EVALUATION OF THE LIVER INTEGRITY AFTER THE INTAKE OF FOODS CONTAINING HIGH ACRYLAMIDE LEVELS

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Summary
The development of food additives and new food processing technologies that can fulfill the requirements of the fast way of living of this century has created a negative impact upon the quality of food and human health. The present experiment evaluates the pathological changes in liver integrity after the intake of foods containing high acrylamide levels (fried potatoes and toast), by the means of biochemical analysis (aspartate aminotransferase - AST, alanine aminotransferase - ALT, and gamma glutamyl transpeptidase - GGT) and histopathological examination at the level of liver. The experimental model included 4 groups of Wistar rats, as follows: reference group (fed with standard food), control group (that received the same diet, except for the drinking water, which was replaced by an acrylamide solution 0.1 µg/L), FP group (fed with fried potatoes and standard food), and T group (that was given toast and standard food). The results of the biochemical analysis reveal that the intake of foods containing high acrylamide levels leads, when compared to the reference group, to the following modifications: increase of AST for control group (69.908 ± 6.2557 vs. 35.526 ± 3.2831), FP group (75.612 ± 7.7026 vs. 35.526 ± 3.2831), and T group (54.284 ± 5.6686 vs. 35.526 ± 3.2831); increase of ALT for control group (46.276 ± 3.7842 vs. 27.986 ± 4.70806), FP group (60.906 ± 8.3831 vs. 27.986 ± 4.70806), and T group (60.592 ± 8.1433 vs. 27.986 ± 4.70806); increase of GGT for control group (11.666 ± 2.514 vs. 9.660 ± 1.3969), FP group (12.066 ± 1.8527 vs. 9.660 ± 1.3969), and T group (10.762 ± 1.054 vs. 9.660 ± 1.3969). The results of the histopathological examination demonstrate the affection of liver for the animals of the control group and FP group. In conclusion, the intake of foods with high acrylamide levels (fried potatoes and toast) has an important negative impact upon the liver integrity.

Key words: acrylamide, fried potatoes, toast, liver integrity.

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Introduction
The extremely rapid rhythm of present life and the interest of food industry to attract consumers by a more intense taste or colour has determined man to invent new food processing technologies, abandoning the notion of living foods, unfortunately with undesirable effects upon human health.

Acrylamide, a process-induced food toxicant with an alarming toxicological profile, has been discovered in 2000 by the Swedish scientists (Eriksson, 2005). The subject has lately been abandoned, due to the lack of evidence of the epidemiological studies. The neurotoxic potential of acrylamide has been known since the very first year of production and use (Le Quesne,
1980; Kuperman, 1958). Later on, the carcinogen action was reconfirmed by experimental research on laboratory animals. Furthermore, toxic effects on the reproductive function have been highlighted, as well as the genotoxic effects (Friedman, 2003; Dybing and Sanner, 2003).

The present experiment evaluates the pathological changes in liver integrity after the intake of foods containing high acrylamide levels (fried potatoes and toast), by the means of biochemical analysis (aspartate aminotransferase - AST, alanine aminotransferase - ALT, gamma glutamyl transpeptidase - GGT), and histopathological examination.

Material and methods

Analysis instruments

The biochemical analysis was achieved with an open system EOS 880 PLUS semi-automatic analyzer.

Experimental model. All the experimental proceedings in this experiment were achieved according to the international ethic reglementations and were approved by the Ethics Commission of the University of Medicine and Pharmacy “Gr. T. Popa” Iași.

A total number of 24 male rats, Wistar strain, having body weights comprised between 180 and 220 g, were divided into 4 groups, as follows:

• reference group - fed with standard food and drinking water.
• control group - received the same diet as the reference group, except for the drinking water, which was replaced by an acrylamide solution 0.1 µg/L (the maximum allowed limit regarding the presence of acrylamide in the drinking water, according to the European Union legislation).
• FP group - the food supply was made up of French fries (given in the morning, around 8.00 o’clock), and the reference group’s diet (at 14.00 o’clock).
• T group – the diet consisted of sliced white bread, toasted for 3 minutes into the electric toaster (8.00 o’clock) and the reference group’s diet (at 14.00 o’clock).

