The Relationship Between Alanerv® Consumption and Erythrocytes’ Redox Status in Post-Acute Stroke Patients Undergoing Rehabilitation

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ABSTRACT

Objectives: Stroke is a pathological condition associated with a redox imbalance. Both the acute and the post-acute phases after a stroke are characterized by a pro-oxidant state, which could be corrected through antioxidant supplementation. The aim of the present study was to evaluate the effect of the ALAnerv® upon the redox status of erythrocytes.

Material and Methods: For this pilot study were enrolled 28 post-acute stroke patients. They were randomly divided into a control group [(-) ALA] and a study group [(+) ALA]. Patients were hospitalized for a period of two weeks. Blood samples were taken at the beginning and at the end of this period. Patients from (+) ALA group received the nutritional supplement ALAnerv®. Catalase, SOD, GPx, GRed and GT activities were assessed on erythrocytes’ lysates. Also, the total antioxidant capacity as well as the concentration of total thiols were evaluated.

Outcomes: During the study period only SOD (1393.48 ± 69.14 U/g Hb vs. 1514.06 ± 60.80) and GRed (4.47 ± 0.37 U/g Hb vs. 5.06 ± 0.24) significantly increased in (+) ALA group. Catalase (173.70 ± 14.62 k/g Hb vs. 123.41 ± 8.71) and GT (5.55 ± 0.26 U/g Hb vs. 4.95 ± 0.23) activities significantly decreased during the study period. Multiple regression indicated that only SOD, GT and GRed activities were influenced by the ALAnerv® consumption. There was no significant increase if the total thiols concentration.

Conclusions: ALAnerv® consumption could be associated with a correction of the erythrocytes’ redox status. There is a need to investigate if administration of ALAnerv® over a longer time period could have a more significant influence on the erythrocytes’ antioxidant system.

Keywords: glutathione reductase, superoxide dismutase, glutathione transferase, catalase, stroke, lipoic acid

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INTRODUCTION

Both acute and post-acute phases following a stroke are characterized by an overproduction of oxygen and nitrogen reactive species (ROS/RNS) (1-4). This is due to the loss of balance between the sources of ROS/RNS and the enzymatic and non-enzymatic antioxidants, in the favour of the former. As a consequence, there is an accumulation of ROS/RNS with deleterious effects on cells and tissues (5).

An important part of the blood antioxidant capacity is provided by erythrocytes. In a clinically healthy individual, these cells comprise almost a quarter of the total number of body’s cells (6,7). Erythrocytes are a special case as they deliver O2 to cells and CO2 to the lungs. During the deoxygenation of haemoglobin the superoxide anion can be formed. As a consequence of their physiological role, erythrocytes are under a permanent exposure to ROS formation. In order to maintain a redox balance they developed an antioxidant system, which consists of antioxidant enzymes, as well as non-enzymatic antioxidants (8).

The antioxidant enzymes include, among others, catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GRed) and glutathione transferase (GT) (8).

Some of the non-enzymatic antioxidants are endogenously synthesized (i.e., reduced glutathione), while the others are taken up from the extracellular space (α-tocopherol, vitamin C, lipoic acid, flavonoids) (8).

Also, the erythrocytes’ metabolism is adapted to ensure the production of some antioxidants, as well as to keep in function the antioxidant enzymes. Thus, glycolysis and the hexose monophosphate shunt are the two major metabolic pathways that use glucose in erythrocytes. The first one is involved in the ATP and NADH production, while the second one ensures the synthesis of NADPH.

All the aforementioned antioxidants cooperate to maintain the redox balance. Thus, SOD converts the superoxide anion to H2O2, which is then cleared through the action of catalase and GPx. GPx uses reduced glutathione which is converted to its oxidized form. This one is converted back to the reduced form through the action of the GRed, a NADPH-dependent enzyme. Also, the exogenous low-molecular antioxidants are involved in the clearance of different types of ROS/RNS.

