Comparison of the Effects of Natural Antioxidants - vitamin E and CĂTINOFORT (*Hippophae rhamnoides* natural extract) - on the body of divers in saturation *(Cezarina Dincu, Magda Nenciu, Alexandru Deftu, Maria Luisa Flonta)*	"Cercetări Marine" Issue no. 43 Pages 358-369	2013

COMPARISON OF THE EFFECTS OF NATURAL ANTIOXIDANTS - VITAMIN E AND CĂTINOFORT (*HIPPOPHAE RHAMNOIDES* NATURAL EXTRACT) - ON THE BODY OF DIVERS IN SATURATION

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ABSTRACT

The work presents the results of research performed in the Clinical Laboratory of the Constanţa Diving Center and emphasizes several aspects on the physiology of human adaptation to hyperbaric conditions. The novelty proposed by the paper is the preventive intervention by dosed and analytically controlled intake of adjuvants with antioxidant properties - vitamin E and CĂTINOFORT sea buckthorn extract. The results substantiate and suggest possibilities of applying these treatments to mitigate the effects of the oxidative stress related and inherent to working in hyperbaric conditions.
KEY-WORDS: hyperbarism, saturation diving, oxidative stress, natural antioxidants, administration, effects

AIMS AND BACKGROUND

The issues of working at great depths and high water pressures are various and complex. Solving them is a worldwide research area which led to the establishment and development of adapted fields, such as hyperbaric physiology and physiopathology, hyperbaric clinical biochemistry etc. As such, new research fields have emerged, more or less integrative: hyperbaric medicine or saturation diving medicine, hyperbaric physiology or saturation diving physiology, hyperbaric microbiology, hyperbaric stress, whose operation manner and results are presented in various papers and journals with specific denominations (Burbakk A., 2003; ***Int. Marit. Health, 2005).

Under such circumstances, this paper aims at introducing in the information circuit of the field some synthetic data based on the results of previous work, meant to contribute to the knowledge of possibilities to prevent the effects of oxidative stress and, at the same time, to emphasize the existing research potential of the Divers Center of Constanța, the single unit of its type in Romania, internationally acknowledged, in the field of control and prevention clinical biochemistry.

Saturation diving

Saturation diving is a special diving technique used by the US Navy since the 1960s, which allows divers to work underwater at great depth for several days, weeks or even months, while the high pressure of the working environment acts continuosly on the human body, any accidental decompression being catastrophic for divers in saturation.

Some examples of using this diving technology are rescue missions or underwater construction projects, for oil and gas submerged platforms. Saturation implies the diver remaining underwater long enough (tipically 24 hours or more) so that the tissues may absorb the maximum gas amount possible at the required depth, for the gas balance between the gas dissolved in tissues and environmental gas pressure should be reached. Once the saturation balance is reached, the extension of the diving period for days, weeks and months will not increase the amount of gas in tissues, thus will not increase the time required for decompression (***Min. Ap. Naț., 2010; Degeratu M., 2008).

According to a definition in literature, saturation diving is the type of long-term diving in which divers are maintained in barochambers at a pressure approximately equal to the pressure corresponding to the working depth, from where they are transported to the underwater site, no more than twice a day, until completing the work. After the completion of the work, decompression is made for the return to normal air pressure (***Min. Ap. Naț., 2010; Dinu D., 2000). Saturation diving is compulsory if the maximum work depth exceeds 180 m.

The submerged activity of man concerning the exposure of the body to stress factors comprises three distinct stages:

- Diving accompanied by exposing the body to external pressure (compression), during which the diver breathes a gaseous mix, bearing a pressure corresponding to the diving depth;
- Stationing at the living level;
- Return to air pressure (decompression), with the related phenomenon of oversaturation of the divers' bodies with a respiratory mix.

Each stage is characterized by a certain duration, depth level and specific parameters which raise hyperbaric physiology issues (***Min. Ap. Naț., 2010**;** Degeratu M., 1999; ***Min. Ap. Naț. Divers Center, 1996).

Saturation diving is either simulated or real.

