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# THE DIMINUTION OF STERYGMATOCYSTIN TOXICITY BY THE ANTIRADICAL ACTION OF SOME FLAVONOID- CONTAINING PLANT PRODUCTS

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***ABSTRACT.*** *Sterigmatocystin is a bifuran mycotoxin, structurally related to aflatoxins, which has a high incidence in plant products from the temperate-continental zone. The present study is part of a more ample experiment that deals with the reduction of this mycotoxin toxicity, which has been included in the first degree carcinogenic category. Taking into account the hypothesis that sterigmatocystin behaves as a free radical coming from epoxy-sterigmatocystin, the experiment pointed out the use of some pharmaceutical preparations containing Hippophäe rhamnoides. The experiment included four groups of five white rats, each. The first group was the control one, while the second one served for the experimental reproduction of chronic sterigmatocystin intoxication. Besides the sterigmatocystin dose, the animals from the third group were given ascorbic acid, a non-enzymatic antioxidant. The fourth group was treated with Hippophäe fructus, along with the sterigmatocystin dose. The animals were then slaughtered, and the blood was used for biochemical investigations with important relevance upon the hepatic function and integrity. The investigated hepatic cytolysis indices, aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase from blood samples emphasized a significant improvement of the liver integrity for the group of animals treated with Hippophäe fructus. The results obtained for the biochemical parameters accountable for the liver proteosynthetic capacity (acetyl cholinesterase and total protein) did not suggest an efficient protective effect of the investigated plant preparation. Plant preparations containing Hipophäe rhamnoides exerted a positive effect on liver integrity, while its benefits on the proteosynthetic function of the liver were insignificant.*

**Key Words**: sterigmatocystin, toxicity, biochemical investigation, liver, plant preparation

***REZUMAT – Reducerea toxicității sterigmatocistinii prin acțiunea antiradicală a unor produse vegetale ce conțin flavonoide.*** *Sterigmatocistina este o micotoxină, legată structural de aflatoxine, semnificativă pentru patologia umană şi veterinară, datorită prezenței sale*

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*crescute în produsele vegetale din zona temperat-continentală. Prezentul studiu face parte dintr-un experiment mai amplu privind reducerea toxicității acestei micotoxine, ce a fost inclusă în categoria carcinogenelor de gradul unu. Luând în considerare ipoteza că sterigmatocistina se comportă ca un radical liber, ce provine din epoxi-sterigmatocistină, acest experiment subliniază importanța folosirii unor preparate farmaceutice, ce conțin Hippophäe rhamnoides. Experimentul a fost realizat pe patru loturi a câte cinci şobolani. Primul lot a reprezentat varianta martor, iar al doilea a fost folosit pentru reproducerea experimentală a intoxicației cronice cu sterigmatocistină. In afară de doza de sterigmatocistină, animalelor din cel de-al treilea lot li s-a administrat acid ascorbic, un antioxidant neenzimatic. Al patrulea lot a fost tratat cu Hippophäe fructus, în afară de doza de sterigmatocistină. Animalele au fost apoi sacrificate, iar sângele lor a fost analizat din punct de vedere biochimic, având relevanță asupra funcției hepatice. Indicatorii hepatici analizați, aminotransferaza şi dehidrogenaza au evitențiat ameliorarea semnificativă a ficatului, în cazul lotului de animale tratate cu Hippophäe fructus. Rezultatele obținute privind parametrii biochimici importanți pentru capacitatea proteosintetică a ficatului (acetil colinesteraza şi proteine totale ) nu au putut demonstra prezența unui efect protectiv eficient al fitopreparatului analizat. Fitopreparatele ce conțin Hipophäe rhamnoides exercită un efect pozitiv asupra integrității ficatului, în timp ce efectele sale benefice asupra funcției proteosintetice a ficatului sunt nesemnificative.*

**Cuvinte cheie:** sterigmatocistină, toxicitate, cercetări biochimice, ficat, fitopreparat

## INTRODUCTION

Sterigmatocystin is a bifuran mycotoxin that behaves like a hepatotoxin, under the form of a free radical, resulted from epoxy-sterigmatocystin (*Figure 1*), the main metabolite from liver of the animals that ingested the parental mycotoxin (Prisăcaru et al., 2004; Prisăcaru et al., 2005).

