

EVALUATION OF THE INFLUENCE OF THE PYCNOGENOL CONTAINING PREPARATIONS UPON THE ANTIOXIDANT POTENTIAL

EVALUAREA INFLUENȚEI PREPARATELOR PE BAZĂ DE PICNOGENOL ASUPRA POTENȚIALULUI ANTIOXIDANT

PRISĂCARU Cornelia¹, PRISĂCARU Anca-Irina¹
e-mail: corneliapris@yahoo.com

Abstract. *Pini maritimae cortex, the vegetal product obtained from the pine tree that grows in the mediterranean area of France, is remarkable through its high content of proanthocyanidins, bioflavonoids that show a strong antiradicalic effect. The extract from the bark of the maritime pine, known under the commercial name of Pycnogenol, is used for its antioxidant and anti-inflammatory effects, as a remedy in preventing and improving the symptoms of cardiovascular and cerebrovascular diseases, cancers, gout. The present experiment evaluates the influence of two pharmaceutical formulations of Pycnogenol (tablets with 0.02000 g extract from the bark of the maritime pine and hydroalcoholic solution containing 85% proanthocyanidin) upon the antioxidant potential of white Wistar rats. The experimental model includes 3 groups of animals: the reference group, Pycnogenol tablets group, and Pycnogenol solution group. The biochemical investigation reveals a significant positive influence of the Pycnogenol solution upon catalase, superoxide dismutase, glutathione peroxidase, but not upon the free sulfhydryl groups. The results also show an improvement for the group treated with Pycnogenol tablets regarding catalase and superoxide dismutase.*

Key words: *Pinus maritima, proanthocyanidins, antioxidant potential, enzymatic and non-enzymatic antioxidants.*

Rezumat. *Pini maritimae cortex, produsul vegetal obținut de la pinul din zona mediteraneană a Franței, se remarcă prin conținutul ridicat în proantocianidine, bioflavonoizi ce manifestă puternică acțiune antiradicalară. Extractul de scoarță de pin maritim, cunoscut sub denumirea comercială de Pycnogenol, se folosește ca antioxidant, antiinflamator, remediu în prevenirea și ameliorarea unor boli cardiovasculare și cerebrovasculare, unor maladii grave (cancere, gută) și a unor maladii specifice bătrâneții sub diferite forma farmaceutice. Experimentul redat în acest articol testează influența a două forme farmaceutice cu Pycnogenol (comprimate cu 0,02000 g extract de pin maritim și soluție hidro-alcoolică titrată conținând 85%proantocianidină) asupra potențialului antioxidant a șobolanului alb din linia Wistar. Experimentul cuprinde 3 loturi de câte 5 animale: lotul de referință, lotul cărui i se administrează prin hrană comprimate cu extractul amintit și lotul ce a fost tratat cu aceeași doză de Pycnogenol sub formă de soluție. Investigația biochimică efectuată pe sângele animalelor evidențiază o influență semnificativ pozitivă a soluției de Pycnogenol asupra CAT, SOD, GPx, dar nu și asupra*

¹ University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

grupărilor –SH libere și o influență pozitivă a comprimatelor cu același extract doar asupra CAT și SOD.

Cuvinte cheie: *Pinus maritima*, proantocianidine, potențial antioxidant, antioxidanți enzimatici și non-enzimatici.

INTRODUCTION

Proanthocyanidins from the bark of the maritime pine (*Pinus maritima*) are compounds that possess a strong free radical scavenging effect, preventing from the deleterious action of the free radicals that are present in concentrations specific to oxidative stress (Liu, 1998). The extract from the bark of the maritime pine, known under the generic name of Pycnogenol, counteracts the destructive action exerted by the free radicals at the cellular level, translated by binding to the cell constituents, including DNA, and by enzymatic inhibitions. By this way, Pycnogenol delays the aging process, prevents or improves the expression of some severe maladies: cancer, cardiovascular and cerebrovascular diseases (Peng, 2000). Pycnogenol prevents from platelet aggregation, has a positive action upon the circulatory system by increasing the resistance of capillary vessels and by protecting the tissue collagen cross-links, exerts anti-inflammatory, immunostimulating and anti-allergy effects (Grimm et al., 2006a; Takano et al., 2011). Starting from these therapeutic virtues of the active compounds from the bark of the maritime pine and from their pharmacokinetic properties (Grimm et al., 2006b) it is considered necessary to test the influence of the intake of different pharmaceutical forms of Pycnogenol upon some enzymatic and non-enzymatic antioxidants from the living organism.