Simulated saturation dives are made with the aim of training divers, testing new diving technologies or for scientific research purposes. Simulated saturation diving is made in a laboratory fitted with barochambers and a simulator which replicate underwater working conditions. In hyperbaric premises (barochambers, diving towers), technology creates and monitors the hyperbaric environment: the pressure and composition of the synthetic respiratory mix, vital requirement throughout the diver's stationing at the living level and during submerged work.

Thus, hyperbarism is an unique environment encountered only in this profession. The hyperbaric environment is felt in the functional systems of the biostructure up to the smallest cell of the human body. The entire body becomes a hyperbaric system. The hyperbaric environment, through its specific and completely different features from natural parameters, fires off the divers' bodies, triggering regulatory and compensatory mechanisms. Adaptation to the new constraint and aggression conditions is made by a complex of physiological and metabolic changes. Sometimes, the human body may develop an injury type of adaptation, sacrificing its first defense weak structure to buffer the impact.

Hyperbarism, to the extent at which it implies using oxygen at high pressures, pushes to the limit the physiological adaptation barriers of the body against oxidative stress, sometimes overcoming the compensating capabilities and triggering crisis response patterns to limit the intensive stress of the pre-damage stage.

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Further, some reactive oxidative species act as cellular messengers in redox signaling. Thus, oxidative stress can cause disruptions in normal mechanisms of the diver's body.

Hyperbaric physiology allowed pointing-out the physiological changes under these circumstances, assessing the functional reserves of the body and, most important, knowing its adaptation manner to such circumstances. Living and working in hyperbaric conditions may sometimes cause psycho-physiological imbalances between the strains of the environment and the subjective and/or objective capability of the diver to bear and often the capacity of the physiological adaptation mechanisms is exceeded, resulting in the occurrence of a series of functional disorders. High pressure causes significant mechanical, biophysical and metabolic effects on the human body (changes in viscosity, enhancement of electrolithic solutions' dissociation, changes in superficial pressure, pH acidifiation, increase in protein hydrolisis etc.), which, in the end, modify all functions of the body. The pattern of the particular saturation divers' physiology must be analyzed from two interpretation views:

1. Changes caused by hyperbarism at cellular and mollecular level, responsible for triggering the regulatory, compensatory and reparatory reactions.
2. Disorder proper of the regulatory systems by affecting them functionally or damaging them by experiencing hyperbarism within these structures (***Min. Ap. Naț., 2010, Burbakk A., 2003; Int. Marit. Health, 2005).

The analysis, monitoring and interpretation of variation ranges of certain chemical - biochemical parameters give the possibility to asses the various effects caused by hyperbaric stress on the duration of return to normal levels (Lambertsen, C.J.,1980; C. Adumitresei, 2008; Gronow G., 2005).

Of all research lines of the Constanța Divers Center Clinical Laboratory, the paper herein is an outline of the following investigations:

- Variation of some thrombocyte coagulation parameters;
- Variation of some hormone concentrations;
- Assessment of the discrete cytolisis likely to occur in different organs during saturation, by enzyme dosing.

The results of these investigations substantiated this paper.

It is acknowledged that platelet hyperaggregation is the most significant mechanism in the pathogenesis of decompression accidents (Philip, R.B., 1979). During decompression, coagulation is activated by blood bubbles at the gas/plasma interface. Maintaining the balance between the platelet aggregating and anti-aggregating factors during saturation is, under these circumstances, a major objective in avoiding decompression accidents.

Literature mentions that hyperbaric hyperoxia increases the production of prostaglandinic and tromboxanic endoperoxides (metabolites of arachidonic acid), with pro-coagulation action (Thorsen Ed., 2001), while the assessment of the coagulation indicators - Quick Test, prothrombin time, I.N.R. (International Normalized Ratio) - showed a correlation between their values in describing potentially pathogenic aspects involved in or induced by the conditions specific for decompression (D. Muf Popescu, 2003).

Endocrine changes caused by hyperbaric stress are highly complex, involving probably the entire endocrine system, yet targeting variably or predominantly different glands, which make them difficult to delineate, even under dry hyperbarism circumstances (Catterton, R.T.,1997).

