

# Evaluation of the Antitoxic Effect of Phthalides from *Apium graveolens* in Acrylamide Intoxication

## I. Evolution of the Hepatic Cytolysis and Proteosynthetic Parameters in Acrylamide Intoxication on the Background of Phthalide Protection

Cornelia PRISACARU<sup>1)</sup>, Anca Irina BURLACU<sup>2)</sup>

<sup>1)</sup> *University of Agricultural Sciences and Veterinary Medicine Iasi, Faculty of Veterinary Medicine; 8 Mihail Sadoveanu Street, Romania; corneliapris@yahoo.com*

<sup>2)</sup> *University of Medicine and Pharmacy "Gr. T. Popa", Faculty of Pharmacy, Department of Environmental and Alimentary Chemistry, 16 Universitatii Street, 700115, Romania; irina.burlacu@yahoo.com*

### Abstract

Acrylamide is a toxic compound formed during thermal processing of foods that contain amino acids, mainly asparagine, and reducing sugars. The toxicodynamics of acrylamide, exerted mainly by its major metabolite glycidamide, is expressed by mutagenic, carcinogenic and immunosuppressive effects. Due to its toxicity and its high prevalence in aliments, the present paper approaches a possible way of reducing the toxic effects of acrylamide by using phytotherapeutical means. In this direction, the antitoxic potential of some phytopreparates obtained from *Apium graveolens* (*Apii aetheroleum*, extractive solutions from *A. radix*, *A. folium* and *A. semen*) were monitorized on the background of acrylamide intoxication. The antitoxic potential of phthalides has been evaluated by determining biochemical parameters: hepatic cytolysis parameters (aspartate aminotransferase, alanil aminotransferase, lactate dehydrogenase) and proteosynthesis parameters (colinesterase, total proteins, albumins). The biochemical investigation confirmed the antitoxic potential fo phthalides from celery, the highest protection being obtained for the phytopreparate from *Apii semen*.

**Keywords:** phthalides, *Apium graveolens*, hepatic cytolysis parameters, proteosynthesis parameters

### Introduction

A chemical compound with a structure characterized by the presence of two non-saturated centers, acrylamide has a special toxicological significance expressed at the level of liver, excretory and central nervous system, with repercussions on the immune system (Awad *et al.*, 1998). The high levels of acrylamide in food products and the exposure risk of the consumers demand for the necessity of finding means of reducing its toxicity, represented by phytotherapeutical, chemopreventive or enzymatical reduction methods, that can accelerate the excretion of the toxic metabolites or that can prevent the formation of chemical aducts, precursors of the biochemical lessions (Boettcher *et al.*, 2005).

The present paper is interested in finding virtual means of decreasing the toxicity of the food noxa by using phthalides containing phytopreparates. Phthalides, synthesized by some Apiaceae plants (*Apium graveolens*, *Levisticum officinale*, *Ligusticum acutilobum*) are penta-atomic lactones condensed with a non-saturated hexagonal ring (Fig. 1). Whether they are alkyl-phthalides or alkylidene-phthalides, these substances posses hepatocitary and renal detoxifying

effect (Istudor, 2001; Stanescu *et al.*, 2004; Prisacaru and Rotaru, 2008). The phthalide content (up to 25%) of the volatile oil obtained from *Apii folium* (*Apii aetheroleum*), illustrated mainly by sedanolid and n-butylphthalide, represent the reason for testing the antitoxic effect of this volatile oil in acrylamide intoxication and comparing it with the effect of some aqueous extractive solutions from *Apii folium* and *Apii radix*.

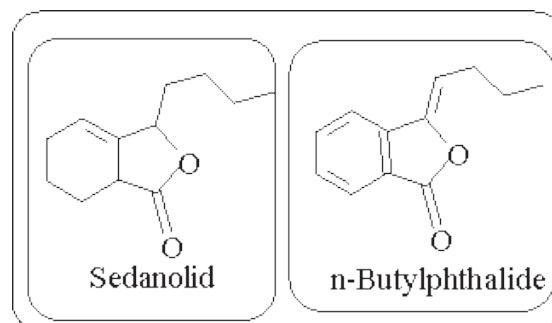


Fig.1. Chemical structure of some phthalides from *Apii aetheroleum*

## Materials and methods

The experimental model presented in Tab. 1 was conceived so as to monitorize the antitoxic effect of phthalides from celery volatile oil (*Apium aetheroleum*) in acrylamide intoxication. At the same time, the experiment aims to gradually evaluate the antitoxic potentials of some extractive solutions obtained from *Apium folium*, *Apium radix* și *Apium semen*.

