European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 05, Issue 01 , 2018

EFFECT OF AN ANTIOXIDANT-BASED FOOD SUPPLEMENT AND PHYSICAL ACTIVITY ON HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN AGING RATS

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Abstract:

Basella alba has hypo-glycemic activity. The present study evaluated the benefits of the natural antioxidant, Basella alba, in treating the effect of age-related oxidative stress. Male Wistarrats of 3- , 12- and 18-months of age received a daily oral supplement of Basella alba for a period of 3 months. At the end of this period, markers of oxidative stress were assessed in the erythrocytes. Basella alba lowered oxidative stress in erthrocytes. These results demonstrated the antioxidant activity of Basella alba probably confers the capacity to reduce oxidative stress in erythrocytes of aging rats. They also suggest a critical role for Basellain reducing blood glucose level and better haemoglobin content.

Keywords: aging,antioxidant, basella alba, erythrocytes, haemoglobin, haematocrit.

Introduction

Studies have suggested that low-fat, plant-based diets reduce body weight, improve cardiovascular risk factors and glycemic control, and, in combination with other lifestyle interventions, reverse atherosclerosis(Kennedy et al., 2001, Barnard et al., 2000). Use of plants as a source of medicine has been an ancient practice and is an important component of the health care system in India as well as other Asian countries. Medicinal plants contain numerous biologically active compounds such as carbohydrates, proteins, enzymes, fats and oils, minerals, vitamins, alkaloids, quinones, terpenoids, flavonoids, carotenoids, sterols, simple phenolic glycosides, tannins, saponins, and polyphenols (Adhikari et al., 2012). Traditional plant medicines might offer a natural key to treat various human ailments. In recent years, there has been an increasing interest by researchers in the use of naturally occurring biologically active compounds of medicinal value (Anandarajagopal et al., 2011). Numerous medicinal plants and their formulations are used for various disorders in ethno-medical practices as well as in the traditional system of India. Increasing attention is being paid by consumers to the health and nutrition aspects such as vitamin content, mineral elements and antioxidants of horticultural products (Scalzo etal., 2005). Interest in the role of antioxidants in human health has promoted research in the field of horticulture and food science to evaluate fruit and vegetable antioxidants and to determine how their content and activity can be maintained or even improved (Ayala-Zavala et. al., 2004). In

the Indian system of medicine, most practitioners formulate and dispense their own recipes; hence this requires proper documentation and research. The supplement can improve the antioxidant defence system in the erythrocytes in aging rats and play a role in glycemic control.

Objectives**:** The purpose of this study was to evaluate the effects of B.alba and swim training on the extent of changes in the oxidative stress indices, in the erythrocytes and certain blood parameters such as haematocrit and haemoglobin levels, and moreimportantly on the hypoglycaemic effect of the two combined interventions as a function of age.

Materials and Methods

Animal care and maintenance

Rats were categorized into four sub-groups. Sedentary control, SE-C; sedentary supplemented, SE-C(S); swim trainees at thermoneutral temperature, SW-T(N) and supplemented swim trainees at thermoneutral temperature, SW-T(N+S).

Supplementation and swim training of animals

Physical activity includedswim exercise training. Rats of the trainee groups were swim exercised for 30 min day^{-1} for 5 days $week^{-1}$ and for a total period of 45 days with a load of 3% body weight to avoid passive floating in the adult, late-adult and middle-aged rats. Group swimming was chosen because rats as a group swim actively and a more vigorous activity is achieved compared to those that swim alone. Initial exposure to water was accompanied by vigorous movements during the first few minutes. The rats of the supplemented groups received B.alba leaf extract dissolved in distilled water at the dose of 800 mg/Kg body weight orally before swim training.

Biochemical studies

Oxidative stress indices in erythrocytes

MDA was measured by the method of Ohkawa et al. (1979) and Prc by the method of Levine et al. (1990).

Antioxidant enzymes in erythrocytes

SOD activity was determined by the method ofMisra and Fridovich, (1972), while CAT activity was measured by the method of Aebi et al. (1984).

Determination of Haemoglobin

Hb content was measured using Hemocor-D Kitwhich utilizes cyanmethemoglobin method. Whole blood was incubated with Hb reagent at RT for 3 min and absorbance was measured colorimetrically at 540 nm. Hb concentration was represented in terms of g/dl.

Determination of Haematocrit (Hct)

The blood collected in EDTA-coated tube was drawn into the heparinized capillary tubes by suction, leaving at least 15 mm unfilled. The tube was then carefully sealed by plasticine and centrifuged at 1000 g for 3 min (RM-12C Microcentrifuge). The RBCs are

heavier (sp. gr. about 1090) than the plasma (sp.gr. about 1030). When blood is placed in a long tube and is centrifuged the cells settle down and pack themselves because of the centrifugal force. The tube shows an uppermost layer of plasma. It is pale straw yellow or amber colored separted by a buffy coat or whitish thin layer consisting of the platelets above and the leucocytes below. The white cell layer is separated from the lower red cell layer by a black line. This line marks the upper limit of the red cell layer. The PCV is read as a percentage directly on the haematocrit reader.