NADH is the main source of reducing equivalents for methaemoglobin reduction to haemoglobin, even if there is also a minor pathway that uses NADPH (9).

Thrombolysis is the only therapeutic intervention currently approved by the FDA (Food and Drug Administration) for the treatment in stroke patients. Moreover, it is effective only if applied in a narrow time window of 4.5 hours from the symptoms’ onset (10).

As a consequence, there are many studies concerning the development of alternative therapies. One such approach is to try to correct the redox balance in both acute and post-acute phases after a stroke (11-13).

OBJECTIVE

This study was intended to evaluate the effect of ALAnerv® consumption on the erythrocytes’ redox status in post-acute stroke patients undergoing rehabilitation. In order to achieve this aim we assessed the activities of the following enzymes: catalase, SOD, GRed, GPx and GT. Total thiols concentration was also evaluated.

MATERIAL AND METHODS

Design and subjects

The study included 28 post-acute stroke patients. The subjects admitted in the study were randomly divided into the control group [(-) ALA], and the study group [(+) ALA], respectively.

The diagnosis of an ischemic or hemorrhagic stroke during the previous 90 days before the enrolment in the study was used as inclusion criterion for both groups. Exclusion criteria were considered the following conditions: cancer pathology, chronic renal failure, chronic inflammatory state, autoimmune and haematological disorders. Also, smoking and chronic alcohol consumption, as well as treatment with vitamins and anti-inflammatory drugs during the two months preceding the beginning of the study were also considered as exclusion criteria. The patients with a previous diagnosis of a cerebrovascular event (cerebral haemorrhage, hemorrhagic infarct, transient ischemic attack) were excluded from the study.
The relationship between ALAnerv® consumption and erythrocytes’ redox status

During two weeks all the patients were hospitalized and were subject to a standard rehabilitation procedure.

Patients from the (+) ALA group received ALAnerv® (2 pills/day) during the study period. Before the beginning of the study, written informed consent was obtained from all patients or from their relatives. This study complies with the established ethical principles (World Medical Association Declaration of Helsinki, version VI, 2002). It was approved by the ethics review boards of the National Institute of Rehabilitation, Physical Medicine and Balneoclimatology and „Elias” Emergency Hospital, Bucharest (Romania).

ALAnerv® composition description

According to the manufacturer specification sheet, one soft gelatine capsule of ALAnerv® contains: α-lipoic acid (300 mg), Borago officinalis (300 mg) which contains 180 mg polyunsaturated fatty acids (linoleic acid and gamma-linolenic acid), D-α-tocopherol on sunflower oil basis (11.177 mg) which contains 7.5 mg vitamin E, thiamine mononitrate 1.259 mg (equivalent of 1.05 mg vitamin B1), riboflavin 1.320 mg (equivalent of 1.2 mg vitamin B2), calcium pantothenate 5.396 mg (equivalent of 4.5 mg vitamin B5), pyridoxine hydrochloride 2.010 mg (equivalent of 1.5 mg vitamin B6), selenomethionine 0.069 mg with 25 μg selenium, fatty acids triglycerides (60 mg), magnesium stearat (14 mg), polyglycerol oleate (10 mg), soya oil and soya lecithine complex (6 mg), food gelatin (177.940 mg), glycerol (82 mg), titanium dioxide (1.520 mg), iron red oxide (0.130 mg).

Rehabilitation program

The detailed rehabilitation program was previously was followed as previously described elsewhere (14). The Barthel Index (BI) scale was used to evaluate the efficiency of the rehabilitation program (15).

Blood samples

From each patient enrolled in this study two blood samples were taken: at the beginning (baseline) and at the end (discharge) of the study. Blood samples were collected from all subjects after an overnight fasting. The samples collected with an anticlotting agent were allowed to stand 20 minutes at room temperature. Plasma and erythrocytes were separated through centrifugation (5000 rpm, 15 minutes, 4°C).

