STUDIES REGARDING THE THERAPEUTICAL EFFECT OF THE EXTRACT FROM THE BARK OF *PINUS MARITIMA*

STUDII PRIVIND ACȚIUNEA TERAPEUTICĂ A EXTRACTULUI DE SCOARȚĂ DE *PINUS MARITIMA*

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Abstract. *The studies by J.A. Masquelier, professor at the University from Bordeaux, regarding the bark of the maritime pine (Pini Maritimae Cortex) emphasized, in 1947, the content in proanthocyanidins, soluble substances with a strong antiradicalic potential. The standardized extract from the bark of the maritime pine from the French mediterranean region, known in the medical field under the commercial name of Pycnogenol®, is used in cardiovascular diseases, cancer, Alzheimer's disease, glaucoma etc. The present experiment evaluates the antioxidant potential of different pharmaceutical formulations of the extract in acrylamide intoxication. The toxicity of acrylamide is based on a free radical, derivative of the free metabolite, glycydamide. The experiment was unfolded on 4 groups of Wistar rats: the reference group, the group intoxicated with acrylamide and other 2 experimental groups that, besided the daily dose of acrylamide, received treatment with hydroalcoholic solution of Pycnogenol, and tablets of Pycnogenol, respectively. After 6 weeks of treatment, blood samples were collected in order to perform the biochemical investigation: serum catalase, superoxide dismutase, glutathione peroxidase and serum concentration of free sulfhydryl groups. The obtained results reveal the significant antioxidant effects of the standardized extract from the bark of Pini maritima.*
Key words*:* acrylamide, Pycnogenol, proanthocyanidins, antioxidant potential

Rezumat. *Studiile lui J.A. Masquelier, profesor la universitatea din Bordeaux, asupra scoarţei de pin maritim (Pini Maritimae Cortex) au evidenţiat în 1947 conţinutul acesteia în proantocianidine, substanţe solubile cu o puternică capacitate antiradicalară. Extractul standardizat de scoarță de pin maritim din zona mediteraneană franceză, impus în lumea medicală sub denumirea comercială de Pycnogenol, este în prezent utilizat în afecțiuni cardiovasculare, cancer, maladia Alzheimer, glaucom etc. Experimentul prezentat testează capacitatea antioxidantă a extractului de pin sub diferite forme farmaceutice în intoxicația cu acrilamidă, substanță ce acționează sub forma unui radical liber derivat de unul din metaboliții săi, glicidamida. Testatarea s-a efectuat pe 4 loturi de şobolani albi Wistar: lotul de referință, lotul intoxicat cu acrilamidă şi 2 loturi experimentale, care, pe lângă doza zilnică de acrilamidă, au primit şi Pycnogenol sub formă de soluție hidro-alcoolică şi comprimate. După 6 săptămâni, atât cât a durat experimentul, s-a recoltat sânge de la animalele experimentului şi s-a supus investigației biochimice: evaluarea activității catalazei serice, superoxid dismutazei, glutation peroxidazei şi determinarea*

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concentrației serice de grupări tiolice libere. Rezultatele obținute evidențiază existența unor semnificative efecte antiradicalare a extractului standardizat de scoarță Pini maritima.
Cuvinte cheie: acrilamidă, Pycnogenol, proantocianidine, potential antioxidant

INTRODUCTION

Pycnogenol® represents the commercial name of the standardized extract from the bark of the maritime pine from the mediterranean region of France (*Pini maritimae cortex*). In 1947, Jacques Arthur Masquelier, professor at the University of Bordeaux, discovered that ceratin active compounds from the bark of *Pinus maritima* hold a strong antioxidant effect. This pharmacological action is due to the proanthocyanidins content, soluble compounds with a flavan-3-ol derived chemical structure characterized by the property of condensation polymerization. The condensation products are found in the grape seeds (*Vitis vinifera)* and are remarkable, as their monomers, through the antioxidant effect (Miron et al., 2002; Istudor, 2001). Due to their antiradicalic action, the proanthocyanidins from the bark of the maritime pine hinder the connection of the reactive oxygen species to the cell components, including DNA (Bowie et Oneill; Packer et al., 1999; Hosseini et al., 2001), and are efficient in preventing or improving the symptoms of severe diseases (cardiovascular diseases, cancer, Alzheimer's disease, inflammatory diseases, bone dystrophies) (Peng et al., 2000; Farid et al., 2004; Sime et Reeve, 2004). Among the food toxicants with high incidence that reclaim antioxidant defence, there can be found acrylamide (2-propenamide), unsaturated substance formed in food products thermally processed by frying, baking, and grilling (fried potatoes, chips, biscuits, toast, coffee etc.) (Burlacu, 2009).

