# THE DIMINUTION OF STERYGMATOCYSTIN TOXICITY BY THE ANTIRADICALIC ACTION OF SOME VEGETAL FLAVONOID CONTAINING PRODUCTS

## DIMINUAREA TOXICITĂȚII MICOTOXINELOR DIFURANICE PRIN ACȚIUNEA ANTIRADICALARĂ A UNOR PRODUSE VEGETALE CONȚINÂND FLAVONOIDE

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***Abstract.****Sterigmatocystin is a mycotoxin derived 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. The present paper is part of a more ample experiment which deals with the reduction of the toxicity of this mycotoxin that has been included in first grade carcinogenic category. Taking into consideration the hypothesis that sterigmatocystin acts as a free radical in the form of epoxy-sterigmatocystin, the experiment presented in this paper stresses upon the use of some pharmaceutical preparates of Hipophäe rhamnoides. The experiment included four groups of five white Wistar rats each. The first group was the reference group, while the second one experimentally reproduced the chronic sterigamtocystin intoxication. Besides the sterigmatocystin dose, the animals from the third group were given ascorbic acid, a nonenzymatic antioxidant. The third group received Hipophäe fructus, along with the sterigmatocystin dose. In the end, the animals were sacrificed and the blood samples were analysed for biochemical investigations with relevance upon the hepatic function and integrity*.

**Key words:** 8,9- epoxi-sterigmatocystin**,** *Hipophäes* fructus, aminotranspherases

***Rezumat****. Sterigmatocistina este o micotoxină difuranică, înrudită structural cu aflatoxinele, a cărei incidență crescută în alimentele de origine vegetală din zona temperat-continentală atrage atenția patologiei umane şi veterinare. Prezenta lucrare face parte dintr-un experiment mai amplu ce vizează reducerea toxicității sterigmatocistinei, micotoxină inclusă în categoria carcinogenilor umani de gradul I. Luând în considerare ipoteza că sterigmatocistina acționează sub forma unui radical liber derivat de la epoxi-sterigmatocistină, experimentul redat în această lucrare vizează utilizarea unor preparate farmaceutice provenite de la Hipophäe rhamnoides. Experimentul a cuprins 4 loturi de câte 5 şobolani albi, linia Wistar. Primul lot a constituit lotul de referință, în timp ce lotul al doilea a servit pentru reproducerea experimentală a intoxicației cronice cu strerigmatocistină. Animalele din lotul al treilea, au primit, pe lângă doza de sterigmatocistină, acid ascorbic, dat fiind rolul său de antioxidant neenzimatic. Celui de al patrulea lot i s-a administrat concomitent cu sterigmatocistina un extract de Hipophäes fructus. La sfârşitul experimentului, animalele au fost sacrificate, iar pe sângele prelevat au fost*

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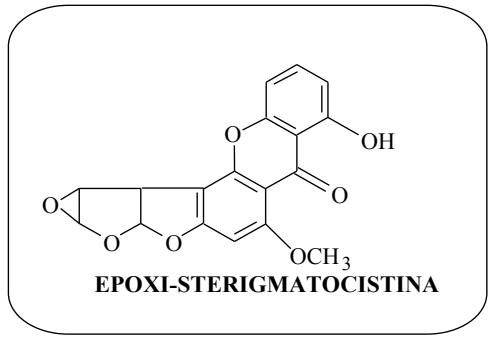
*efectuate investigaţii biochimice cu relevanţă pentru integritatea şi funcţia hepatică. Indicatorii de citoliză hepatică investigate (aspartat aminotransferaza, alanil aminotransferaza) au evidenţiat o ameliorare a integrităţii hepatocitului pentru animalele din lotul protejat cu fitopreparat pe bază de Hipophäe fructus.*

**Cuvinte-cheie**: 8,9-epoxi-sterigmatocistina, *Hipophäe* fructus, aminotransferaze

## INTRODUCTION

Sterigmatocystin, a difuran-coumarin derivative, is a mycotoxin produced by molds from the genera *Aspergillus, Biplaris, Eurotium, Emericella*, but the main source remains *A. versicolor* (Keller, N.P. et al. 1995). Chemically related to aflatoxin $B_1$from which it differs by the presence of the xanthone nucleusinstead of the coumarin one, sterigmatocystin reveals toxicokinetic and toxicodynamic aspects similar to its chemically related toxin. The toxic effects of sterigmatocystin, expressed by the means of carcinogenicity, teratogenicity and immunosupression, are caused by the metabolic activation at the level of the hepatic microsomes, where the mycotoxin is converted by the enzymatic system of cytochrome $P_{450\text{-}480}$-monooxygenase into the 8,9-epoxyde derivative (Shimada,T., et al, 1996, Prisăcaru, 1998).