MATERIAL AND METHOD

The present experiment makes a comparative analysis regarding the influence of the active compounds from the maritime pine upon some biochemical parameters with antioxidant effect (table 1).

Two pharmaceutical formulations have been tested: tablets with 0.020 g extract from the bark of the maritime pine and hydroalcoholic solution available in 30 mL bottles, containing 2.000 mg of extract (85% proanthocyanidin). The two pharmaceutical formulations have been tested on female white rats, Wistar breeding, 2 months old, having an average body weight of 312 g.

All the experimental proceedings achieved on laboratory animals (Wistar rats) in this study were in agreement with the international ethics regulations and were approved by the Ethics Commission of the University of Agricultural Sciences and Veterinary Medicine of Iași.

The experimental model included 3 groups (5 animals/group), as follows: the first group - the reference group, that was housed and fed in the same standard conditions as the other two experimental groups for 4 weeks; the second group (Pycnogenol tablets group) - the group that was given standard food with Pycnogenol tablets containing 0.020 g extract from the bark of the maritime pine (in dose of 0.1428 mg extract/100 g body weight/day); the third group (Pycnogenol solution group) - the group that was administered the Pycnogenol solution, containing the same dose of extract expressed as 85% proanthocyanidins, e.g. 0.1428 mg extract/100 g body weight/day (Table 1). Both the Pycnogenol solution and Pycnogenol tablets were

administered with food, each day, during the morning. In order to respect the correct doses, each rat was housed in individual cages during the administration of food.

Table 1

Experimental model

Groups	Pycnogenol tablets (0.02 g extract of <i>Pinus maritima</i>) (dose of extract/100 g/day)	Pycnogenol solution (85% proanthocyanidins) (dose of extract/100 g/day)	Biochemical parameters
Reference group	-	-	Catalase Superoxide dismutase
Pycnogenol [®] tablets group	0.1428 mg extract as titrate powder	-	Glutathione peroxidase
Pycnogenol [®] solution group	-	0.1428 mg extract as titrate solution	Free sulfhydryl groups

In the end of the experiment that lasted 4 weeks, blood samples were collected and submitted to biochemical analysis: serum catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and free sulfhydryl groups.

RESULTS AND DISCUSSIONS

The evolution of CAT, enzyme found in mitochondria and peroxisomes of all aerobic cells, is presented in fig. 1. The study of these data reveal a significant increase of the activity of this enzyme from 527.05 U/ml, value registered for the reference group, to 567.43 U/ml, the value of CAT for the group that received the treatment with Pycnogenol tablets 0.020 g. A significant increase, but with 4 units less, appears for the group protected with Pycnogenol solution (85% proanthocyanidins). The value for the activity of serum CAT for this group is 551.56 U/ml.

The second enzyme tested in this experiment (SOD) is a metallo enzyme (Zn-Cu-SOD) that annihilates the superoxide radicals (O₂⁻). Observing the variation of the activity of this enzymatic antioxidant, as it is shown in fig. 2, there can be noticed an evolution similar to catalase, but in a more discrete manner. There has to be remarked that, in the case of SOD, the maximum of the activity is obtained in the serum of the group treated with Pycnogenol hydroalcoholic solution. The activity of SOD increases from 459.1 U/ml (the reference group) to 467.8 U/ml. The evolution of SOD for the group that was given Pycnogenol tablets is also positive, being only 1.05 units smaller than the value of SOD for the previous group.

The results obtained for the third tested enzymatic antioxidant (GPx) are given in fig. 3. From the study of these data, there can be noticed a different evolution when compared to the other two antioxidant enzymes. Therefore, the enzyme registers a decrease from 77.2 U/min/ml (the reference group) to 74 U/min/ml (the group treated with Pycnogenol tablets). In contrast with this unexpected behavior, the evolution of GPx in the serum of the animals treated

with Pycnogenol solution is positive (80.26 U/min/ml), the activity of the enzyme surpassing with 8 units the value of the reference group.