Blood glucose

Whole blood glucose was measured using Accu-Chek Active- blood glucose monitor.

Statistical Analyses

Data are shown as mean ± S.E.M of five animals /sub-group. Changes between the sub-groups and age groups of swim trained rats in thermoneutral waters and supplemented trainees were compared with their respective sedentary. Data was further analyzed by two-way ANOVA and tested by Bonferroni post-hoc test using GraphPad Prism 5 and $p < 0.05$ was considered significant.

Results

Blood parameters

Glucose : The blood glucose level showed a significant reduction in the swim trainees by 12% (3-mo), 12% (12-mo) and 10% (18-mo) and by 24% (3-mo), 22% (12-mo) and 17% (18-mo) in supplemented trainees compared to their respective sedentarys by the end of the swim training regime (Table 1).

Table 1. Effect of swim training and B.alba supplementation on blood glucose, Hb, Hct

Sub-groups	3 [A]	12 [B]	18 [C]
	Blood Glucose (mg/dl)		
SE-C	98 ± 0.46[a]	108 ± 0.92 [a]	120 ± 0.92 [a]
SE-C(S)	95 ± 0.21[a]	104 ± 2.30[a]	113 ± 0.69[b]
SW-T(N)	86 ± 0.46[b]	95 ± 1.30[b]	108 ± 0.46[c]
SW-T(N+S)	74 ± 2.30[c]	84 ± 0.46[c]	99 ± 0.46[d]
	Haemoglobin (g/dl)		
SE-C	12.09 ± 0.01 [a]	11.30 ± 0.05 [a]	10.75 ± 0.06 [a]
SE-C(S)	13.16 ± 0.01[a]	12.45 ± 0.02[a]	12.50 ± 0.08[a]
SW-T(N)	15.52 ± 0.01[b]	14.03 ± 0.02 [b]	13.59 ± 0.03 [bc]
SW-T(N+S)	17.65 ± 0.01[c]	16.78 ± 0.04[c]	14.61 ± 0.03[c]
	Haematocrit (%)		
SE-C	37.50 ± 0.69 [a]	34.30 ± 0.27 [a]	33.6 ± 0.46 [a]
SE-C(S)	39.66 ± 0.27[a]	37.50 ± 1.25[b]	35.0 ± 0.69[a]
SW-T(N)	41.00 ± 0.47 [b]	40.00 ± 0.60 [c]	39.0 ± 0.46 [b]
SW-T(N+S)	43.00 ± 0.47[b]	42.00 ± 0.47[c]	41.0 ± 0.69[b]

Values are mean ± SEM (n=5 rats/sub-group). SE-C, Sedentary control; SE-C(S), supplemented sedentary; SW-T(N), swim trainees in thermoneutral water; and SW-T(N+S), supplemented trainees in thermoneutral water. A two-way ANOVA with Bonferroni's post-hoc test was used to compare between the age groups and significance is represented in upper case, and between the sub-groups in lower case. Those having dissimilar upper and lower cases are significantly different at $p < 0.05$.

Hb and Hct

Significant increase in Hb was seen in the swim trainees by 28%, 24% and 26% and by 46%, 48% and 35% in supplemented trainees with respect to their sedentary counterparts of 3-, 12- and 18-months of age (Table 1).

Significant increase in haematocrit was seen in the swim trainees by 9%, 16% and 16% and by 15%, 22% and 22% in supplemented trainees with respect to their sedentary counterparts of 3-, 12- and 18-months of age (Table 1).

Antioxidant enzymes in erythrocytes

Superoxide dismutase

Increases in SOD activity by 33%, 28% and 19% and by 50%, 48% and 38% were observed in the erythrocytes of swim-trained and supplemented swim trainees compared to their respective sedentary counterparts in the 3-, 12- and 18-month old rats(Table 2).

Catalase

There was a significant increase in the CAT activity in both the swim trained and supplemeted swim trainees by 17%, 13% and 17% and by 23%, 27% and 20% respectively with respect to their sedentary counterparts of all the three age groups (Table 2).

Oxidative stress indices

Lipid peroxidation (LPO)

Malondialdehyde in the erythrocytes was assayed as a measure of LPO. MDA decreased with swim exercise by 36%, 25% and 21% and a further decrease by 54%, 41% and 38% in the supplemented swim trainees when compared to their sedentary animals of adult, late-adult and middle-aged rats (Table 2).

Protein oxidation (PO)

The PrC level decreased by 52%, 40% and 33% in the swim trainees and by 63%, 49% and 44% in their supplemented ones with respect to their respective sedentarys of all 3-, 12- and 18-mo-old (Table 2).