The toxicological active form of sterigmatocystin is represented by its epoxidic metabolite that is easily converted into the free radical. The sea-buckthorn berries are apreciated in therapy due to their antioxidant potential. Among the compounds with antiradicalic effect there is to be mentioned the L-ascorbic acid protected by the presence of flavonoids that have a sinergic activity. The high concentrations of vitamin C (up to 1500-1800 mg%) along with the flavonoids and tocopherols make *Hipophäe fructus* one of the vegetal products with the highest antioxidant activity (Kensler, T.W. et al., 1997, Brad, I. et al, 2002, Prisăcaru, C., 2010). Taking into consideration these arguments, there can be considered the fact that the necesity of anihilating the oxidative stress generated by the epoxidic metabolite of sterigmatocystin includes as priority the therapeutical use of phytopreparates from *Hipophäe fructus*




**EPOXI-STERIGMATOCISTINA**

**Fig. 1** - The chemical structure of the main metabolite of sterigmatocystin

## MATERIAL AND METHOD

The experimental model (table 1) was achieved in order to evaluate the antioxidant activity of the active principles from the berries of *Hipophäe rhamnoides* in the oxidative stress produced by the epoxidic metabolite of sterigmatocystin. The experiment comprises 4 groups of 5 Wistar rats each, having a mean weight of 185,6 g. The first group was the reference group that has been maintained in standard conditions regarding food and habitat. The second group was conceived as the control group of the sterigmatocystin intoxication and was treated with a dose of 10 ppm of mycotoxin per day that was introduced in daily food. The third group (experimental group 1) was administered both sterigmatocystin (10 ppm) and 3,5% hydroalcoholic solution of *Hipophäes fructus* (XX drops). The animals of the fourth group received, along with the daily mycotoxin dose (10 ppm) and the *Hipophäes fructus* phytopreparate (xx drops), the additional protection of a 5% aqueous solution of ascorbic acid in daily doses of XV drops, administered in drinking water. The experiment was unfolded on a period of 8 weeks. In the end, blood samples were collected in order to evaluate the biochemical parameters relevant for the hepatic cytolisis (aspartate aminotransferase - AST, alanine aminotransferase - ALT) and oxidative stress (catalase - CAT, superoxid dismutase - SOD, and gluathione peroxidase - G-Px).

*Table 1*

**Experimental model**

| Groups | STG [ppm] | *Hipophäes fructus*(Hydroalc. sol. 3,5%) | AA (Aqueous sol.5%) | Biochemical parameters |
|---|---|---|---|---|
| Reference group | - | - | - | AST, ALT CAT, SOD, G-Px, |
| Control group | 10 | - | - | |
| Experimental group 1 | 10 | XX guttes | - | |
| Experimental group 2 | 10 | XX guttes | XV guttes | |

Legend: STG = sterigmatocystin; AA= ascorbic acid (5% aqueous solution); CAT = catalase; SOD = superoxide dismutase; G-Px = glutathione peroxidase; AST =aspartate aminotransferase; ALT = alanine aminotransferase.

## RESULTS AND DISCUSSIONS

The results obtained from the quantification of the hepatic cytolysis parameters (table 2) emphasize aleatory and different evolutions. The activity of alanine aminotransferase is not conclusive for the present experiment, illustrating decreased values both for the group intoxicated with sterigmatocystin and the groups protected with phytopreparates obtained from *Hipophäes fructus*. Unexpectedly, the lowest values for ALT (14,43 UI) are registered for the group of animals that were exclusively given the difuran mycotoxin that is known to have as target organ the liver. Contrary to the unexpected evolution of ALT, the second parameter illustrating the hepatic cytolysis, AST, shows a significant increase for the group agressed by the epoxidic radicals of sterigmatocystin (39,73

UI compared to 31,53 UI, the value for the reference group). The activity of AST from the blood samples of the animals treated with hydroalcoholic extract of *Hipophäes fructus* is discreetly decreased compared to the intoxicated group, suggesting the antiradicalic intervention of the active principles from the sea-buckthorn berries. Unfortunately, the evolution of AST for the group that was further treated with ascorbic acid leads to a highly raised value (39,7 UI), value that is almost identical to the value obtained for the intoxicated group (39,73 UI), thus infirming the antitoxic-antioxidant role of the sea-buckthorn berries.

