Studii şi Cercetări	Martie 2014	Biologie	23/1	13-15	Universitatea"Vasile Alecsandri" din Bacău

RESEARCHES REGARDING THE HEPATOPROTECTIVE EFFECT OF SOME PHYTOPREPARATIONS OBTAINED FROM *HIPOPHÄE RHAMNOIDES* AND *CYNOSBATI FRUCTUS*

Cornelia Prisăcaru, Anca-Irina Prisăcaru

Key words: *oxidative stress (OS), sterigmatocystin, Cynosbati fructus, Hipophäe fructus, alanine aminotransferase (ALT), aspartate aminotransferase (AST), catalase (CAT), superoxid dismutase (SOD).*

INTRODUCTION

Oxidative stress represents a pathological condition caused by the accumulation of high concentrations of oxygen reactive species in the cells, which can be the cause of endogenous overproduction of free radicals (FR), consequence of certain unhealthy processes or of the access of some environmental noxes in the organism's cells.

The high concentrations of oxygen reactive species turn into aggressors because of the oxidative degradation they exert on some molecules from the cellular structure (proteins, lipids, nucleic acids) [9]. The most aggressive oxygen free radicals considering their oxidant potential are: hydroxyl radical HO, superoxide radical O_2, peroxyle radical O_2. Along with them an oxidative action is performed also by the highly reactive molecules as: singlet oxygen $^1O_2$, hydrogen peroxide H_2O_2 and peroxynitrite OONO. The free radicals derived from oxygen (FRO_2) and the oxidant molecules are known as oxygen reactive species (ORS). They can be characterized as ions or molecules whose peripherical electronic structures have odd ions. Because of the existence of this solitary ion ORS are very active chemically, trying to complete the electronic doublet, reason why they have such a short life [9, 5, 6]. Sterigmatocystin, a difuran mycotoxin, with high incidence in the cereals and the alimentary products from the Moldova Plateau [8], acts as a grade 1 carcinogen to humans, presenting itself as the free radical of its metabolite, epoxy-sterigmatocystin (figure 1). Considering that the cold season from the temperate area of Moldavia lasts longer every year and the incidence of furo-furanic mycotoxins is every time higher, discovering and using various chemopreventive phytoaliments becomes necessary. This chemopreventive potential might be present at the vegetal products rich in vitamin antioxidants: the pseudoberries of *Hipophäe rhamnoides* and the fruit of *Rosa canina (Cynosbati fructus).* The box thorn pseudoberries (*Hipophäe fructus),* are considered to have important therapeutic features, mostly antitoxic/antioxidant qualities because of the high content of ascorbic acid (400 -1 500 mg %) and bioflavonoids with which form an unbeatable phytocomplex in front of ORS. The antitoxic/antioxidant complex from this effective remedy is completed and intensified by ergosterol and carotenoids (β-carotene, lycopene, physalin, cryptoxanthin and zeaxanthin) [3, 10, 1, 8]. The rose hip fruits *Cynosbati fructus*, make themselves known in phytotherapy because of their great antitoxic/antioxidant effects determined by vitamin C (500-1000 mg%), carotenoids (600-10 000 mg %) and tocopherol [4].

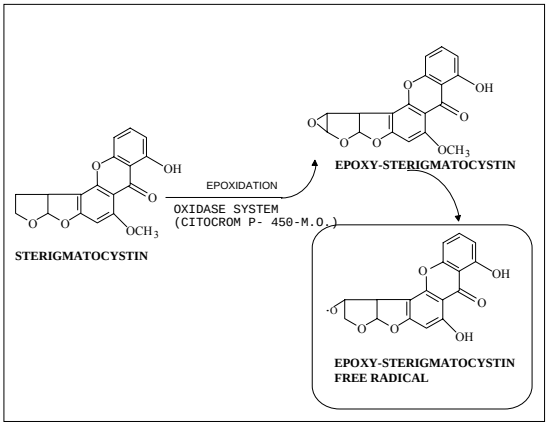

Fig. 1. Toxic form of sterigmatocystin and its origins

MATERIAL AND METHOD

The experimental model as it is presented in table 1, includes 5 groups of 4 months old Wistar white rats with the mean body weight of 391.2g. The first group represented the reference group and they were only given the habitual food. The second group was used for the experimental reproduction of the chronic sterigmatocystin intoxication (control group) and the animals in this group were given 8 ppm of mycotoxins daily. The animals from the third group