The drop of circulating thyroid hormones' concentrations (T_3 and T_4), along with changes in plasma proteins and drop of the haematocrit, suggests the primary affection of the thyroid gland function by high environmental pressure, probably by dropping the TRH (thyrotrophin-releasing hormone) levels (Andersen Y., 2002). Assessing the effects of oxidative stress by the variation of thyroid hormones' concentrations during monitored saturation diving results from the examination of certain physiological peculiarities thereof related to the hyperbaric environment (Dietrich J.W., Brisseao K., Boem O., 2008):

- Cellular cytolysis is caused by the attachment of free radicals, peroxides, superoxides, caused by the effects of hyperbaric hyperoxia, on the lipid architecture of cell membrane and by the gaseous microemboly likely to occur during decompression in the capillary and microcirculatory area of any tisues (Doran G.R., 1985); the effects were assessed by the variation in activity of specific enzymes. With reference to the capacity of enzymes to be assimilated as indicators of the settlement of induced hyperbaric stress, the results obtained are indicative by their unanimous, yet differentiated increase (Dincu C., 2011). As such, transaminases do not have any function in the blood plasma, thus an increased activity may be due to the affection of certain tissues in which they are naturally found, namely in liver cells and muscle tissue (Șerban M., 2003).

- Gamma-Glutamyl-Transferase (GGT) is present in all organs and has a quite low tissue specificity, yet, as it is involved in the detoxification processes in the liver, its increase in activity indicates most often liver toxicity (Șerban M., 2003).

- **Lactate-Dehydrogenase (LDH)** is also found in various organs and tissues, as such its variations offer limited information (Șerban M., 2003). The activity increase found during analyses may be related, according to previous data, to a certain liver or muscle sensitivity.

- Alkaline-Phosphatase (ALP) has not raised, until recently, a practical interest, yet investigating the activity of this enzyme is becoming more actual due to the information it may provide on blood and infectious diseases (Şerban M., 2003). For the purposes of the paper herein, the increased values may be related to potential digestive disorders caused by hyperbaric stress state.

The use of the antioxidants in the prevention of the effect of oxidative stress, in various domains and applied in various forms, is frequently mentioned in literature: food and nutrition, public health, diving medical practice. Acute oral intake of antioxidants (E.g. vitamin E) by divers can reduce alterations in cardiovascular function, particularly acute endothelial disfunctions that are caused by saturation diving (Obad et al., 2006; Ikeda M., Nakabayashi K., 2004, Obad et al., 2007).

Vitamin E has a complex biologic active potential, within which its antioxodant function is the most acknowledged. Other features include the modulation of enzyme activity, genetic expression and enhancing neurological functions. As an antioxidant, vitamin E acts as a peroxyl-radical catcher, thus preventing the propagation of free radicals in tissues based on the reaction of forming the tocopheryl-radical compound in tissues, which will be oxidized by a proton donor (E.g. vitamin C), returning to the reduced form. Being liposoluble, vitamin E is embedded in tissue - cell membranes - thus protecting them from oxidative stress damages (Herrera; Barbas C., 2001). Vitamin E is involved in neurobiological functions, inhibition of platelet aggregation (Muller D.P., 2010.) and protection of lipids by preventing the oxidization of polynonsaturated acids.

CĂTINOFORT, a strong natural sea buckthorn extract, referred to in this text as the ”natural extract”, is a food supplement having the following properties: vitaminizer, remineralizer, immunomodulator, antioxidant, antianemic, antiatherosclerotic, it completes the nutritional deficiencies, it protects the liver cells, it strengthens the body’s resistance to physical and intellectual effort by its increased energetic contribution (Papuc C., 2008; Papuc, C., 2009.). For this reason, the extract was deemed suitable for the purposes herein, to emphasize its benefic effect for the protection of divers’ health.

The 10 year retrospective observations (2001-2010), which showed the alteration of certain parameters of coagulation, hormone concentration and enzyme activity (DINCU C., 2011, 2012) and recent data on antioxidant adjuvant intake (DINCU C., 2012) account for the pertinence of this synthesis paper.