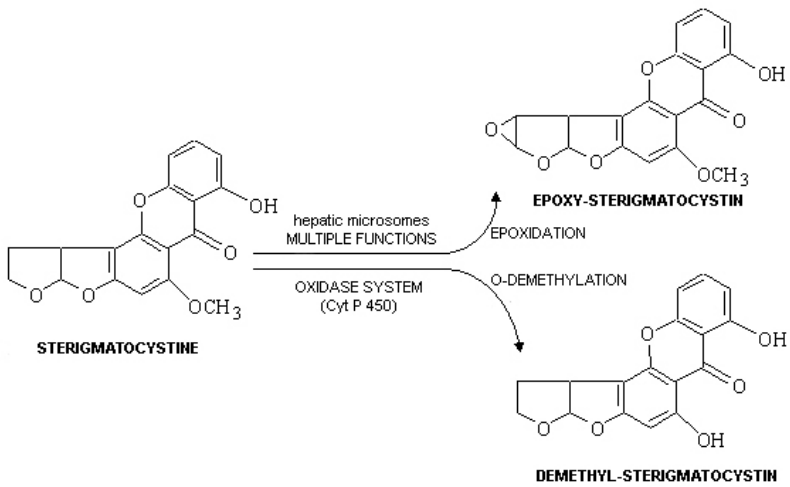


**Fig. 1 - The primary metabolization of sterigmatocystin**

The high incidence of sterigmatocystin in food products and vegetal fodder from the Moldavian region (Prisăcaru, 1998) compels the necessity of discovering new vegetal low-costing antitoxic remedies, which are easy to get. The high content of *Hippophäe rhamnoides* in antiradical compounds like ascorbic acid, flavonoids and carotenes (Brad, 2002) makes this plant useful for preventing the binding of epoxy-sterigmatocystin radical to the elite structures of the cell (DNA, RNA, enzymes) (Kensler, 1997).

## MATERIALS AND METHODS

The experimental model presented in this paper aims at pointing out the eventual antioxidant/antitoxic effect of a 5% extractive solution from *Hippophäe rhamnoides* in the case of sterigmatocystin intoxication. In order to estimate this effect accurately, a 5% ascorbic acid solution was used as standard, mainly because of the high content of *Hippophäe rhamnoides* in this vitamin. It is also considered that the antitoxic effect of this plant is, at a significant rate, due to the enolic function of this antioxidant.

The experiment has included four groups of Wistar white rats, 4 months old, with an average body weight of 176 g. The first group was made up of five rats and represents the control group. The second group was made up of five rats, in order to reproduce the sterigmatocystin intoxication. The animals were given 8 ppm of hepatotoxic mycotoxin per day. The third group was made up of five rats, for evaluating the antiradical effect of *Hippophäe rhamnoides*. These five animals were given 10 ml of 5% *Hippophäe rhamnoides* infusion per day in the drinking water, besides the 8 ppm dose of sterigmatocystin. The five rats from the fourth group were concomitantly treated with the sterigmatocystin dose and an injectable 5% solution of ascorbic acid (10 ml per day in the drinking water).

At the end of the experiment that lasted six weeks, blood samples were collected from the slaughtered animals and tested biochemically.

In order to assess the integrity of the hepatocyte, the activity of three enzymes was tested: aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH). For evaluating the proteosynthetic activity of liver, the activity of total proteins and acetyl cholinesterase (ChE) has been investigated.

The aqueous extractive solution of *Hippophäe fructus* has been prepared according to the present Pharmacopoeia ([xxx], 1993). The animals were administered the fresh, *ex tempore* prepared infusion in the drinking water.

The activities of the seric enzymes and the total proteins concentration were determined by standardized tests (Cucuianu et al., 1991; Filip, 1993; Şerban et al., 1993).

## RESULTS AND DISCUSSION

The results obtained after the quantification of the activities of the three enzymes, used as cytolysis indexes, are presented in *Table 1, Figures 2 and 3*. These results pointed out a significant increase of the alanine aminotransferase activity for the sterigmatocystin intoxicated group (36.45 UI), compared to the control group (18.38 UI). In the group that received the concomitant treatment

with sterigmatocystin and the 5% *Hippophäe rhamnoides* infusion, the enzyme activity had a significantly low value (22.78 UI), compared to the sterigmatocystin treated group.