The animals were housed in separate cages, in order to control their diets. The experiment was conducted over a period of 90 days. At the end of the experiment the animals were anesthetized with ketamine i.p. (75mg/kg) and blood samples were collected by the puncture of the cord with a Vacuette ® system and submitted to biochemical analysis. When the absence of the vital signs (respiration, cord beats, reflexes) was ascertained, the animals were sacrificed in order to collect samples of liver for assessing the histopathological changes.

Evaluation of the animals’ behavior. The protocol for the monitoring of animal behavior considered the assessment of the motor activity of the animals using the open field test performed every 2 weeks, throughout the period of 90 days. The test consists in monitoring the number of lines crossed over by each rat during a period of 5 minutes, by using a camera coupled to a computer (fig. 1).

Biochemical investigation. After the collection, the blood samples meant for the biochemical determinations were left at room temperature for coagulation, and then centrifuged for 15 minutes, at 3000 rotations per minute. The obtained serum was immediately subjected to the biochemical investigation, which evaluated the cellular integrity in the sense of deceleration of the pathological modifications of the hepatocyte membrane permeability, by determining:

• the activity of the aspartate aminotransferase (AST);
• the activity of the alanine aminotransferase (ALT);
• the activity of the gamma glutamyl transpeptidase (GGT).

Histopathological investigation

In order to achieve the histopathological examination, the collected samples of liver were fixed in 10% buffered formaline, progressively dehydrated in
solutions containing an increasing percentage of ethanol (70, 80, 95, and 100%, v/v), embedded in paraffine, sectioned at 5 μm thickness, deparaffinized, and stained with hematoxylin-eosin (HE).

The histopathological examination aimed to emphasize the morphological changes at the level of liver after the administration of a diet containing high acrylamide levels and after the administration of a 0.1 μg/L acrylamide aqueous solution.

**Statistical interpretation.** Statistical data were processed by program StatsDirect version 2.7.2 (2008). The accepted significance threshold is 95%, i.e. p<0.05. The value of p is inversely proportional to the statistical significance. Statistical interpretation of data considered the differences corresponding to a significant threshold as follows: p> 0.05 statistically insignificant; p <0.05 statistically significant; p <0.01 highly statistically significant; p<0.001 very highly statistically significant.

**Results and discussions**

**Evaluation of the animals’ behavior**

The animals of the control group, which consumed *ad libitum* drinking water containing acrylamide 0.1 μg/L (the maximum allowed limit regarding the presence of acrylamide in the drinking water, according to the European Union legislation), showed ataxy and a statistically significant decrease of the motor activity at 10 and 12 weeks in comparison with the initial time and the reference group (fig. 1).

The animals from FP group, which consumed French fries (with a content of acrylamide ranging between 2000-3000 μg/kg daily portion) revealed a decrease of the motor activity, however not significant from a statistic perspective (fig. 1).

The animals of T group, which consumed toast, with a low acrylamide content (100-500 μg/kg daily portion) did not change their motor activity (fig. 1).

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**Biochemical analysis**

**Determination of aspartate aminotransferase**

The results obtained for the activity of AST are given in fig. 2. The study of these results reveals important variations from the statistical point of view, the interpretation of which may lead to important data related to the toxicodynamics of acrylamide which was supplied both in drinking water and as thermally-processed food (fried potatoes and toast).

There can be noticed an accentuated increase of this cellular enzyme’s activity from 35.526 UI for the reference group to 69.908 UI for the group which received acrylamide in drinking water. Its presence in the serum of the animals which received acrylamide in the drinking water in a double concentration in comparison with the reference group represents the proof of the AST migration from the hepatocytes cytoplasm to the intercellular space due to the increase of the membrane’s permeability (fig. 2). The permeabilization of the hepatocyte’s membrane may represent the consequence of the production of certain lesions following the connection of glycidamide with the functional groups of the membrane proteins. The AST activity registers an extremely increased value in the serum of the animals pertaining to the FP group, group which was fed with French fries (75.612 UI) (fig. 2). This increase suggests the fact that the intake of French fries leads to an advanced negative influence in comparison with the intake of acrylamide solution. A lower value of the
AST activity against the aforementioned can be seen in the group which was fed with toast (54.284 UI). However, this value too is significantly higher than that of the reference group, fact which suggests the presence of acrylamide in toast in highly harmful dosages for the safety of the consumers.