MATERIAL AND METHODS

The synthetic character of the paper does not require an experimental support, however, for the coherence of information, some of the methodologic aspects of the “source“ papers (DINCU C., 2011, 2012) will be mentioned.

All observations were made at the Constanța Divers Center, with the equipment of the in-house Clinical Laboratory.

The research subject and, at the same time, the source of biological material, the divers team, comprised 16-20 men aged between 30 and 45, all with the same professional qualifications and certified as healthy and able-bodied for diving by the Military Medical Expert Commission. The team was divided in groups comprising four people (the barochamber capacity), of which two were considered “witness“ and two “experiment subject“, the latter being given dosed amounts of antioxidant adjuvants throughout the observations, namely: vitamin E 400 I.U. prestart and after diving and 800 I.U. during stationing in the barochamber; the encapsulated sea buckthorn extract was given nine capsules per day, in three doses, throughout the entire observation period.

The working materials, the blood samples, were collected before and after diving.

The working methods for each of the investigated parameters comprise:

- Quick Test and I.N.R. (International Normalizated Ratio) investigations of the thrombocyte coagulation parameters were made with a standardized SYMEX CA-500 Coagolometer, with control plasma included;
- The evaluation of the discrete cytolysis by enzymatic activities was performed with a VIDA/BIOMEDIO analyzer by the “enzyme linked fluorescent assay” ELFA and after following the methods according to the analytic prospect of the devices: ALT, E.C.26.12. SIEMENS DF-43 A/2008; AST E.C.26.11. SIEMENS DF-414/2008; GGT E.C.23.22., SIEMENS DF 45A/2008;
- The variation of hormonal concentrations was measured according to R. Bănică, 2007.

Of all the data collected, the variations of mean values for witnesses (control) and experiment were selected, in two distinct situations, before submergence and after submergence. The related histograms were accompanied by several statistical aspects: variation range of results, mean M, standard deviation SD and variability coefficent VC.

RESULTS AND DISCUSSION

Thrombocyte coagulation parameters - Quick Test and I.N.R. (International Normalized Ratio)

As previously shown, the need to avoid the consequences of decompressions caused by the interactions at the gas-liquid (blood) interface and the production of endoperoxides with pro-coagulating action requires a proper knowledge of the coagulation indicators’ variation.

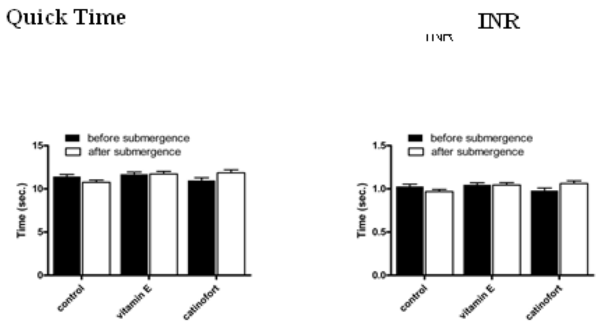


Fig. 1 - Variation ranges of mean values of the Q.T. and I.N.R. coagulation thrombocyte indicators

Table 1 a) - Statistical information synthesis - Quick Test

	Witness		Vitamin E		Sea buckthorn extract	
	Before	After	Before	After	Before	After
Variation range	10.3-12.7	9.8-12.2	11.0-12.7	10.5-12.3	9.90-11.80	10.80-13.00
mean±SD	11.8±0.92	11.06±0.93	11.7±0.52	11.7±0.67	10.96±00.68	11.85±0.8
VC%	7.9	8.4	4.5	5.7	6.23	6.7
NORMAL RANGE 1.0-15.0 s						

Table 1 b) - Statistical information synthesis - I.N.R.

