# STUDY ON THE ANTIRADICAL ACTION OF ASEA (FOOD SUPPLEMENT) IN CASE OF SUBACUTE ACRYLAMIDE INTOXICATION

## STUDII PRIVIND ACȚIUNEA ANTIRADICALARĂ A UNUI SUPLIMENT ALIMENTAR (ASEA) PE FUNDALUL INTOXICAȚIEI SUBACUTE CU ACRILAMIDĂ

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***Abstract.*** *ASEA is "the first stable mixture with redox signaling molecules (RSM)" with the highest ORAC index (free radical absorption capacity). It is not a drug intended to cure a certain disease, but a mixture of redox signaling molecules (RSM) with high addressability, high efficacy level (100%) and zero toxicity. Considered to be a food supplement, ASEA acts on every cell stimulating the intracellular balance disrupted after the appearance of oxidative stress (OS) which is characteristic to most acute and chronic disease. This contraindication free food supplement is recommended for the disorders of all organs, apparatus and to all ages, its efficacy being remarkable in the case of autoimmune diseases. ASEA, this stable mixture of redox signaling molecules was developed and put on the American market from 2009. It is obtained of clean water, after a three-phase process: the dissolution of a certain amount of pure sodium chloride (NaCl) in water (I), the solution obtained goes through a special electrolysis process where the bonds between chloride, sodium, hydrogen and oxygen atoms are broken and they are transformed into free, floating radicals (II) and their recombination and the formation of stable molecules with redox signal (III). The present experiment tries to compare the antioxidant potential of this stable mixture with the antiradical capacity of certain phytopreparates as Pycnogenol (maritime pine bark extract), a phytopreparate with an ORAC index lower than that of ASEA, but with recognized efficacy, well tested in the European therapy. The experiment was conducted on 4 groups of Wistar rats and lasted 6 weeks. The results were assessed using the biochemical evaluation of certain oxidative stress indices.*
***Key words****: redox signaling molecules (RSM), free radicals (FR), antioxidants (AO), superoxide dismutase (SOD),* glutathione peroxidase *(G-Px), free sulfhydryl groups*

***Rezumat.*** *ASEA este „primul amestec stabilizat cu molecule de semnalizare redox (MSR)" care posedă cel mai înalt indice ORAC (capacitatea de captare a radicalilor liberi). Nu este un medicament care vindecă o anumită boală, ci este un amestec de molecule de semnalizare redox (RSM) care prezintă o adresabilitate mare, eficiență ridicată (100%) şi toxicitate zero. Considerat supliment alimentar, ASEA, acționează la nivelul fiecărei celule, stimulând instalarea echilibrului intracelular perturbat în urma producerii stresului oxidativ (SO), caracteristic majorității maladiilor acute şi cronice. Acest*

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*supliment alimentar lipsit de contraindicaţii se recomandă în patologiile tuturor oraganelor şi aparatelor, tuturor vârstelor, eficienţa sa fiind remarcabilă în maladiile autoimune. ASEA, acest amestec stabilizat de molecule de semnalizare redox, este elaborat şi comercializat în SUA din 2009. Este obţinut din apă curată în 3 faze: dizolvarea unei anumite cantităţi de clorură de sodiu pură (NaCl) în apă (I), supunerea soluţiei obţinute unui proces special de electroliză, proces ce presupune ruperea legăturilor dintre atomii de clor, natriu, hidrogen şi oxigen cu transformarea acestora în atomi liberi, flotanţi (II) şi recombinarea acestor cu formarea de molecule stabile cu semnal redox (III). Experimentul prezent încearcă să compare potenţialul antioxidant al acestui amestec stabilizat cu capacitatea antiradicalară a unor fitopreparate de tipul Pycnogenolului (extract de scoarţă de pin maritim), fitopreparat cu indice ORAC inferior celui declarat pentru ASEA, dar cu eficienţă incontestabilă, bine testatată în terapia europeană. Experimentul s-a efectuat pe 4 loturi de şobolani albi, linia Wistar, timp de 6 săptămâni, iar rezultatele au fost apreciate prin evaluarea biochimică a unor indicatori de stres oxidativ.*
***Cuvinte cheie**: molecule de semnalizare redox, radicali liberi, antioxidanti, superoxid dismutaza, glutation peroxidaza, grupări sulfhidril lbere*