Table 2. Effect of swim training and B.alba supplementation on erythrocytes

	Age (months)		
Sub-groups	**3** ^{A}	**12** ^{B}	**18** ^{C}

SOD (U/g Hb)			
SE-C	2425 ± 7.5[a]	2646 ± 9.0 [a]	2944 ± 8 [a]
SE-C(S)	2530 ± 7.5[b]	2736 ± 5.2[b]	3064 ± 15[b]
SW-T(N)	3234 ± 2.8[c]	3375 ± 10 [c]	3528 ± 12 [c]
SW-T(N+S)	3648 ± 4.6[d]	3926 ± 7.8[d]	4086 ± 19[d]
CAT (mmol/mg protein/min)			
SE-C	0.178 ± 0.005 [a]	0.210 ± 0.004 [a]	0.317 ± 0.006 [a]
SE-C(S)	0.181 ± 0.005[a]	0.213 ± 0.003[a]	0.327 ± 0.005[a]
SW-T(N)	0.209 ± 0.004 [b]	0.238 ± 0.005 [b]	0.372 ± 0.004 [b]
SW-T(N+S)	0.219 ± 0.009[b]	0.267 ± 0.007[c]	0.381 ± 0.003[b]
MDA(nmol/g Hb)			
SE-C	7.24 ± 0.5 [a]	13.46 ± 0.2 [a]	13.46 ± 0.4 [a]
SE-C(S)	5.48 ± 0.7[b]	11.69 ± 0.2[b]	13.11 ± 0.2[a]
SW-T(N)	4.64 ± 0.6 [c]	9.87 ± 0.1 [c]	10.57 ± 0.3 [b]
SW-T(N+S)	3.31 ± 0.7[c]	7.75 ± 0.2[d]	8.24 ± 0.9[c]
PrC(nmol/mg protein)			
SE-C	14.7 ± 0.2 [a]	18.1 ± 0.2[a]	23.1 ± 0.4 [a]
SE-C(S)	10.0 ± 0.2[b]	16.5 ± 0.8[b]	20.6 ± 0.1[b]
SW-T(N)	7.00 ± 0.6 [c]	10.8 ± 0.3 [c]	15.5 ± 0.2 [c]
SW-T(N+S)	5.42 ± 0.6[d]	9.20 ± 0.2[d]	13.0 ± 0.2[d]

Values are mean ± SEM of five animals /sub-group. Sedentary control, SE-C; sedentary supplemented, SE-C(S); Swim trained at thermoneutral temperature, SW-T(N); supplemented swim trainees at thermoneutral temperature, SW-T (N+S). A two-way ANOVA with Bonferroni's post-hoc test was used to compare between the age groups and significance is represented in upper case, and between the sub-groups in lower case. Those having dissimilar upper and lower cases are significantly different at $p<0.05$.

Discussion

Interest in the role of antioxidants in human health has promoted research in the field of horticulture and food science to evaluate fruit and vegetable antioxidant capacity. The present study was aimed at understanding the combined effects of exercise-training and B.alba supplementation in retarding age-related oxidative modification of lipids and proteins and analyzing the status of OS and antioxidative defence in the erythrocytes.

Aging is associated with elevated levels of OS. In this study, preliminary results are provided in support of our hypothesis that exercise training and B.alba supplementation can alleviate OS to a better extent in the aging rats than any one of the single interventions.

B.alba is also a potent antibiotics, antihypertensives and blood building agents. B.alba is used traditionally in countries like Thailand for anti-inflammatory, cytotoxicity and antioxidant activities of anti-inflammatory remedies. The leaf extracts (methanolic extract and aqueous extract) of Basella alba have been investigated for in-vitro anti-inflammatory activity by human red blood cell membrane stabilization method and it has been concluded that aqueous extract has significant in vitro anti-inflammatory activity compared to the methanolic extract (Siriwatanametanen et al., 2010). Further these investigators have shown

that in-vitro anti inflammatory activity of the extracts are concentration-dependent, with the activity increasing with concentration. Studies on erythrocytes have shown that leaf extracts havemembrane stabilization effect by inhibiting hypotonicity-induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extracellular release (Abhay, 2010).
The present study showed increased haemoglobin levels with supplementation of B.alba. The noticeable increase in the Hb of supplementees is consistent with that reported by Bamidele et al. (2010) on male albino rats supplemented with aqueous leaf extract of B.alba. Considering the composition of B.alba leaf extracts that include proteins, fats, vit C and vit K along with minerals such as calcium, magnesium and iron (Grubben and Denton, 2004; Duke and Ayensu, 1985) that are well known hematinics are essential for the formation of red blood cells (Ganong, 2005; Alada et al., 2004). Similar increases in Hb concentration and packed cell volume have been reported for protein-rich diets in humans and animals (Alada et al., 2004; 2000).

In the present study, we observed that, treatment with B.alba leaf extract reduced the blood glucose compared to the unsupplemented sedentary. However, a combination of supplementation and swim training was more effective in alleviating blood glucose level. These results are similar to the studies on male diabetic albino rats supplemented with Basella rubra, where Basella extract showed hypoglycemic action by significantly reducing blood glucose level in the diabetic rats compared with the non-diabetic controls (Nirmala et al., 2009).

Collectively, the results on the responses of the aging rats muscles to exercise and supplementation with Basella suggests that B.alba as a antioxidant rich in polyphenols and flavanoids, can reduce the oxidative stress while improving the adaptive capacity to exercise of aerobic type. In conclusion, the preliminary studies and the results that have been reported here on the benefits of combining swim training and B.alba leaf extract supplement alleviating oxidative stress with concomitant amelioration of the antioxidant enzymes especially in the middle-aged.

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