Fig. 2. The activity of aspartate aminotransferase

**Determination of alanine aminotransferase**

The evolution of ALT, an enzyme with strictly cytosolic location, leads to significant increases of the enzyme’s activity, fact which suggests the permeabilization of the hepatocyte’s membrane and its extravasation into the intercellular space (fig. 3).

The ALT activity registers an ascension from 27.986 UI, a value registered for the reference group, to 46.276 UI for the group which consumed acrylamide in its drinking water, and even to 60.592 UI and 60.906 UI for those groups which were fed with toast, and French fries respectively. The most increased value of the ALT activity is reached in the serum of the animals having an intake of French fries (fig. 3), correlating with the AST evolution.

SD* = standard deviation; RSD ** = relative standard deviation

Fig. 3. The evolution of alanine aminotransferase

**Determination of gamma glutamyl transpeptidase**

The gamma glutamyl transpeptidase, an enzyme located mostly in the kidneys, liver, biliary tract and pancreas interferes in the proteic synthesis, facilitating the transfer of the gamma glutamyl from one peptide to another, or to an aminoacid. This plasmatic enzyme is mostly of hepatic origin, however small quantities may have a renal, pancreatic or intestinal origin.

At the same time, it has a role as membrane enzyme, interfering in the glycoproteins transport activity. The major biochemical function is focused on the scission of glutathione into glutamic acid and cysteinil glycine. Acting as a transpeptidase, the GGT fixes the amino acid to be transported through the membrane on the glutamic acid, forming the gamma glutamyl amino acid dipeptide, which diffuses into the cells.

This membrane enzyme existing as five isoenzymes is used as an index for enzymatic induction, as the marker of the hepatic and biliary disorders, of the ethanol-based hepatic disorders and as an essential parameter in the monitoring of the disintoxication by means of the alcohol consumption reduction or cessation (Cucuianu et al., 1998).
The diagnosis-related value of this membrane enzyme has increased by its usage as a marker in the assessment if the oncogenesis capacity of some mycotoxins and other food-related noxious substances, through the Tatematsu test for cancer promotion (Tatematsu et al., 1977).

The values of GGT activity for the experimental groups are given in fig. 4. As shown by this data, the activity of the GGT is of 9.660 IU for the reference group, a value which is deemed normal. The GGT activity registers a significant increase for the control group, the value in the serum of these animals being of 11.666 IU, fact which is correlated with the evolution of the hepatic cytolysis indices in this group. In the serum of the animals which were given toast, the enzyme’s activity registers an average value of 10.762 UI, a lower value than that of the control group, however significantly higher than that of the reference group (fig. 4). The activity of the GGT reaches its top value in the serum of the animals fed with French fries, where the average value is of 12.066 UI, such accentuation of the enzyme’s activity being characteristic to the acute lesions of the hepatocyte, of viral or toxic etiology (fig. 4).

**Histopathological investigation**

The histopathological analysis of the liver for the reference group revealed a normal histoarchitectonic, with no morphological changes (fig. 5).

Unlike the reference group, the micrographies obtained for the control group emphasize a maintained lobular organisation, hepatocitar granulovacuolar degenerescence focus (subcapsular and around the centrolobular vein), inconstant zones with congestive and dilated sinusoidal capilara (fig. 6).

The histopathological evaluation of the samples collected from the FP group (group which was fed with fried potatoes) rare foci of piecemeal necrosis (fig. 7), moderate proliferation of Kupffer cells, and limphocytes in portobiliar spaces are noticed.

The results of the histopathological examination at the level of liver for the group fed with toast do not show significant morphological changes. These results can be explained by the reduced acrylamide intake from these foods during the three months period.

In consequence, the histopathological results at the level of liver correlate with the variations of transaminases, revealing the hepatotoxic potential of acrylic amide in chronic intoxication.

![Fig. 4. The activity of the gamma glutamyl transpeptidase](image)

SD* = standard deviation; RSD ** = relative standard deviation

![Fig. 5. Liver – reference group (HEx20)](image)
Conclusions
The intake of foods with high acrylamide levels (fried potatoes and toast) has an important negative impact upon the liver integrity, demonstrated by the significant increase of aspartate aminotransferase, alanine aminotransferase, and gamma glutamyl transpeptidase, and by the results of the histopathological examination.

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References