## INTRODUCTION

In the previous century, somewhere around the 1950s, proving the existence of free radicals as a result of all oxidation processes, including the ones in living organisms, represented a turning point for the manner in which pathology and therapy were approached. The expression "oxidative stress" (OS) appeared (Olinescu and Greabu, 1990; Halliwell and Gutterridge, 1989; Percival, 1998; Burlacu and Prisăcaru, 2006) and the phenomenon was initially associated to chronic diseases and it was characterized by the presence of FR, mainly chemically reactive species of oxygen (RS $O_2$), in the cell, their attack on the key components of cells leading to cellular apoptosis. The action of free radicals in severe diseases as cancer, diabetes, arthrosis, Parkinson and Alzheimer disease etc. is obvious (Prisăcaru and Burlacu, 2009; Prisăcaru, 2010; Prisăcaru et al., 2011). Immediately after, it appeared the problem of finding the antioxidants, those chemical substances capable of preventing the formation of FR or inhibiting their high reactivity level; the focus fell initially, according to the tendencies of the time, on chemically synthesized antioxidants. When the organism's unexpected answer to synthetic antioxidants pointed out in some of the cases their toxicity and in other cases, their inefficiency, vegetal antioxidants started being sought, according to the model of the ones in living organisms (glutathione, flavonoids, vitamins C, A, E etc.).

The combination of the results obtained from phytochemical, pharmacognostic and toxicological studies with the results from phytotherapeutic studies and medical clinique have led to the apparition of numerous vegetal pharmaceutical forms with antioxidant action (Burlacu and Prisăcaru, 2006; Prisăcaru and Burlacu, 2009; Prisăcaru, 2010; Prisăcaru et al, 2011). The interest in the commercial aspect of the ones in the industry of food supplements led to the apparition on the Romanian market of numerous unpatented "forms of food

supplements", that are not subjected to any type of control to certify the content of their active principles, their purity, or the simplest information of pharmacokinetics and pharmacodynamics etc.

Under the name of "food supplements" we often discover mixtures of vegetal products, sometimes, much too many to be capable of fulfilling the role of pharmacodymanic synergism and which can represent not only objects of charlatanry but also a risk to human health. These phytopreparates are pointed out with advertisements that underline the effects of some universal panacea accompanied by numerous financial advantages: bonuses, free products, the possibility of getting a subscription to a smaller price etc. One of the products in this category is ASEA, a stable mixture of redox signaling molecules created and commercialized in USA from 2009. It is obtained from clean water after a 3 phases process: dissolution of a certain quantity of pure sodium chloride (NaCl) in water (I), subjecting the resulting solution to a special electrolysis process that includes breaking the bonds between the atoms of chloride, sodium, hydrogen and oxygen and transforming them in free, floating atoms (II) and their reorganization so to create stable molecules with redox signal (III) (http://asea.myvoffice.com).

## MATERIAL AND METHOD

The present experiment (Table 1) tries to compare the antioxidant potential of this stable mixture (ASEA) with the antiradicalar capacity of some phytopreparates as Pycnogenol (maritime pine bark extract), a phytopreparate with an ORAC index lower than that of ASEA, but with recognized efficacy, well tested in the European therapy (Peng et al., 2010; Farid et al., 2004; Sime and Reeve, 2004). The experiment was conducted on 4 groups of white Wistar rats and lasted 6 weeks. The first group that included 5 rodents represented the reference group; they were accommodated and fed in standard conditions. The five rats from the second group represented the control group and they were subjected to a sub-acute intoxication with acrylamide (*dose pro die* of X drops). The third group was administered, apart from the daily acrylamide dose a number of X drops of hydro alcoholic solution of Pycnogenol (standardized solution with 85% of procyanidins).