**Table 1**

**Evolution of the cytolysis indexes**

| Groups | ALT [UI] | AST [UI] | LDH [µmol/ml] |
|---|---|---|---|
| Group 1 | 18.88±1.929 | 33.53±3.706 | 2.99±0.598 |
| Group 2 | 36.45±1.306 | 54.60±3.017 | 9.33±5.276 |
| Group 3 | 22.78±2.999 | 34.52±3.212 | 4.29±2.320 |
| Group 4 | 30.79±0.997 | 44.37±2.907 | 5.99±2.997 |

ALT = alanine aminotransferase; AST = aspartate aminotransferase; LDH = lactate dehydrogenase

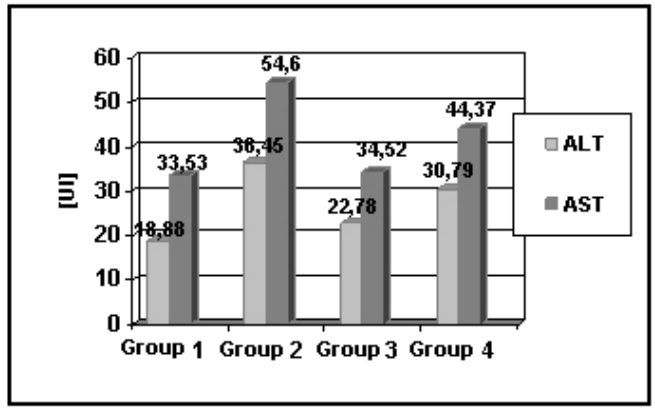


**Fig. 2 - The values of the aminotransferase activities**

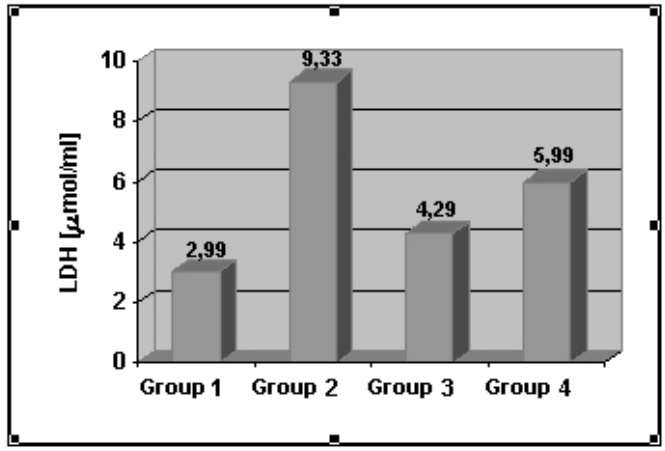


**Fig. 3 - The values for the lactate dehydrogenase activities**

The evolution of the alanine aminotransferase activity, considered to be a cytolysis marker, has shown the antitoxic/antiradical effect of the active principles from *Hippophäe rhamnoides*.

As concerns the group that received the ascorbic acid solution, as antioxidant treatment, the alanine aminotransferase value was closer to the alanine aminotransferase value of the sterigmatocystin group (30.79 UI), suggesting that the total active principles from *Hippophäe fructus* have exerted a higher effect than vitamin C alone.

The evolution of the second aminotransferase, aspartate aminotransferase group suggested a significant increase in the blood samples of the sterigmatocystin group (54.60 UI), compared to the reference group (33.53 UI). In the group that benefited of the *Hippophäe fructus* infusion protection, the enzyme value was significantly reduced, compared to the exclusively sterigmatocystin treated group (34.52 UI). For the animals treated with ascorbic acid solution, the enzyme value was of 44.37 UI, a higher value compared to the control group; these results could not ignore the antiradical effect of this vitamin.

The antioxidant/antitoxic effect of the active principles from *Hippophäe fructus* was sustained by the variation of the third enzyme, lactate dehydrogenase (*Tables 1 and 3*). The value of 2.99 μmol/ml, recorded for the control group, has increased to 9.33 μmol/ml for the sterigmatocystin group and then decreased significantly to 4.29 μmol/ml, for the group protected with the *Hippophäe fructus* infusion. The group protected with ascorbic acid has recorded the value of 5.99 μmol/ml, an equidistant value situated between the control group and the group protected with the *Hippophäe fructus* infusion.

An opposite situation is revealed by the results obtained after investigating the parameters that give information about the proteosynthetic capacity of the liver.