*Table 2*

**The evolution of the hepatic cytolysis parameters**

| **Groups** | **Alanine aminotransferase (ALT) [UI]** | | | **Aspartate aminotransferase (AST) [UI]** | | |
|---|---|---|---|---|---|---|
| | minimum | Mean | MAXIMUM | minimum | Mean | MAXIMUM |
| Reference group | 11.30 | 16.90 | 19.20 | 28.50 | 31.53 | 37.90 |
| Control group | 11.20 | 14.43 | 18.50 | 29.80 | 39.73 | 54.30 |
| Experimental group 1 | 12.30 | 15.36 | 16.60 | 29.00 | 37.00 | 54.40 |
| Experimental group 2 | 11.90 | 15.66 | 17.20 | 31.00 | 39.70 | 51.00 |

The evaluation of the oxidative stress markers reveals a different image, thus confirming the antioxidant potential of the active principles from *Hipophäes fructus*. Catalase (CAT), as there can be seen in table 3, fig. 4, decreases its values in the case of the group that was given the difuran derived mycotoxin with 40 units (246.00 U/L) compared to the reference group (280,3 U/L), thus suggesting the presence of the oxidative stress produced by the epoxidic metabolite of sterigmatocystin.

The activity of CAT from the serum of the animals treated with hydroalcoholic solution from sea-buckthorn berries reaches normal values, the highest level being obtained for the experimental group 2 that benefit from the additional treatment with ascorbic acid. These variations suggest the fact that the antioxidant phytocomplex from the sea-buckthorn berries and ascorbic acid anihilate the oxidative stress generating free radicals.

*Table 3*

**The evolution of catalase**

| **CATALASE (U/L)** | | | |
|---|---|---|---|
| | Minimum | Mean | MAXIMUM |
| Reference group | 220.0 | 280.3 | 339.0 |
| Control group | 125.0 | 246.0 | 315.0 |
| Experimental group 1 | 170.0 | 281.6 | 351.0 |
| Experimental group 2 | 112.0 | 287.3 | 337.0 |

Evaluating the activity of superoxide dismutase as shown in table 4 , there can be noticed an evolution that emphasizesthe consumption of the enzyme by the free radicals produced by the presence of the difuran mycotoxin in the intoxicated group and discrete improvements for the groups protected with the hydroalcohlic extract of *Hipophäe rhamnoides*. The improvement of the SOD activity for the group protected both with the phytopreparate from the sea-buckthorn berriesand the ascorbic acid solution is not significant (296.8 U/L compared to 293.9 U/L, value of the intoxicated group), therefore there cannot be taken into consideration a significant antioxidant potential.

*Table 4*

**The evolution of superoxide dismutase**

| SUPEROXIDE DISMUTASE (U/L) | | | |
|---|---|---|---|
| | minimum | Mean | MAXIMUM |
| Reference group | 311.0 | 324.9 | 369.0 |
| Control group | 211.0 | 293.5 | 371.0 |
| Experimental group 1 | 258.0 | 303.5 | 332.0 |
| Experimental group 2 | 217.9 | 296.8 | 366.0 |

The evolution of the third parameter relevant for the oxidative stress (table 5), glutathione peroxidase, leads to values that sustain the presence of the oxidative stress in the control group and the antioxidant effect of the phytopreparate from the sea-buckthorn berries, effect that becomes stronger when associated with the exogen ascorbic acid.

*Table 5*

**The evolution of glutathione peroxidase**

| GLUTATHIONE PEROXIDASE (U/L) | | | |
|---|---|---|---|
| | minimum | Mean | MAXIMUM |
| Reference group | 58.0 | 80.33 | 88.0 |
| Control group | 50.5 | 75.96 | 85.3 |
| Experimental group 1 | 49.5 | 80.36 | 84.9 |
| Experimental group 2 | 44.5 | 77.57 | 89.5 |

## CONCLUSIONS

1. The quantification of the two parameters relevant for the hepatic cytolysis (AST and ALT) leads to insignificant aleatory results;

2. The evolution of serum catalase emphasize the fact that the antioxidant phytocomplex from the sea-buckthorn berries and the additional ascorbic acid intake decrease the oxidative stress produced by the epoxidic metabolite of sterigmatocystin;

3. The evolution of superoxide dismutase reveals a reduced and insignificant antioxidant effect for the group treated with sea-buckthorn berries and ascorbic acid;

4. The values of glutathione peroxidase confirm that the antioxidant phytocomplex from *Hipophäe rhamnoides* is efficient.

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