Table 1. Experimental model

GROUPS	Sterigmatocystin [ppm]	Ascorbic acid Solution 5%	*Hipophäe fructus* Solution 5%	*Cynosbati fructus* Solution 5%
Reference group (I)	-	-	-	-
Control group (II)	8	-	-	-
Experimental group 1 (III)	8	*10 guttes*	-	-
Experimental group 2 (IV)	8	-	*10 guttes*	-
Experimental group 3 (V)	8	-	-	*10 guttes*

were given, apart from the daily dose of sterigmatocystin, a 5% aqueous solution of ascorbic acid (the *pro die* dose being of 10 *guttes*), being known its role of non-enzymatic antioxidant. The third group received along with sterigmatocystin, a 5% extractive solution of *Hipophäe fructus*, 10 drops per day. For assessing and comparing the antitoxic potential of the two vegetal products, the last group of rats received an additional 5% extractive solution of *Cynosbati fructus* in identical doses. At the end of the experiment, the animals were subjected to biochemical investigations for quantifying the two transamylases acting as parameters of hepatic cytolysis (the liver being considered the target organ of difuran mycotoxins), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as well as the two oxidative stress parameters, catalase (CAT) and superoxid dismutase (SOD).

RESULTS AND DISCUSSIONS

The results recorded after quantifying the hepatic cytolysis parameters are presented in table 2. Analyzing the evolution of the two aminotransferases, both alanine aminotransferase and aspartate aminotransferase record an accentuated and significant increase in the blood of the animals from group II, which had been treated with difuran mycotoxin (38.945 UI). This increase is the result of the attack of the free radical of the epoxy sterigmatocystin on the proteins from the hepatocyte membrane accompanied by the apparition of cellular lysis [7]. An obvious strengthening of the activity of aminotransferases is noticed at the groups treated with an aqueous solution of ascorbic acid (28.611 UI), *Cynosbati fructus* solution (26.999 UI) and *Hipophäe fructus* (27.984 UI).

Table 2. The evolution of aminotransferases

Groups	ALT [UI]	AST [UI]
Reference group (I)	24.561	31.554
Control group (II)	38.945	41.123
Experimental group 1 (III)	28.611	30.998
Experimental group 2 (IV)	27.984	33.569
Experimental group 3 (V)	26. 999	32.174

After trying to gradually intensity the chemopreventive effect of the tested solutions, we can conclude that the rose hip extract solution presents the highest antioxidant/antitoxic potential, followed by the box thorn berry solution, while the lowest chemopreventive potential was present at the aqueous solution of ascorbic acid.

Studying the evolution of catalise and superoxid dismutase, enzymes considered to be OS parameters,
as it results from table 3, we notice a significant decrease at the control group (CAT = 409.199 U/mL, SOD =700.346 U/mL) compared to the reference group (CAT = 559.112 U/mL, SOD =789.114 U/mL). Values similar to those of the reference group are recorded by SOD and CAT enzymes at the groups treated with rose hip and box thorn extract solution which suggests the existence of certain antitoxic/antioxidant features at the active principles of these fruits. The discreet improvement of OS parameters at the group treated with ascorbic acid, suggests the therapeutic superiority of the natural rose hip and box thorn extracts.

Table 3 Variation of oxidative stress parameters

Groups	CAT [U/mL]	SOD [U/mL]
Reference group (I)	559. 112	789.114
Control group (II)	409.199	700.346
Experimental group 1 (III)	530.175	733.919
Experimental group 2 (IV)	561.712	781.599
Experimental group 3 (V)	555.161	783.981

CONCLUSIONS

The evolution of aminotransferases point out the important chemopreventive potential of *Cynosbati fructus* and *Hipophäe fructus* extracts; the ascorbic acid solution presents an effective chemopreventive effect but not as intense as the rose hip and box thorn extracts;

The activity of hepatic cytolysis parameters and OS parameters decreases dramatically in the blood of the animals from the control group (treated only with sterigmatocystin);

The evolution of CAT and SOD suggests the doubtless existence of the antioxidant/antitoxic potential of the active principles from rose hip and box thorn;

The discreet improvement of OS parameters at the group treated with ascorbic acid suggests the therapeutic superiority of natural rose hip and box thorn extracts.