Erythrocytes’ preparation

The erythrocytes were washed three times with four volumes of a phosphate buffer – saline solution made by mixing one volume of sodium phosphate buffer (100 mM, pH 7.4) with nine volumes of NaCl (0.9%). Then, a 0.5% lysate was obtained through adding over 100 μL of erythrocytes 100 μL of cold redistilled water previously treated with Chelex® 100. Finally, the 50% lysates were stored in 1.5 mL Eppendorf tubes at -80°C until analysis.

Reagents

Reduced L-glutathione, 5,5’-dithio-bis-(2-nitrobenzoic acid), ethanol (95%), Tris, cacodylic acid, diethylene triamine pentaaetic acid (DTPA), pyrogallol, H₂O₂ (37%), 1-chloro-2,4-dinitrobenzene (CDNB), potassium phosphate (KH₂PO₄), potassium ferricyanide, and potassium cyanide were purchased from Sigma-Aldrich (St. Louis, Mo., USA). All the reagents were Ph. Eur. (p.a.).

GRed and GPx were assessed using kits brought from Randox (Randox Laboratories Limited, UK).

### TABLE 1. Demographic, comorbidities and medication of the study groups.

<table>
<thead>
<tr>
<th></th>
<th>(-) ALA</th>
<th>(+) ALA</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>67.1 (2.9)</td>
<td>64.0 (2.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Sex ratio (females/males)</td>
<td>7/7</td>
<td>7/7</td>
<td>NS</td>
</tr>
<tr>
<td>Time from stroke (range, days)</td>
<td>36.1 ± 5.5 (12-86)</td>
<td>48.6 ± 7.4 (21-91)</td>
<td>NS</td>
</tr>
<tr>
<td>Stroke subtypes (IS/HS)</td>
<td>12/2</td>
<td>11/3</td>
<td>NS</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension N (%)</td>
<td>11 (78.6)</td>
<td>10 (71.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus N (%)</td>
<td>2 (14.3)</td>
<td>6 (42.9)</td>
<td>0.044</td>
</tr>
<tr>
<td>Dyslipidemia N (%)</td>
<td>7 (50.0)</td>
<td>5 (35.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Coronary ischemic disease N (%)</td>
<td>4 (28.6)</td>
<td>5 (35.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Carotid atherosclerosis N (%)</td>
<td>7 (50.0)</td>
<td>3 (21.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statins N (%)</td>
<td>11 (78.6)</td>
<td>8 (57.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Antiplatelet agents N (%)</td>
<td>3 (21.4)</td>
<td>3 (21.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Antithrombotic agents N (%)</td>
<td>6 (42.9)</td>
<td>4 (28.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Anticoagulant agents N (%)</td>
<td>2 (14.3)</td>
<td>4 (28.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Antidepressive agents N (%)</td>
<td>6 (42.9)</td>
<td>9 (64.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Antiacids N (%)</td>
<td>7 (50.0)</td>
<td>8 (57.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Anti hypertensive agents N (%)</td>
<td>7 (50.0)</td>
<td>8 (57.1)</td>
<td>NS</td>
</tr>
<tr>
<td>ACE inhibitors N (%)</td>
<td>7 (50.0)</td>
<td>6 (42.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Beta blockers N (%)</td>
<td>9 (64.3)</td>
<td>7 (50.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Acetylsalicylic acid N (%)</td>
<td>7 (50.0)</td>
<td>3 (21.4)</td>
<td>NS</td>
</tr>
</tbody>
</table>

* (-) ALA vs. (+) ALA; IS, ischemic stroke; HS, hemorrhagic stroke; NS, non significant.
The cyanomethaemoglobin standard used to assess haemoglobin’s concentration was purchased from Spinreact (Spinreact, Spain).

Biochemical assays

All the assays were performed on erythrocyte lysates. The results were divided by the haemoglobin concentration. All the biochemical tests were done in duplicate and were expressed as mean values.