*Table 1*

**Experimental model**

| **Groups** | ***Acrylamide Water solution 1.5%*** | **Pycnogenol hydro alcoholic solution (85 % procyanidin) *pro die* dose** | **ASEA *pro die* dose** |
|---|---|---|---|
| Reference group | - | - | - |
| Control group 1 | X *guttes* | - | - |
| Control group 2 | X *guttes* | X *guttes* | - |
| Trial group 1 | X *guttes* | - | X *guttes* |

The last group (considered the trial group that provides information on the antioxidant potential of the tested product) was treated with the toxic dose of acrylamide and X drops of ASEA solution.

At the end of the experiment, in order to assess and compare the antitoxic potential of the ASEA solution on the blood collected from the animals included in the trial it was determined the catalase activity (CAT), the activity of superoxide dismutase (SOD), of glutathione peroxidase and the serum concentration of sulfhydryl groups.

## RESULTS AND DISCUSSIONS

The results obtained after quantifying the activity of serum catalase and after statistically processing the information, are presented in table 2 and fig. 1. From the analysis of this data results a significant growth of its activity at the group of rats treated exclusively with acrylamide solution (645.921 U/mL), compared to that of the reference group (612.515 U/mL), which suggests the consumption of the enzyme after the attack of the free radicals of glycidamide, the acrylamide attack type, followed by the stimulation of enzyme synthesis with an abrupt growth. The figures illustrating catalase activity from the serum of the group protected with hydro alcoholic solution of *Pycnogenol* is equal to 629.990U/mL, the figure being equally distant between the value of the reference group (612.515 U/mL) and the one of the group treated exclusively with the SO producing toxic substance (645.921 U/mL) which suggests the antiradicalar effect of the procyanidins deriving from maritime pine bark. The analysis of catalase activity from the serum of the animals treated with antioxidant saline solution, ASEA, points out a value similar to the one of the group treated with Pycnogenol, but lower than that of the reference group (623.166 U/mL). The study of the second oxidative stress indicator, superoxide dismutase, indicates a sinuous trajectory of enzymatic activity, which decreases significantly from the reference group (683.132 U/mL) to the control group 1 (568.274 U/mL), after which it grows at the groups protected with ASEA (662.33 U/mL) and especially at the one protected with Pycnogenol (705.25 U/mL).

A similar evolution, almost identical, is also recorded by the third parameter glutathione peroxidase, an enzyme with an activity value equal to 121.112 μmol/min/mL; the activity decreases significantly to 89.492 μmol/min/mL for the group intoxicated with acrylic amide and then returns to values similar to those of the reference group. The highest intensity of the enzyme's activity, glutathione peroxidase, is reached in the serum of the animals that benefited from the administration of ASEA, which can suggest its significant antioxidant role.

*Table 2*

**Evolution of catalase and serum superoxide dismutase activity**

| Groups | CAT [U/mL] | SOD [U/mL] |
|---|---|---|
| Reference group | 612.515 | 683.132 |
| Control group 1 | 645.921 | 568.274 |
| Control group 2 | 629.990 | 705.25 |
| Trial group 1 | 623.166 | 662.33 |

*Table 3*

**Oscillation of serum glutathione peroxidase and thiolic groups**

| Groups | G-Px [μmol/min/mL] | Free thiolic groups [μmol/ mL] |
|---|---|---|
| Reference group | 121.112 | 303.262 |
| Control group 1 | 89. 492 | 219.621 |
| Control group 2 | 110.662 | 319.439 |
| Trial group 1 | 188.131 | 321. 002 |