The last experimental group offered information regarding the role of phthalides from celery seeds in acrylamide intoxication, the animals being protected with 3% *Apium semen* infusion. The experiment lasted for a period of 11 weeks and ended with the collection of blood samples. The biochemical exploration was fulfilled as battery tests that aimed to evaluate the permeabilisation degree of the hepatocytary membrane and to estimate the changes in the proteosynthetic function of the liver in acrylamide intoxi-

Tab. 1. The experimental model relating to the antitoxic effect of phthalides from *Apium graveolens*

Groups	Name	Acrylamide ( $\mu\text{g}/\text{kg}$ body weight)	<i>Apium Aetheroleum</i> [ppm]	<i>Apium folium</i>	<i>Apium radix</i>	<i>Apium semen</i>
Group 1	Reference (Ref group)	-	-	-	-	-
Group 2	Control (Control group)	25	-	-	-	-
Group 3	Experimental 1 (Aeth group)	25	10	-	-	-
Group 4	Experimental 2 (Folium group)	25	-	Ad libitum	-	-
Group 5	Experimental 3 (Radix group)	25	-	-	Ad libitum	-
Group 6	Experimental 4 (Semen group)	25	-	-	-	Ad libitum

The experiment was conducted using six groups of five white 3-month old Wistar rats, with a medium body weight of 221,4 g. The first group was the reference group, the animals being maintained in the same habitat conditions as the other groups, with the difference that their

cation and phthalide protection (Tab. 2). Evaluation of the parameters indicated in the Tab. below was performed using a semiautomatic analyzer in open system EOS 88 PLUS.

Tab. 2. Scheme of the biochemical investigations

Test Batteries	Tested Parameters
1 Hepatic Cytolysis Parameters	aspartate aminotransferase (ASAT sau AST)
	alanine aminotransferase (ALAT sau ALT)
	lactate dehydrogenase (LDH)
2 Proteosynthesis Parameters	Cholinesterase (ChE)
	Total seric proteins (PT)
	Seric albumins (ALB)

food was not treated with any of the tested substances. The second group was given 25  $\mu\text{g}$  acrylamide/kg body weight *pro die* by gavage. The third group (experimental 1) offered informations about the antitoxic effect of the celery volatile oil, the intoxicated animals being treated with 10 ppm *Apium aetheroleum*.

The animals of the fourth group (experimental 2) were treated so as to emphasize the antitoxic potential of *Apium folium* extractive solution. In agreement with this aim, the rats of this experimental group were given, besides the *pro die* acrylamide dosis, 3% *Apium radix* infusion *ad libitum*.

## Results and discussions

The data obtained as a result of the investigated hepatic cytolysis parameters, statistically processed, are given in Tab. 3.

The study of aspartate aminotransferase activity in the animal serum of the six experimental groups emphasize variations that offer important information (Fig. 2). Therefore, the activity of this celular enzyme follows a significant increase in the serum of the animals treated with daily acrylamide doses. The presence in the serum of acrylamide treated rats of high concentrations of this enzyme having a strictly cytosolic localization in normal physiological conditions is translated by the occurrence of an advanced permeabilisation of the hepatic membrane, that allowed the flowing of the enzyme into the serum (Cucuianu *et al.*, 1998; Prisacaru *et al.*, 2008).

Continuing the study of the evolution of the enzyme activity for the groups treated with phytopreparates obtained from *Apium graveolens*, values higher than those of the reference group but lower than the values for the acrylamide intoxicated group are obtained. The activity of aspartate aminotransferase shows values nearest to the ref-

Tab. 3. The value of the hepatic cytolysis parameters

CYTOLYSIS PARAMETERS			
Groups	$x \pm \sigma$ (DS)		
	AST (UI)	ALT (UI)	LDH ( $\mu\text{mol/ml}$ )
Group 1	31.291 $\pm$ 4.244	24.969 $\pm$ 2.687	6.666 $\pm$ 0.415
Group 2	39.536 $\pm$ 4.222	34.859 $\pm$ 4.097	15.998 $\pm$ 0.207
Group 3	35.311 $\pm$ 2.284	28.39 $\pm$ 3.143	6.559 $\pm$ 0.488
Group 4	35.897 $\pm$ 3.455	30.010 $\pm$ 1.466	6.358 $\pm$ 0.351
Group 5	33.532 $\pm$ 4.357	30.318 $\pm$ 3.159	6.509 $\pm$ 0.832
Group 6	35.8958 $\pm$ 2.8812	25.951 $\pm$ 3.160	6.458 $\pm$ 0.378

reference group for the animals treated with *Apii radix* infusion, suggesting the presence of the antitoxic effect of the celery phytopreparate in acrylamide intoxication.