The activity of CAT was evaluated according to the method of Aebi (16). The decomposition of H$_2$O$_2$ was followed at 240 nm for 30 seconds. The activity was calculated using the rate constant of a first-order reaction (k). The results are expressed as k/g Hb.

The activity of SOD was evaluated according to the method of Marklund (17). The inhibition of pyrogallol autoxidation was followed for 3 minutes at 420 nm. One unit of SOD is defined as the amount of enzyme that inhibits 50% the autoxidation rate of pyrogallol. The results are expressed as U/g Hb.

The activity of GT was measured according to the method of Habig (18). The conjugation of CDNB with reduced glutathione was followed for 3 minutes at 340 nm. The activity was calculated using the molar extinction coefficient of 9.6 mM-1cm-1. One unit of GT is defined as the amount of enzyme that catalyzes the conjugation of 1 μmol of CDNB during 1 minute. The results are expressed as U/g Hb.

The kit used to assess the GPx activity is based on the method developed by Paglia (19). The NADPH consumption is followed at 340 nm for 3 minutes. The reaction is initiated by the addition of cumene hydroperoxide which is used by GPx to oxidize reduced glutathione. The oxidized form of glutathione is converted back to its reduced form using NADPH in the reaction catalyzed by GR. The results were calculated according to the manufacturer’s specifications and are expressed as U/g Hb.

The kit used to assess the GRed activity is based on the method developed by Goldberg (20). NADPH is used for the reduction of the oxidized form of glutathione. The decrease in absorbance at 340 nm is followed for 5 minutes. The results were calculated according to the manufacturer’s specifications and are expressed as U/g Hb.

The concentration of total thiols was assessed by a previously described method (21). The results are expressed as μmol/g Hb.

The haemoglobin concentration was evaluated using the method described by Drabkin (22).

For all the spectrophotometric assays performed it was used a Shimadzu UV-Vis mini 120 spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

Statistics

Results are presented as mean ± SEM (standard error of the mean). Two-tailed P values <0.05 were considered statistically significant. The data were analyzed using GraphPad InStat 5 software.

The distribution of the data was evaluated with the Kolmogorov-Smirnov test. The Wilcoxon and Mann-Whitney tests were used to compare the means between the two moments.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Discharge</th>
<th>Change (%)</th>
<th>P value*</th>
<th>P valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiols (μmol/g Hb)</td>
<td>(-) ALA</td>
<td>7.62 (0.04)</td>
<td>6.59 (0.02)</td>
<td>-1.3 (7.5)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(+) ALA</td>
<td>7.51 (0.01)</td>
<td>7.69 (0.02)</td>
<td>+1.4 (6.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Catalase (k/g Hb)</td>
<td>(-) ALA</td>
<td>101.87 (20.68)</td>
<td>100.13 (24.13)</td>
<td>+1.5 (10.0)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(+) ALA</td>
<td>173.70 (14.62)</td>
<td>123.41 (8.71)</td>
<td>-23.1 (11.2)</td>
<td>NS</td>
</tr>
<tr>
<td>SOD (U/g Hb)</td>
<td>(-) ALA</td>
<td>1075.95 (169.17)</td>
<td>1431.80 (101.52)</td>
<td>+9.3 (3.0)</td>
<td>0.031</td>
</tr>
<tr>
<td></td>
<td>(+) ALA</td>
<td>1393.48 (69.14)</td>
<td>1514.06 (60.80)</td>
<td>+47.2 (25.4)</td>
<td>0.039</td>
</tr>
<tr>
<td>GT (U/g Hb)</td>
<td>(-) ALA</td>
<td>5.17 (0.29)</td>
<td>3.74 (0.28)</td>
<td>+12.1 (4.0)</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>(+) ALA</td>
<td>5.55 (0.26)</td>
<td>4.95 (0.23)</td>
<td>-10.1 (3.8)</td>
<td>0.014</td>
</tr>
<tr>
<td>GPx (U/g Hb)</td>
<td>(-) ALA</td>
<td>27.10 (2.24)</td>
<td>27.92 (2.81)</td>
<td>+3.9 (6.8)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(+) ALA</td>
<td>26.99 (2.30)</td>
<td>25.14 (1.48)</td>
<td>-4.4 (6.3)</td>
<td>NS</td>
</tr>
<tr>
<td>GRed (U/g Hb)</td>
<td>(-) ALA</td>
<td>4.31 (0.30)</td>
<td>3.88 (0.24)</td>
<td>-6.6 (3.7)</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>(+) ALA</td>
<td>4.47 (0.37)</td>
<td>5.06 (0.24)</td>
<td>+15.4 (5.3)</td>
<td>0.027</td>
</tr>
</tbody>
</table>