The activity of seric cholinesterase, an enzyme synthesized by the liver, has recorded an aleatory evolution, which came in contradiction with the evolution of cytolysis indexes, revealing the antiradical/antitoxic effects of the active principles from *Hippophäe fructus* (*Table 2 and Figure 4*). The cholinesterase activity from the control group had the value 255.95 UI that decreased until 210.17 UI, for the sterigmatocystin intoxicated group. Paradoxically, the enzyme activity for the group treated with the *Hippophäe fructus* infusion was below the value of the enzyme activity for the group intoxicated with sterigmatocystin (199.55UI), while for the group protected with vitamin C, the cholinesterase activity was dramatically reduced to 182.78UI.

The results for the total proteins (*Table 2, Figure 5*) have shown a remarkable increase in the blood of the animals treated exclusively with sterigmatocystin (36.39 g‰), suggesting a reduction of the proteosynthetic activity of the liver. An amelioration of the proteosynthetic function of the liver was shown by the slightly augmented values of the total proteins for the group

protected with the *Hippophäe fructus* infusion (40.99 g ‰) and with the ascorbic acid injectable solution (41.55 g ‰).

**Table 2**

**Variation of the parameters referring to the proteosynthetic capacity**

| Groups | Ch E [UI] | Total seric proteins [g‰] |
|---|---|---|
| Group 1 | 255.95±11.422 | 53.67±1.99 |
| Group 2 | 210.17±17.203 | 36.39±3.02 |
| Group 3 | 199.55±13.24 | 40.99±2.97 |
| Group 4 | 182.78±19.69 | 41.55±3.09 |

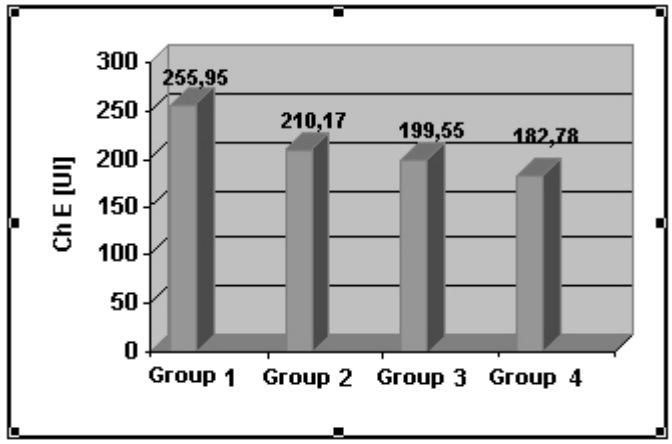


**Fig. 4 - The value for the seric cholinesterase activity**

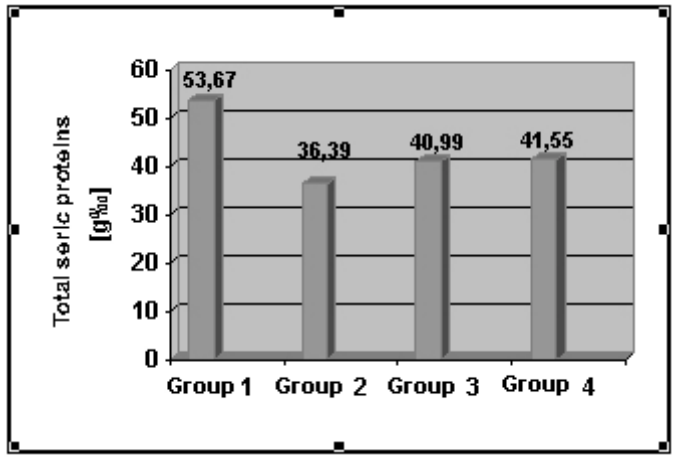


**Fig. 5 - Total protein concentration**

## CONCLUSIONS

The variation of the seric transaminases (AST, ALT) suggests the presence of a significant antitoxic effect of the *Hippophäe fructus* plant preparation in the intoxication with sterigmatocystin.

The antioxidant/antitoxic effect of the active principles from *Hippophäe fructus* plant preparations is superior to the ascorbic acid solution alone; this superiority is explained by the presence of flavonoids.

The evolution of lactate dehydrogenase confirms the chemopreventive intervention of the *Hippophäe fructus* extractive solution at the impact with sterigmatocystin.

The cholinesterase variation infirms the good activity of *Hippophäe fructus* plant preparation and ascorbic acid.

The total protein concentration may represent an argument in sustaining the antiradical/antitoxic effect of *Hippophäe fructus.*

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