Industrially produced and used since the XIXth century, known for its toxic effects, acrylamide gets into the medical forefront only in 2002, when the studies of a group of researchers from the University of Stockholm emphasize its presence in foods (Dybing et Sannner, 2003; Burlacu et al., 2007). The main mechanism of formation is represented by the Maillard reaction, having as forerunners amino acids, mainly asparagine, and reducing sugars (Mottram et al., 2002). Neurotoxic substance, possessing a carcinogen and mutagen potential, acrylamide manifests its toxicity due to its free epoxide radical, glycidamide, that forms adducts with hemoglobin, proteins with highly specialized functions and DNA (Watts, 2004). The elucidation of its formation mechanisms and its toxicodynamics opens the possibility to discover ways of neutralizing or reducing the toxic effects of acrylic amide.

MATERIAL AND METHOD

The present experiment joins the direction of finding ways of reducing the toxicity of acrylamide by phytotherapeutical methods (table 1). The standardized extract of the maritime pine formulated as 2 pharmaceutical phytopreparations (Pycnogenol® hydroalcoholic solution and tablets) has been used as antioxidant agent. The experiment was unfolded on white rats, Wistar strain, 4 months old, with an average

body weight of 287.5 g, divided into 4 groups of 5 animals each. The first group represented the reference group, while the second group (control group) offered informations regarding the acrylamide intxication. The animals of this groups were administered acrylamide in dosis of 10 µg/kg b.w. Each animal of the third group (experimental group 1) was exposed to acrylamide and treated with 0.4285 mg pine extract as titrated powder obtained from the tablets of Pycnogenol®. The last group with acrylamide induced toxicity (experimental group 2) was protected with the same dose of pine extract, using the hydroalcoholic solution of Pycnogenol®. The experiment lasted 6 weeks and ended with the biochemical investigation of the collected blood samples. The biochemical investigation quantified parameters that reflect the antitoxic potential of the organsim: serum catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and free sulfhydryl groups (free –SH groups).

Table 1

Experimental model

Experimental groups	Acrylamide [µg/kg b.w./day]	Pycnogenol-tablets [mg/ animal/ day]	Pycnogenol-solution [mg/ animal/ day]	Investigated parameters	Duration of experi-ment
Reference group	-	-	-	CAT, SOD, G-Px, free –SH groups	6 weeks
Control group	10	-	-		
Experimental group 1	10	0.4285	-		
Experimental group 2	10	-	0.4285		

RESULTS AND DISCUSSIONS

The study of the CAT levels reveal a descending variation from the reference group towards the control group and even to the groups protected with the extract from the bark of the maritime pine. The activity of CAT lowers from 623.5 U/mL (the value for the reference group) to 610.80 U/mL (the value for the control group) (fig. 1). This decrease can be justified by the presence of the free radicals of glycidamide, the acrylamide attack form, that leads to the consumption of the antioxidant enzyme. The marked decrease of CAT for the groups treated with the extract of *Pinus maritima* is surprising, as a protective intervention had been expected. On the contrary, the valus of CAT decreased even under the value for the control group: 567.1 U/ml for the group treated with the Pycnogenol tablets and 603.3 U/ml for the group protected with the hydro alcoholic solution (fig. 1).

Examining the activity of SOD, a predictable evolution, completely different from that of CAT, can be noticed (fig. 2). Therefore, the activty of SOD decreases from 714 U/mL, the value of the reference group, to 605.8 U/mL, value registered for the control group, as a consequence of the consumotion of SOD determined by the presence of the free radicals of glycidamide. Improved values, although situated under those of the reference group, are obtained for the group protected with Pycnogenol® tablets (612.8 U/ml) and with the hydroalcoholic solution (688 U/ml).