TABLE 2. The dynamic of the assessed redox status markers in the study groups.

* baseline vs. discharge (Wilcoxon paired test); b difference in change (%) between baseline vs. discharge (Mann-Whitney test); NS, non significant.
of the study and the percentage of variation, respectively. The differences between the two groups in respect to comorbid states, stroke subtypes incidence, as well as medication were evaluated with the Chi-square test. Multiple regression analysis was performed to evaluate the relation between independent variables (baseline values of the assessed parameters, ALAnerv® treatment, and incidence of diabetes mellitus) and a dependent variable (discharge values of the assessed parameters).

### OUTCOMES

For the present pilot-study we enrolled 28 post-acute stroke patients. They were randomly divided into (-) ALA and (+) ALA groups. During the two-weeks hospitalization period, all patients were subject to a standard rehabilitation procedure.

All the demographic data, comorbidities and medication are presented in the Table 1. The only comorbid state for which we found a significant difference between the two groups was diabetes mellitus. For this reason, we introduced this condition as an independent variable in our model of regression analysis.

The results obtained for the assessed biochemical parameters are presented in Table 2. At baseline there was no statistically significant difference between the two study groups.

In the (+) ALA group we found significant increase only for SOD (1393.48 ± 69.14 U/g Hb vs. 1514.06 ± 60.80, P = 0.039), and GRed (4.47 ± 0.37 U/g Hb vs. 5.06 ± 0.24, P = 0.027). On the other hand, catalase (173.70 ± 14.62 k/g Hb vs. 123.41 ± 8.71, P = 0.020), and GT (5.55 ± 0.26 U/g Hb vs. 4.95 ± 0.23, P = 0.014), decreased significantly during the two-weeks period.

In the (-) ALA group SOD (1075.95 ± 169.17 U/g Hb vs. 1431.80 ± 101.52, P = 0.031), and GT (5.17 ± 0.29 U/g Hb vs. 5.74 ± 0.28, P = 0.012) significantly increased, while GRed significantly decreased (4.31 ± 0.30 U/g Hb vs. 3.88 ± 0.24, P = 0.012).

Statistically significant percentages of variation between the two study groups were found for all the assessed biochemical parameters, except for GPx.

In order to identify the effect of different independent variables (baseline values, ALAnerv® treatment, and diabetes mellitus) on the discharge values of all the assessed biochemical parameters we used multiple regression (Table 3).

Our model of multiple regression indicated that the difference of diabetes mellitus incidence between the two groups did not account for the difference in the variation of the assessed parameters.

The dynamic of all the parameters was influenced by both the baseline value and ALAnerv® treatment.

### DISCUSSION

Erythrocytes play a central role in the antioxidant capacity of the blood through a plethora of antioxidant enzymes, as well as low-molecular antioxidants like reduced glutathione, α-tocopherol, and vitamin C among others etc.

These cells act as mobile ROS/RNS scavengers throughout the body and provide antioxidant protection to other tissues.

Correction of the stroke-associated redox imbalance through the use of different antioxidants is an approach under study during the past years (11,13).

The present pilot study was developed to evaluate the effect of ALAnerv® consumption on redox status in post-acute stroke patients undergoing rehabilitation. Beside the evaluation of serum markers of oxidative stress, we investigated also the erythrocytes’ redox status.