	Witness		Vitamin E		Sea buckthorn extract	
	Before	After	Before	After	Before	After
Variation range	0.92-1.14	0.88-1.09	0.98-1.15	0.94-1.10	0.98-1.15	0.94-1.10
mean±SD	1.07±0.07	1.03±0.07	1.05±0.05	1.045±0.06	1.1±0.0 5	1.25±0.05
VC%	6.8	7.4	5.0	5.7	4.9	5.2
NORMAL RANGE 0.9-1.3 s						

The analysis of the charts and table data shows that the variation of coagulation thrombocyte indicators Q.T. and I.N.R. during saturation diving has some common and other distinct characteristics:

- although the values are small, these variations are significant for the assessed parameters in seconds or fractions thereof;
- the variation range of individual data is within normal limits;
- the dispersion of data is homogenous within the normal variation range;
- the pro-coagulant effects of oxidative stress are obvious by the numerical drop of indicators when exiting saturation (control batch);
- similarly obvious are the effects of the natural antioxidant input, which determined either a stabilization of mean values, or an increase thereof, which results in the protection of the body and inhibition of pro-coagulating effects; the range of the variation coefficient V.C. around the mean value supports this theory;
- comparing the effects triggered by the two natural adjuvants used, it was concluded that the sea buckthorn extract had a stronger effect, determining a double percentage increase compared to the one induced by vitamin E.

Hormonal parameters

The observations on the variation of certain hormone concentrations, caused by saturation diving and the related stress, comprised the triad TSH (Thyroid Stimulating Hormone), T4 (Thyroxine) and T3 (Triiodothyronine), whose secretion is correlated from the genetic and operational point of view. Thus, any variation in the concentration caused by stress agents will affect the information transfer on this path (biochemical), with follow-ups on the normal function of various organs (heart, liver, kidneys). Consequently, unlike the previous data concerning a single physiological aspect (blood coagulation), the synthetic data on hormones comprise complex situations, which point-out a differentiated biological response in accordance with the hormone type observed and the antioxidant used.

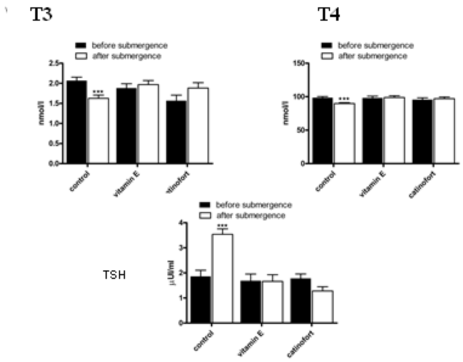


Fig. 2 - Variation ranges of mean values of hormone concentrations

Table 2 - Statistical information synthesis for hormones

a) TSH

	Witness		Vitamin E		Sea buckthorn extract	
	Before	After	Before	After	Before	After
Variation range	0.52-2.9	2.3-4.75	0.74-2.44	0.72-2.43	1.2-2.3	0.8-1.8
mean±SD	1.65±0.71	3.65±0.77	1.70±0.35	1.66±0.45	1.78±0.39	1.28±0.37
VC%	19.0	23.0	20.0	27.0	21.0	29.0
NORMAL RANGE 0.2-3.0 Miu/ml						

b) T4

	Witness		Vitamin E		Sea buckthorn extract	
	Before	After	Before	After	Before	After
Variation range	88.0-107.0	81.0-99.0	89.0-106.0	90.0-106.0	89.0-104.0	91.0-105.0
mean±SD	95.5±6.1	87.66±3.7	98.2±6.0	98.8±5.7	95.66±6.95	97.1± 5.54
VC%	4.2	8.7	6.0	6.0	6.3	5.7
NORMAL RANGE 45.0-112.0 mg/l						