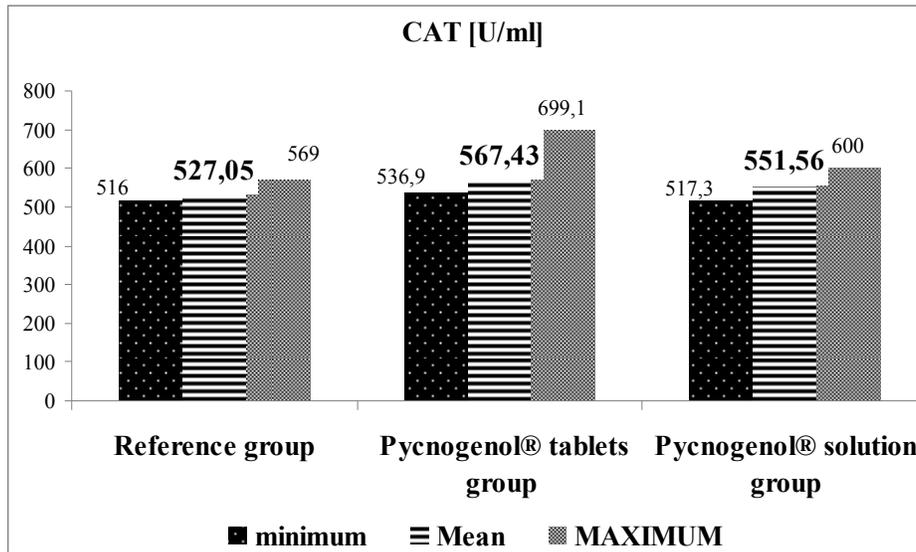


Fig. 1 - Evolution of CAT activity for the 3 experimental groups

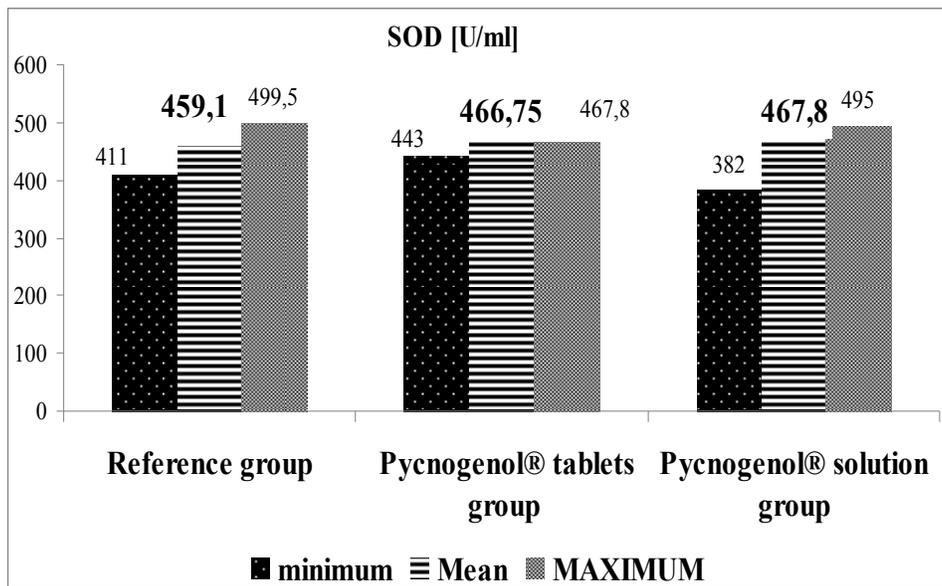


Fig. 2 - Evolution of SOD activity for the 3 experimental groups

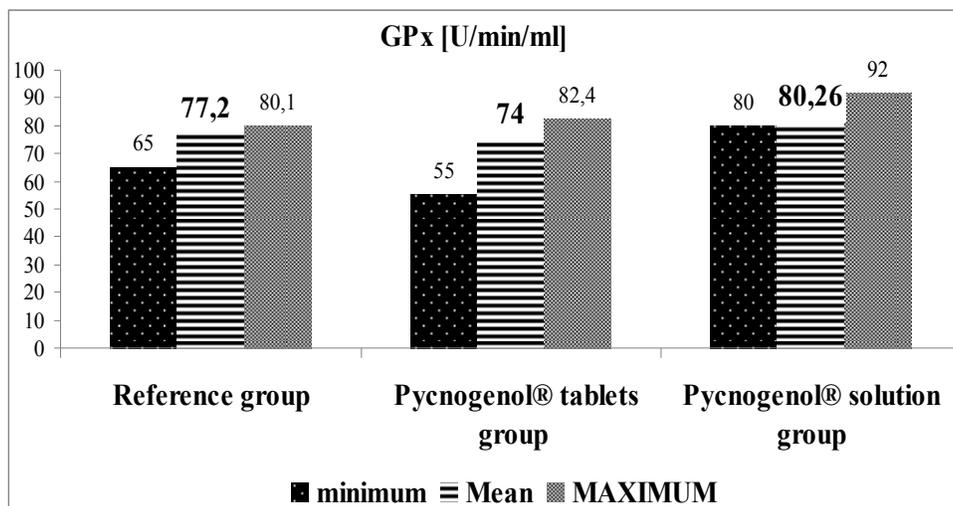


Fig. 3 - Evolution of GPx for the 3 experimental groups

The quantification of the concentrations of the free sulfhydryl groups from the serum of the animals of the three experimental groups are shown in fig. 4. Analyzing these data, there can be noticed a decrease of the concentration of the free sulfhydryl groups for both treated groups. Therefore, the value decreases with 2 units for the group treated with tablets containing the extract of the maritime pine (344 $\mu\text{mol/ml}$) and with 4.7 units for the group