Studying the activity of the second cellular aminotransferase, alanil aminotransferase (Fig. 3.), major variations

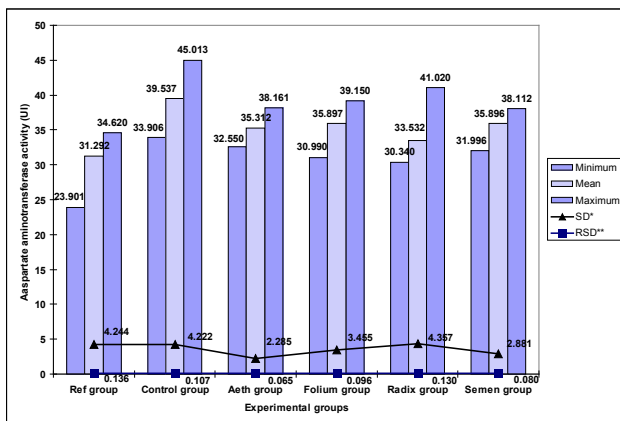


Fig. 2. Evolution of aspartate aminotransferase activity

between the reference and intoxicated group are noticed on the one hand, and also between the acrylamide treated group and the groups protected with *Apium graveolens* phytopreparates, on the other.

Investigation of the third cytolysis parameter was conceived as an evaluation of acrylamide aggression in time. The activity of lactate dehydrogenase, as it is revealed in Fig. 4., outlines an exacerbation for the control group. This dramatic augmentation of the enzyme activity suggests the advanced permeabilization of the hepatic membrane (Burlacu and Prisacaru, 2008). Moreover, if this augmentation is correlated with the important increase of the other cellular enzymes, other sources of membranar lysis can be suggested (cord, kidneys etc.). The variation of LDH activity for the groups protected with *Apium graveolens* phytopreparates is almost linear, the values of the enzyme activity recording an oscillation around the reference group value, confirming their antitoxic effect.

Among the parameters able to provide information on eventual disorders of the proteosynthetic activity of the liver is colinesterase, a hepatic secretion enzyme, whose diagnostic utility is augmented by its slow turnover. Fol-

lowing the evolution of this enzyme for the experimental groups, aleatory and inconclusive variations are observed (Fig. 5.).

The oscillation of this enzyme does not only provide zero information regarding the possible improvement of the proteosynthetic action of the liver by the phthalides from the studied vegetal products, but also it doesn't reveal the fact that the proteosynthetic function of the liver is disordered by the acrylamide metabolisation effort with the formation of the toxic active form. Nevertheless, the evolution of colinesterase, which presents high values for the groups protected with phthalides but mainly for the acrylamide treated groups, can be correlated with a nephrotic sindrom, an aspect that has been referred to in literature (Plesca *et al.*, 200; Prisacaru and Burlacu, 2008).

The evaluation of the total proteins, as it is shown in Fig. 6., reveals a discrepancy of values between the refer-

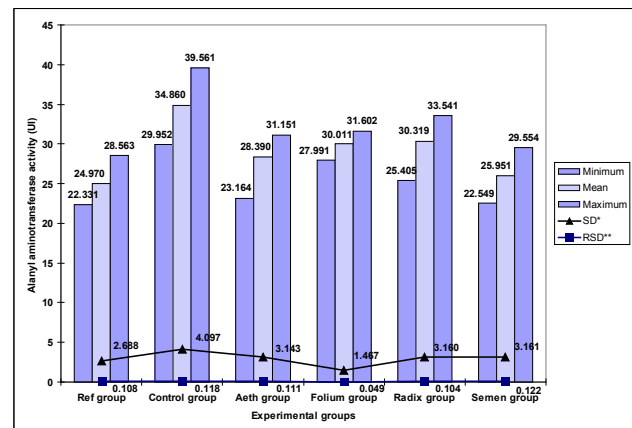


Fig. 3. Variation of alanil aminotransferase activity

ence group and the acrylamide intoxicated group, proving the decrease of the proteosynthetic potential of the liver under the impact of acrylamide and its toxic metabolite, glycidamide. The significant high values compared to the intoxicated group demonstrate the antitoxic effect of *Apium graveolens* volatile oil and of the extractive solutions from *Apii folium* and *Apii radix*, but most of all, of the *Apii semen phytopreparate*.