The nutritional supplement used in our study is a complex mixture. It contains several compounds with potential antioxidant activity.

### TABLE 3. Results of the multiple regression analysis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline value</th>
<th>ALAnerv® treatment</th>
<th>Diabetes mellitus incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiols (μmol/g Hb)</td>
<td>0.233/0.058/NS</td>
<td>0.311/0.078/NS</td>
<td>-1.473/0.366/NS</td>
</tr>
<tr>
<td>Catalase (k/g Hb)</td>
<td>0.044/0.207/NS</td>
<td>29.957/18.009/NS</td>
<td>-5.166/16.425/NS</td>
</tr>
<tr>
<td>SOD (U/g Hb)</td>
<td>0.599/0.112/0.0004</td>
<td>224.060/90.942/0.036</td>
<td>-193.465/93.723/NS</td>
</tr>
<tr>
<td>GT (U/g Hb)</td>
<td>0.644/0.165/0.001</td>
<td>-0.947/0.328/0.011</td>
<td>-0.184/0.352/NS</td>
</tr>
<tr>
<td>GPx (U/g Hb)</td>
<td>0.856/0.170/0.0001</td>
<td>-2.334/2.792/NS</td>
<td>-0.671/2.966/NS</td>
</tr>
<tr>
<td>GRed (U/g Hb)</td>
<td>0.578/0.119/0.0002</td>
<td>1.236/0.258/0.0002</td>
<td>-0.349/0.292/NS</td>
</tr>
</tbody>
</table>

* B coefficient/SE of B/P value
(α-lipoic acid, D-α-tocopherol). It contains also a source of selenium (selenomethionine), which is essential for the function of different selenium-dependent enzymes (i.e., GPx).

It is well documented the fact that erythrocytes take up lipoic acid and reduce it to dihydrolipoic acid, which is further released into the bloodstream (23). The reduction is dependent on the presence of NADPH which is the product of hexose monophosphate shunt. The same study indicated that GRed and thioredoxin reductase are involved in this reduction. In our study we found a significant increase of GRed activity during the two weeks of the study. Also, there is a significant difference in respect to the percentage of variation of GRed activity between the two study groups. The multiple regression model indicated a positive correlation between the GRed activity and ALAnerv® consumption. On the other hand, there are studies indicating that under conditions of restricted glucose, lipoic acid could have a detrimental effect upon erythrocytes’ redox status (24).

Dihydrolipoic acid acts as a potent reducing agent and reduces the oxidized forms of different antioxidants, including vitamin C and glutathione (25,26). In our study we found an increasing trend for the concentration of total thiols, which did not reach statistical significance. This could be the consequence of the short study duration.

A study indicated that dietary supplementation of cyclosporine A – treated rats with a combination of α-tocopherol and lipoic acid lead to a correction of the redox status evidenced by increased activities of both catalase and SOD (27). Our results are in partial agreement with those of the previous study, as we found a significant increase only for SOD activity. This difference could also be explained in terms of time difference between the two studies. In the case of the cyclosporine A study, the rats received both antioxidants for a period of eight weeks before cyclosporine A administration. Also, as in the case of GRed, the activity of SOD we found a positive correlation with ALAnerv® consumption.

Erythrocytes’ GPx activity is a reliable indicator of selenium nutritional status (28). Early studies indicated that correction of erythrocytes’ GPx activity in response to selenium supplementation occurs after at least 3 months (29). Our results are in agreement with these results, as we did not find any significant change in the case of GPx activity.

Finally, the multiple regression model that we proposed indicated that diabetes mellitus did not influence any of the assessed biochemical parameters. Moreover, only SOD, GT and GRed activities were significantly influenced by ALAnerv® consumption.

In conclusion, our study indicates that consumption of ALAnerv® is associated with a partial correction of erythrocytes’ redox status. On the other hand, it raises the possibility of obtaining a better correction of the redox status following a prolonged period of supplementation.

Conflict of interests: none declared.

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