that was administered the Pycnogenol solution (341.3 $\mu\text{mol/ml}$), when compared with the reference group (346 $\mu\text{mol/ml}$).

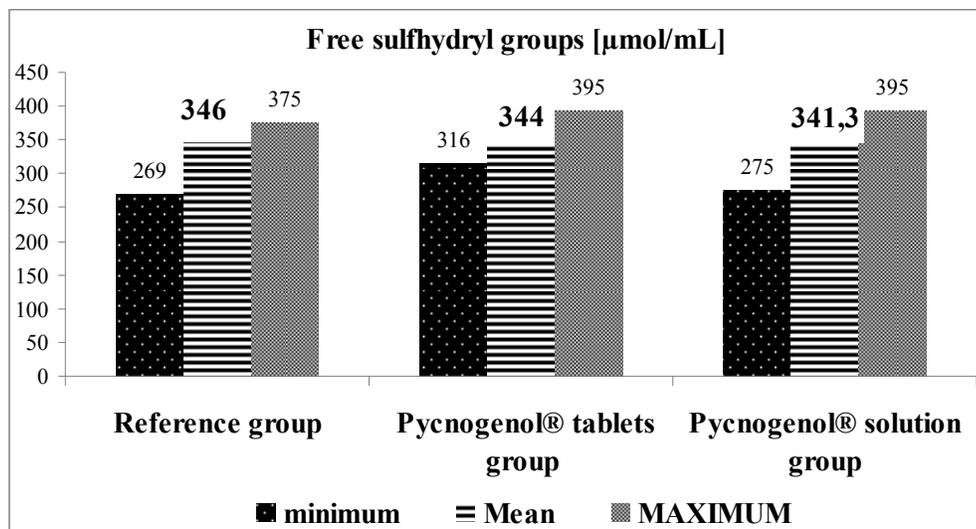


Fig. 4 - Evolution of free sulfhydryl groups for the 3 experimental groups

CONCLUSIONS

1. Administration of the extract of *Pinus maritima* as tablets (0,020 g) influences positively the activity of catalase and superoxide dismutase.
2. The administration of the extract of *Pinus maritima* as hydroalcoholic solution (85% proanthocyanidins) stimulates the activity of catalase, superoxide dismutase, and glutathione peroxidase.
3. The active compounds from the bark of the maritime pine do not influence the concentration of free sulfhydryl groups.

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