The evaluation of albuminemia reveals, as it is shown in Fig. 7, new aspects of acrylamide toxicodynamics that are not completely cleared up for the moment. The diagrama

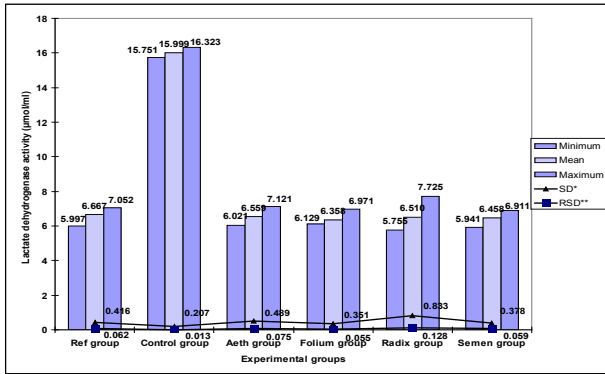


Fig. 4. Variation of lactat dehydrogenase activity

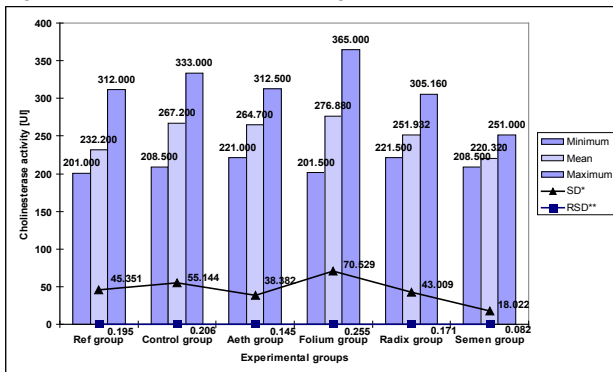


Fig. 5. Evolution of the colinesterase activity

of the albumin seric concentration follows an ascendent direction for the acrylamide intoxicated group, this unexpected increase possible being the consequence of a compensatory effect due to the transporter role of toxins or the consequence of a nephrotic syndrom that can be correlated with the evolution of colinesterase. Albuminemia of the groups protected with *Apium graveolens* phytopreparates follows an uneven and insignificant curve for the groups treated with *Apium folium*, *A. radix* and *A. aetheroleum* infusion. In contradiction to these insignificant values, the albuminemia of the animals treated with *Apium semen* phytopreparate is appropriate to the values of the reference

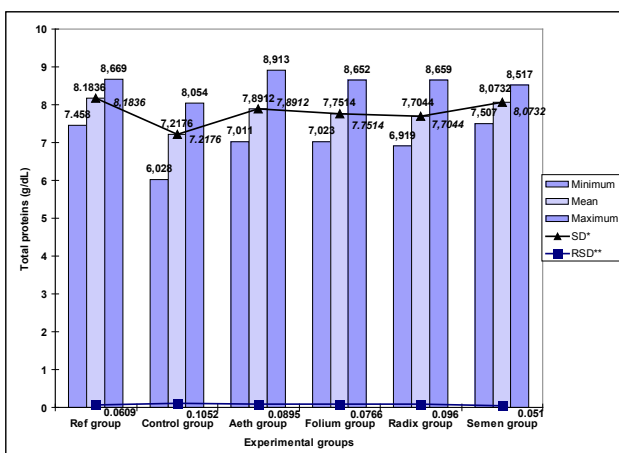


Fig. 6. Concentration of total proteins

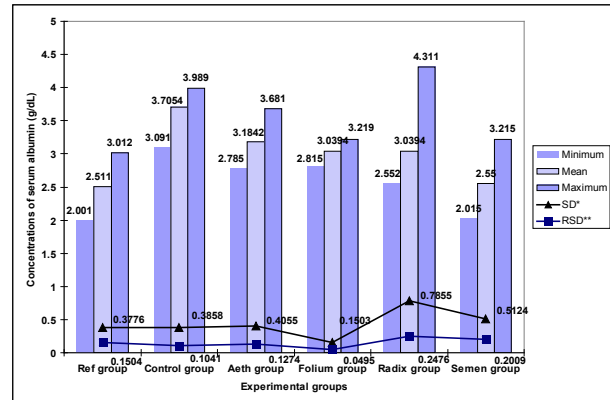


Fig. 7. Serum concentrations of albumin

group. It can be concluded again that the phytopreparate obtained from *Apium graveolens* seeds have the highest antitoxic effect from all the phytopreparates tested in this experiment.