c) T3

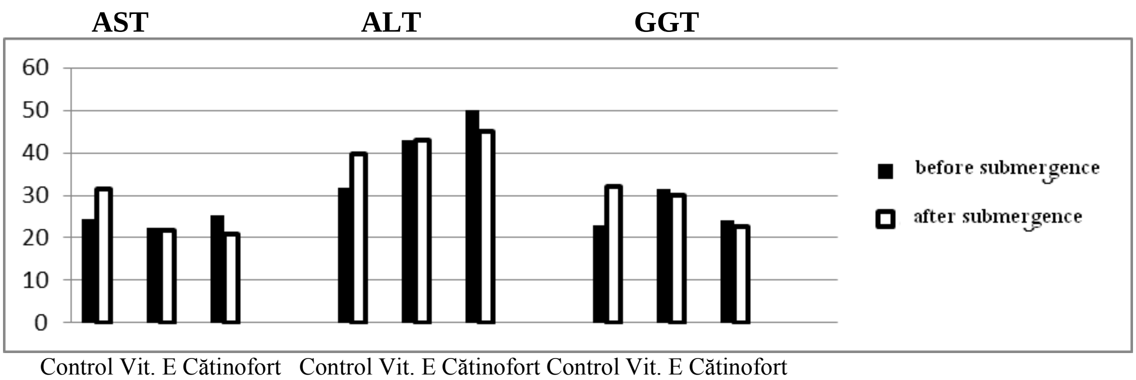
	Witness		Vitamin E		Sea buckthorn extract	
	Before	After	Before	After	Before	After
Variation range	1.6-2.6	1.3-2.4	1.5-2.2	1.6-2.3	1.2-2.1	1.5-2.4
mean±SD	2.2±0.28	1.55±0.2	1.89±0.24	1.96±0.22	1.56±0.3	1.9±0.3
C.V.%	13.0	19,0	12.7	11.6	12.0	11.0
NORMAL RANGE 0.2-3.0 Miu/ml						

Overall observations comprise: integration of the variation range of individual values within normal limits and homogenous or relatively homogenous dispersion around the mean values; pointing-out the oxidative stress effects when exiting saturation by the increase of the Thyroid Stimulating Hormone (TSH) and decrease of T4 and T4 thyroid hormones' concentrations; similarly obvious are the effects of the natural antioxidant intake, which causes the reduction of the variation range, minimizes data dispersion and balances mean values. Particularly, vitamin E has a mainly balancing effect on concentrations (TSH, T4), while the sea buckthorn extract diffentiates more obviously concentrations values before and after submergence, thus proving a protective efficiency higher that vitamin E. This intervention is remarkable in the Thyroid Stimulating Hormone (TSH) compared to the witness (control) batch values.

Enzyme activity indicators

Hyperbaric physio-pathology research also covers the investigation of the enzyme fitting, based on their distribution in most tissues and organs and their sensitivity to the effects of oxidative stress related to saturation diving. The general trend was increasing, with a rate depending on the intensity of the stressors and the resistance capacity specific for each enzyme.

The analyzed enzymes were the following:
AST (Aspartate-Aminotransferase), ALT (Alanine-Transferase), GGT (Gamma-Glutamyl-Transferase), ALP (Alkaline-Phosphatase) and LDH (Lactate-Dehydrogenase).



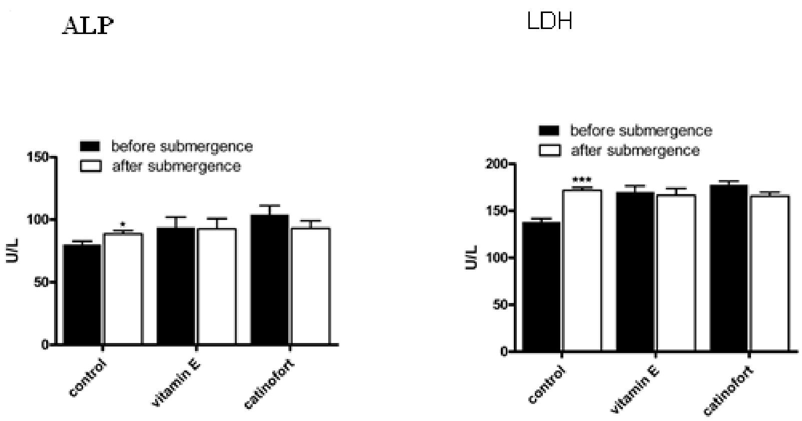


Fig. 3 - Variation ranges of mean values of enzyme activity

Table 3 - Statistical information synthesis for enzymes

a) AST (Aspartate-Aminotransferase)