ABSTRACT

The present paper is part of a wider experiment focused on reducing the toxicity level of some mycotoxins considered to be grade 1 carcinogen to humans. Among them there is sterigmatocystin, a mycotoxin that derives from difuran, structurally related to aflatoxins, which withdraws the attention of the human and veterinary pathology by having a high incidence in vegetal aliments from the temperate-continental climate. Considering the hypothesis that sterigmatocystin acts as a free radical coming from epoxy-sterigmatocystin, the experiment presented in this paper deals with the use of some pharmaceutical preparates coming from *Hipophäe rhamnoides* (boxthorn) and *Cynosbati fructus* (rosehip). The vegetal product from the boxthorn pseudoberries, *Hipophäe rhamnoides,* is thought to have special antitoxic/antioxidant properties because of the high level of ascorbic acid (400-1500mg %) and the bioflavonoids that protect the antioxidant vitamin itself from the redox reactions. The antitoxic/antioxidant biocomplex from this effective remedy is completed and increased by the presence of ergosterol and carotenoids (β-carotene, lycopene, physalin, cryptoxanthin and zeaxanthin). The pseudo rosehip berries *Cynosbati fructus* are considered to have real antitoxic/antioxidant effects because of vitamin C (500-1000 mg %), carotenoids (600-10000 mg %) and vitamin E. The experiment included 5 groups of 5 Wistar white rats each. The first group represented the reference group, while the second group was used for the experimental reproduction of the chronic sterigmatocystin intoxication (control group). The animals in the third group were given, apart from the daily dose of sterigmatocystin, ascorbic acid, being known its role of non-enzymatic antioxidant. The third group was given sterigmatocystin and, simultaneously, an extractive 5% solution of *Hipophäe fructus*. For assessing and comparing the antitoxic potential of the two vegetal products, the last group of rats were given an additional extractive 5% solution of *Cynosbati fructus*. At the end of the experiment, the animals were subjected to biochemical analyses by quantifying the two transaminases acting as hepatic cytolysis parameters, alanine aminotransferase – ALT and aspartate aminotransferase – AST as well as the three oxidative stress parameters, catalase - CAT, superoxid dismutase – SOD and the free thion groups. The results obtained underline the significant antitoxic/antioxidant effects of both studies phytopreparates.

REFERENCES

1. BAORU YANG AND HEIKIKI KALLEO, 2006 – Analysis of TAG of Seeds and Berries of *Hipophäe rhamnoides* of different Origins by Mass Spectrometry and Tandem Mass Spectrometry, Pro Quest Medical, 381-392;
2. GOEL H.C., PRASAD J., SINHA S., 2002 – Radioprotection by a herbal preparation of *Hipophäe rhamnoides*, RH_3 against whole body lethal Phytomedicine 9, 1, 21-25;
3. GUPTA R., 2006 – Protective effects of fruits extract *Hipophäe rhamnoides* L. against arsenic toxicity in Swiess albino mine, Human and Experimental Toxicology, 25: 285-295;
4. ISTUDOR V., 2001 - *Farmacognozie, Fitochimie, Fitoterapie*, vol II, Editura Medicală, Bucureşti, 357-358; (Pharmacognosy, Phytochemistry, Phytotherapy, Medical Publishing house, Bucharest);
5. OLINESCU R., GREABU M., 1987 – Chemiluminiscenţa şi bioluminiscenţa, Ed. Tehnică, 73; (Chemiluminescence and bioluminescence, Technical Publishing house);
6. PACKER L., 1984 – Oxygen Radicals in Biological Systems – Methods in Enzymology, Academic Press, 107;
7. PLEŞCA-MANEA, L., CUCUIANU M., CRÎSNIC I.AND BRUDAŞCĂ I., 2003 - Clinical Biochemistry; Physiological Substantiation, (in Romanian). Argonaut Press, Cluj-Napoca: 182-191;
8. PRISĂCARU CORNELIA, 1998 – Incidenţa micotoxinelor în produse alimentare de origine vegetală şi animală din zona Podişului Moldovei şi toxicocinetica şi toxicodinamia acestora, Teză de doctorat, UMF, Iaşi, 186-189; (The incidence of mycotoxins in the vegetal and animal alimentary products from the Moldova Plateau and their toxicokinetics and toxicodynamics, PhD thesis);
9. WEIER J. L., 1989 – Oxy Radicals in Molecular Biology and Pathology, Ed. P. A. Cerutti, J. M. Cord, Ar. Liss. Publ, New York, 268 – 273;
10. YU-LING-TANG, JIANG-HUA SUN, CHENG-HUA YHAO AND YHONG-NING YHANG, 2005 -Sex pheromone components of the sandtorn carpenterworna, *Holcocerus hippophaeecolus*, J. of Chemical Ecologz, Vol, 31, No 1, 39-48.

AUTHORS' ADDRESS

PRISĂCARU CORNELIA - "Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine Iaşi,
e-mail: corneliapris@yahoo.com

PRISĂCARU ANCA IRINA - Institute of Macromolecular Chemistry "*Petru Poni*" Iasi, e-mail: irina.prisacaru@yahoo.com