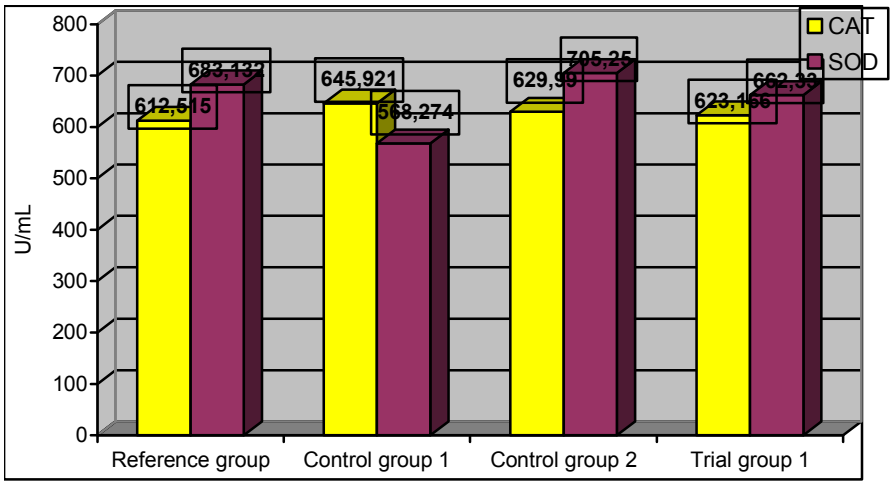


**Fig. 1 -** Variation of catalase and serum superoxide dismutase activity

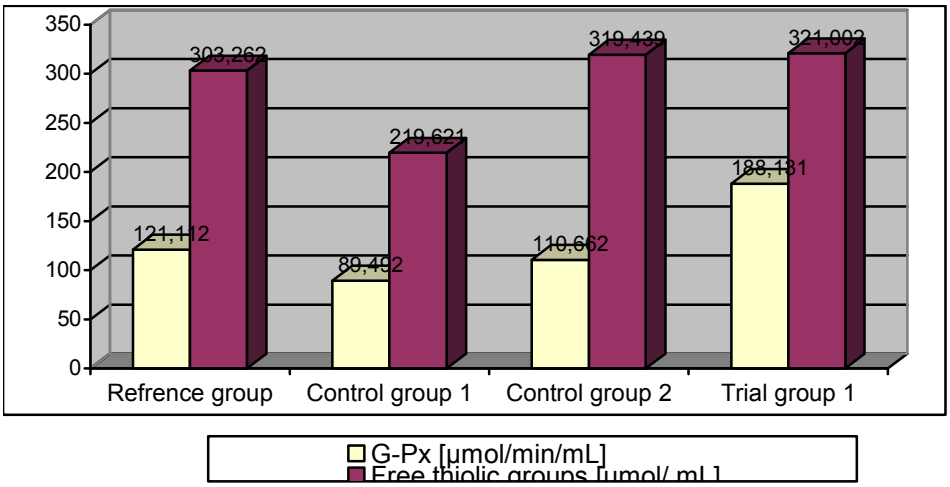


**Fig. 2 -** The evolution of serum glutathione peroxidase and free thiolic groups

The study of the results of the analysis of the free thiolic groups, as it is presented in table 3 and figure 2 confirm the role of the procyanidins from the marine pine bark, as a valuable antioxidant and detoxifying element. If the concentration of thiolic groups is of 303.262 µmol/ mL for the reference groups, for the group subjected to the action of the FR from the organism of the animals included in the control group 1 it decreases in an obvious manner at 219.621 µmol/ mL, while the value of mercapto groups increases significantly in the serum of the animals included in the groups treated with ASEA (319.439 µmol/ mL) and especially in the serum of the animals protected with Pycnogenol (321.002 µmol/ mL).

## CONCLUSIONS

1. The analysis of catalase activity from the serum of the animals treated with antioxidant saline solution, ASEA, indicates a value similar to the one of the groups treated with Pycnogenol and lower than that of the reference group;

2. The study of superoxide dismutase, indicates a sinuous trajectory of enzymatic activity, which decreases significantly from the reference group to the control group 1, after which it grows at the groups protected with ASEA and especially at the one protected with Pycnogenol;

3. The highest intensity of the enzyme's activity, glutathione peroxidase, is reached in the serum of the animals that benefited from the administration of ASEA, which can suggest its significant antioxidant role.

4. The concentration of mercapto groups increases significantly in the serum of the animals included in the group treated with ASEA (319.439 μmol/ mL) and especially in the serum of the animals protected with Pycnogenol (321.002 μmol/ mL).

5. The stabilized mixture with redox signaling molecules present antiradicalar action but according to the parameters studied, it is inferior to the antioxidant action of Pycnogenol.

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