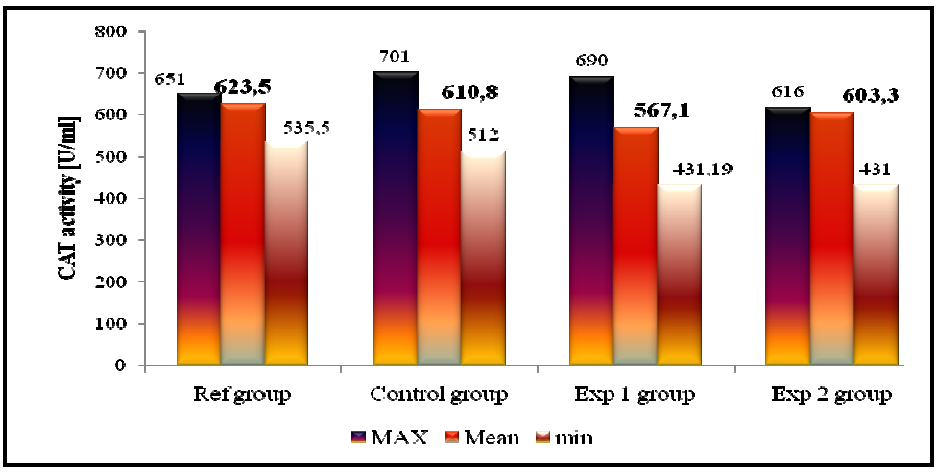

Fig. 1 – The evolution of serum CAT activity

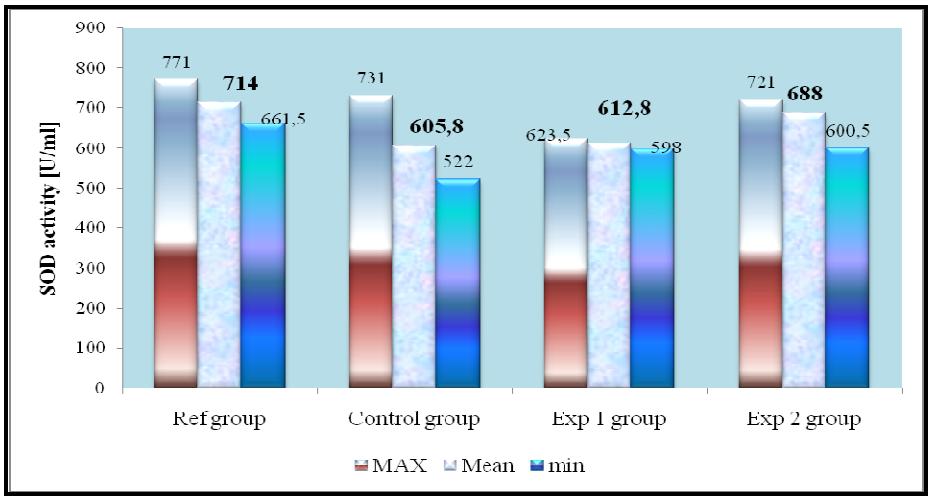

Fig. 2 – The evolution of SOD activity

The evolution of glutathione peroxidase, presented in fig. 3, emphasize the protective effect of the extract from the bark of the maritime pine formulated both as tablets and hydroalcoholic solution. This phenomena is argued by the increase of the enzyme activity for the group protected with Pycnogenol tablets (91.43 µmol/min/mL) and for the group treated with Pycnogenol solution, for which the activity raises even to 93.3 µmol/min/ml, bothe values being superior to that of the reference group (91.43 µmol/min/ml).

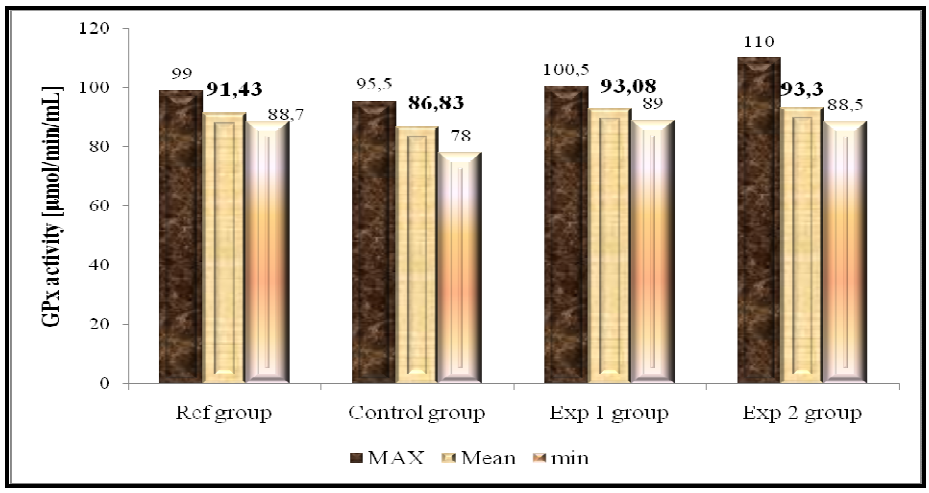

Fig. 3 – The evolution of GPx activity

The analysis of the free sulfhydryl groups, as shown in fig. 4, sustains the double role of glutathione (antioxidant and detoxifying). If the value of the free sulfhydryl groups follows an important decrease for the control group (287 µmol/mL), the antioxidant action of proanthocyanidins from the 2 phytotherapy formulations of Pycnogenol determine a significant improvement of the values in the serum of the animals treated with the hydroalcoholic solution (301 µmol/ mL) and mainly in the serum of the animals treated with Pycnogenol tablets (305 µmol/mL).