	Witness		Vitamin E		Sea buckthorn extract	
	Before	After	Before	After	Before	After
Variation range	15.0-33.0	21.0-39.6	15.0-33.0	15.0-29.0	15.0-33.0	15.0-29.0
mean±SD	24.33±6.6	31.5±6.6	22.33±6.0	21.83±4.4	25.33±5.93	20.83±4.4
CV%	27.2	21.0	26.0	20.2	26.0	20.2
NORMAL RANGE 20.0-40.0 U/L						

b) ALT (Alanine-Transaminase)

	Witness		Vitamin E		Sea buckthorn extract	
	Before	After	Before	After	Before	After
Variation range	21.0-44.0	45.0-61.0	36.0-61.0	37.0-60.0	36.0-65.0	37.0-44.0
mean±SD	31.83±9.35	59.6±7.7 3	43.0±10.0	43.0±9.45	50.0±8.2	45.1±7.3
CV%	19.0	13.0	4.5	3.0	23.0	21.0
NORMAL RANGE 5.0-65.0 U/L						

c) GGT (Gamma-Glutamyl-Transferase)

	Witness		Vitamin E		Sea buckthorn extract	
	Before	After	Before	After	Before	After
Variation range	14.0-28.0	21.0-39.0	18.0-39.0	17.0-37.0	19.0-28.0	17.0-27.0
mean±SD	23.0±4.54	32.0±5.0	31.5±4.6	30.0±4.5	24.2±4.3	22.6-±4.5
CV%	21.0	21.0	14.0	15.0	18.0	19.0
NORMAL RANGE 5.00-85.00 U/L						

d) ALP (Alkaline-Phosphatase)

	Witness		Vitamin E		Sea buckthorn extract	
	Before	After	Before	After	Before	After
Variation range	68.0-92.0	80.0-99.0	76.0-122.0	74.0-120.0	76.0-122.0	74.0-120.0
mean±SD	78.2±7.5	85.8±6.6	88.8±18.2	87.7±18.3	103.83±18.2	92.66±18.30
CV%	9.5	7.6	5.2	9.7	19.0	19.7
NORMAL RANGE 50-136 U/L						

e) LDH (Lactate-Dehydrogenase)

	Witness		Vitamin E		Sea buckthorn extract	
	Before	After	Before	After	Before	After
Variation range	120.0-155.0	165.0-184.0	147.0-185.0	139.0-184.0	147.0-185.0	139.0-184.0
mean±SD	137.8±11.85	175.5±6.75	170±14.6	166.5±16.5	170.0±14.5	166.0±16.5
CV%	7.6	7.7	8.5	10.0	8.6	9.9
NORMAL RANGE 100-190 U/L						

The results show that the body's sensitivity and response to the action of stress factors, as well as to the antioxidant intake manifest specifically by the variation of enzyme activity. Under stress conditions, enzyme activity may increase, but, for the purposes of the paper herein, it is significant that all enzymes examined responded to the antioxidant adjuvants used by the trend of balancing or reducing their activity.

The difference stands-out: vitamin E balances the before submergence - after submergence levels, reduces the variation ranges around the mean; on the other hand, CĂTINOFORT reduces significantly enzyme activity. The data obtained show a stabilizing effect for vitamin E and an inhibitory effect for CĂTINOFORT. Most likely, the intervention mechanisms are different, the former by blocking radical scavenging, while the latter by modulating the activities. Consequently, a potential synergism among the two adjuvants could be of interest for future research.

As well as the other indicators examined (coagulation, hormones), the data on enzyme activity are within normal ranges and their distribution is either homogeous or relatively homogenous.

CONCLUSION

Both antioxidants - vitamin E and CĂTINOFORT sea buckthorn extract - showed a certain potential to reduce the scale of oxidative stress effects related to saturation diving. This potential manifests itself differently in enzymes. The data obtained show a stabilizing effect for vitamin E and an inhibitory effect for CĂTINOFORT. Most likely, the intervention mechanisms are different, the former by blocking radical scavenging, while the latter by modulating the activities.

Both antioxidant adjuvants can be used to prevent oxidative stress effects in working under hyperbaric regime conditions.

Consequently, a potential synergism among the two adjuvants could be of interest for future research.

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