### Conclusions

The evolution of the hepatic cytolysis parameters emphasize a significant decrease for the group treated exclusively with acrylamide, suggesting the membranare lysis.

The variation of the AST activity for the protected groups reveals the existance of a good antitoxic effect of the phthalides from *Apium radix* and a moderate antitoxic effect for the *Apium graveolens* volatile oil, leaves and seeds.

The variation of lactat dehydrogenase activity records an exacerbation of this enzyme for the acrylamide intoxicated animals, suggesting the agresivity of acrylamide not only at the level of liver, but at the level of other organs that house LDH intracellularly.

The variation of colinesterase reveals an uneven and insignificant curve, that doesn't offer information about the proteosynthetic capacity of liver, but that correlates with the existance of a nephrotic syndrom.

Proteinemia records variations that emphasize the depression of the proteosynthetic function of the liver for the acrylamide intoxicated animals and reveal the benefic intervention of the phthalides from *Apium graveolens*, especially *Apium semen*.

The evaluation of albuminemia confirms the antitoxic effect of phthalides from *Apium semen* and suggests a possible correlation of hiperalbuminemia of the acrylamide intoxicated group with a nephrotic syndrom.

### References

Awad, M. E., M. S. Abdel-Rahman and S. A. Hassan (1998). Acrylamide toxicity in isolated rat hepatocytes. *Toxicol. In vitro*. 12:699-704.

Boettcher, M. I., T. Schettgen, B. Kutting, M. Pischetsrieder and J. Angerer (2005). Mercapturic acids of acrylamide and glycidamide as biomarkers of acrylamide and glycidamide

- as biomarkers of the internal exposure to acrylamide in the general population. *Mutat. Res.* 589:166-176.
- Burlacu, A. I. and C. Prisacaru (2008). Evaluation of the antitoxic effect of some phthalides containing vegetal products. 45th Congress of the European Societies of Toxicology, Rhodes, Greece, 05-08 Oct. 2008. Vol 180S: 562.
- Cucuianu, M., I. Crisnic and L. Plesca-Manea (1998). *Clinical Biochemistry; Physiological Substantiation*. Dacia Press, Cluj-Napoca. 122-146.
- Istudor, V. (2001). *Pharmacognosy, Phytochemistry, Phytotherapy*. Medical Press, Bucharest. Vol. II: 223-226.
- Plesca-Manea, L., M. Cucuianu, I. Crisnic and I. Brudasca (2003). *Clinical Biochemistry; Physiological Substantiation*, (in Romanian). Argonaut Press, Cluj-Napoca. 182-191.
- Prisacaru, C. and L. Rotaru (2008). Monitorization of the antitoxic effect of some volatile oils containing phthalides (*Apii aetheroleum* and *Levistici aetheroleum*), (in Romanian). *Rev. Med. Chir. Soc. Med. Nat. Iasi.* III(2), Suplim. Nr. 1:412-416.
- Prisacaru, C., A. I. Burlacu and L. Rotaru (2008). Evaluation of biochemical changes from oxidative stress specific to ochratoxicosis induced by *Apii aetheroleum* administration. *Lucrări științifice USAMV, Sectia MV, Iasi*, 51(10):140-143.
- Prisacaru, C. and A. I. Burlacu (2008). Evaluation of antitoxic effect of some phthalide containing vegetal products (*Apii aetheroleum*, *Levistici aetheroleum*). *Lucrari stiintifice USAMV, Sectia MV, Iasi*. 51(10):143-147.
- Stanescu, U., M. Hancianu, A. Miron and C. Aprotosoaic (2004). *Medicinal Plants from A to Z: Monographies of therapeutically interested products*. "Gr.T.Popa" Press, Iasi. Vol. II: 348-350.