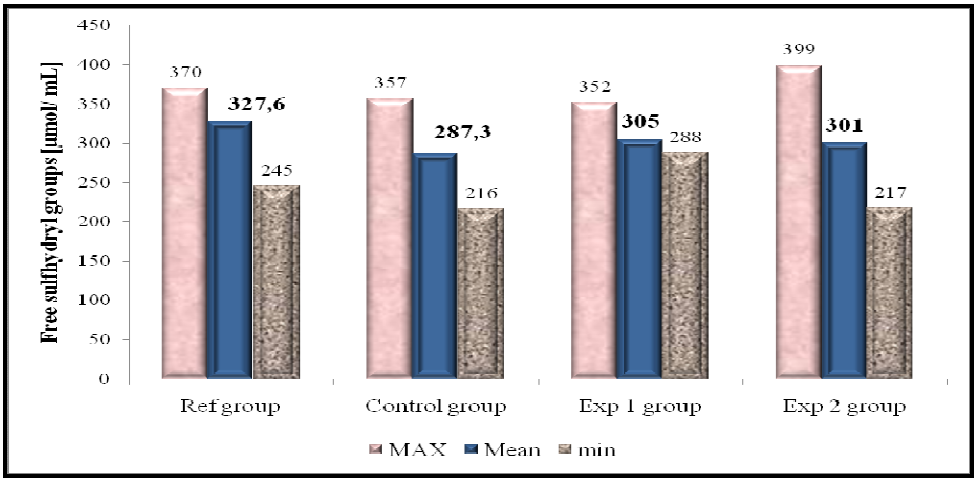

Fig. 4 – The evolution of GPx activity

CONCLUSIONS

1. The evolution of serum catalase is aleatory and doesn't demonstrate the antiradicalic effect of the maritime pine extract.

2. The evolution of SOD and GPx follows similar variations that reveal the antioxidant capacity of the proanthocyanidins from Pycnogenol.

3. The evaluation of the free sulfhydryl groups levels from the serum of the animals sustains the antiradical potential of the phytopreparations obtained from *Pinus maritima*.

REFERENCES

1. **Bowie A., Oneill L. A., 2000** - *Oxidative stress and nuclear factor-k B activation.* Biochem., 59, p. 13-23.
2. **Burlacu Anca Irina, 2009** – *Cercetări privind prezența acrilamidei în alimentele prelucrate termic*. Teză de doctorat, UMF „Gr. T. Popa".
3. **Burlacu Anca Irina, Cuciureanu Rodica, Prisăcaru Cornelia, 2007** – *Acrylamide – toxic compund formed during the thermical process of aliments.* Lucrări Ştiințifice, Secția Medicină Veterinară, 50 (9), p. 129-133.
4. **Dybing E., Sannner T., 2003** – *Forum: Risk Assessment of Acrylamide in Foods.* Toxicological Sciences, 75, p. 7-15;
5. **Farid R., Mirteizi A., Mirheidari M., Rezaieyazdi S., Mansouritorghabeh H., Zibadi S., Watson RR., 2004 -** *Pycnogenol as an adjunct in the management of childhood astma,* Asthma, 41, p. 825-832;
6. **Hosseini S., Pishnamazi S., Sadrzadeh S.M.H., Farid R., Watson RR., 2001** - *Pycnogenol in the management of asthma.* J. Med. Food, 4, p. 201-209;
7. **Istudor V., 2001** - *Farmacognozie, Fitochimie, Fitoterapie*, vol II. Editura Medicală, Bucureşti, p. 275;
8. **Miron A., Stănescu U., Hăncianu M., Aprotosoaie C., 2002 -** *Bazele farmaceutice, farmacologice şi clinice ale fitoterapiei,* vol II. Ed. „Gr. T. Popa" UMF, Iaşi, p. 185;
9. **Mottram D.S., Wedzicha B.L., Dodson A.T., 2002** – *Acrylamide is formed in the Maillard reaction.* Nature. 419, p. 448-449;
10. **Packer L., Rimbach G., Virgili F., 1999** – *Antioxidant activity and biologic properties of a procyanidin-rich extract from pine (Pinus maritime) bark. Pycnogenol*. Free Radic Biol Med, 27, p. 704-724;
11. **Peng Q., Wei Z., Lau B.H., 2000** - *Pycnogenol inhibits tumor necrosis factor-lafa-induced nuclear factor Kappa B activation and adhesion molecule expression in human vascular endothelial cells.* Cell. Mol. Life Sci., 57(5), p. 834-841;
12. **Sime S., Reeve VE., 2004** - *Protection from inflammation, immunosupression and carcinogenesis induced bz UV radiation in mice by topical Pycnogenol.* Photochem Photobiol, 79, p. 193-198;
13. **Watts R., 2004** – *Neutropenia.* In: Wintrobes Clinical Hematology, Philadelphia ed, p. 1777- 1795.