicine* **33**: 27-32.

Fairbanks, V.F. and George, G.K. (1986) *Biochemical aspects of haematology*, In: textbook of clinical chemistry (Tietz, N.W. ed), *W.B.Saunders Company,* Philadelphia, pp1533-1534.

Fisher, G., Schwartz, D.D., Quindry, J.C., Barberio, M. D. and Foster, E.B. (2011) Lymphocyte enzymatic antioxidant responses to oxidative stress following high intensity interval exercise. *Journal of Applied Physiology*. **110 (3)**: 730-737.

Galvano, F., La Fanci, L., Lazzarino, G., Fogliano, V., Ritieni, A., Ciapellano, S et al., (2004) Cyanidins: metabolism and biological properties. Journal *of Nutri-*

tional Biochemistry **15**: 2-11

Gutteridge, J.M.C. and Wilkins, S. (1982). Copper-dependent hydroxyl radical damage to ascorbic acid. Formation of thiobarbituric acid reactive product. *FEBS Letters*. **337**: 327-330.

Halliwell, B. (1994) Free radicals and antioxidants. A personal view. *Nutrition Reviews* **52**: 253-256.

Jackson, M.J. (1999) Free radicals in skin and muscle: damaging agents or signals for adaptation? *Proceedings of Nutrition Society* **58**: 673-676.

Ji, L.L. (1999) Antioxidants and oxidative stress in exercise. *Proceedings of Society of Experimental Biology & Medicine* **222**: 283-292.

Kaplan, J.H Groves JN (1972) Liver and blood cell catalase activity in tumor-bearing mice. *Cancer Research*. **32**: 1190-1194.

Kerksick, C. and Willoughby, D. (2005) The antioxidant role of glutathione and N-acetyl cysteine supplements and exercise-induced oxidative stress. *Journal of International Society of Sports Nutrition* **2 (2):** 38-44.

Köhkönen, M.P. and Heinonen, M. (2003) Antioxidant activity of anthocyanins and their aglycons. *Journal of Agriculture & Food Chemistry* **51**: 628-633.

Lekhi, C., Gupta, P.H. and Singh, B (2007) Influence of exercise on oxidant stress products in elite Indian cyclists. *British Journal of Sports Medicine* **41**: 691-693.

Lukaski, H.C., Hoverson, B.S., Gallagher, S.K. and Bolonchuk, W.W. (1990) Physical training and copper, iron and zinc status of swimmers. *American Journal of Clinical Nutrition* **51**: 1093-1099

Misra HP, Fridovich I (1972) The role of superoxide ion in auto-oxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological. Chemistry*. **247**: 3170-3175.

Packer, L. (1997) Oxidants, antioxidant nutrients and the athlete. *Journal of Sports Science* **15**: 353-363.

Perry, L.M. (1980) *Medicinal plants of East and Southern Asia*. MIT Press, Cambridge.

Powers, S.K. and Jackson, M.J. (2008) Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiological Reviews* **88**: 1244-1276.

Rice-Evans, C.A., Miller, N.J. and Paganga, G (1996) Structure antioxidant activity relationships of flavonoids and phenolic acids. *Free Radicals in Biology & Medicine* **20**: 933-956.

Romero, F.G., Lira, F.S., Marques, F.A., Muzy, P.C., Peres, A.N.R. and Caperuto, E.C. (2010) PAKS supplement improves imune status and body composition but not muscle strength in resistance trained individuals. *Journal of International Society of Sports Nutrition* **7**:36

Rossi, A.L., Blostein-Fujii, A. and DiSilvestro, R.A. (2000) Soy beverage consumption by young men: increased plasma total antioxidant status and decreased acute, exercise-induced muscle damage. *Journal of Nutraceuticals, Functional & Medical Foods* **3(1)**: 279-291.

Schroder, H., Navarro, E., Tramullas, A., Mora, J. and Galiono, D (2000) Nutrition antioxidant status and oxidative stress in professional basketball players: effects of a three compound antioxidative supplement. *International Journal of Sport Medicine* **21**: 146-150.

Schumacher, Y.O., Schmid, A., Grathwohl, D., Bultermann, D. and Berg, A. (2002) Hematological indices and iron status in athletes of various sports and performances. *Medicine & Science in Sports & Exercise* **34 (5):** 869-875.

Williams, M. (2005) dietary supplements and sports performance: amino acids. *Journal of International Society of Sports Nutrition* **2 (2)**: 63-67.

Zoppi, C.C., Hohl, R., Silva, F.C., Lazarim, F.L., Neto, J.M.F.A. Stancanneli, M. And Macedo, D.V. (2006) Vitamin C and E supplementation. Efect in professional soccer players under regular training. *Journal of International Society of Sports Nutrition* **3 (2):** 37-44.