RESERCH ON THE INFLUENCE OF APIDIET IN ACRYLAMIDE-INDUCED HEPATOPATHY IN WISTAR RATS ON THE SERUM PROTEINS PROFILE – EVALUATED BY ELECTROPHORESIS

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Summary
The aim of the present experiment was to investigate the influence induced by apidiet in in acrylamide-induced hepatopathy in rats. The objectives of the experimental study were to investigate the following biochemical profile of seric proteins, by electrophoretic analysis. In order to reduce the influence of factors affecting progression of hepatic lesions, we have administrated apidiet products. Toxic hepatopathy was experimentally induced administrating of acrylamide (water solution, 50mg/L concentration) by gastric gavaj. Co-administration of api-diet in group which received acrylamide, compared to standardized food group, determined: 1) an increase of albumins level (30.14±0.69 versus 41.7±1.34, p<0.0001) and of albumins / globulins ratio (0.43±0.01 versus 0.72±0.04, p<0.0001) and 2): a decrease of globulins concentration (69.85±0.69 versus 58.41±1.12, p<0.0001); alfa-1 globulin concentration (28.64±0.22 versus 23.83±1.62, p<0.0001) and betta-globulin concentration (26.47 ± 0.44 versus 19.27±1.32, p<0.0001). In conclusion, the administration of apitherapc producs in case of acrylamide toxic hepatic disease proved efficient in normalizing the investigated parameters.

Key words: apitherapy, acrylamide, serum proteins, electrophoresis

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Introduction
The seric proteins electrophoresis is a very-commonly used method used to separates and quantifies serum proteins in medical biochemistry laboratories. It is used for detection of gamma-monoclonal antibodies producing tumours, abnormalities in seric proteins concentrations, nutritional state (Tiey, 1995), as well as for the monitoring of treatment efficacy in case of hepatic diseases. In the human body, proteins fulfil different functions> they exhibit structural or plastic role (they are the main constituents of the cytoplasm and of the cellular organists, humours and biological liquids), as well as functional roles: catalysis, intervening in the enzymatic and hormonal function necessary for most biochemical reactions necessary for metabolic processes specific to living structures; immunological reactions (host defence); transportation of substances necessary for life-maintainence; contractility and mechanical resistance; protection and detoxification; physic-chemical – the are involved in the regulations of water and electrolytes exchanges between the interior of the cells and the extracellular environment, supplying with substances necessary for biosynthesis of numerous compound that organisms needs, such as aminoacids.
resulted from protein chemical degradation, either in plasma or muscles, with no origin-specificity, which are the precursor of porphyrins, nucleic acids, different hormones, creatine, creatinine, carnosin, anserine, glutathione, purine and pyrimidine nucleotides, neurotransmitters (Banu, 2004).

Pollen contains all of essential and non-essential amino acids with a role in human nutrition (Andriţoiu, 2005). High biological value of pollen results from its increased and various contain in essential amino acids, needful to life. Amino acids are (after Caillas, cited by Mărghiţaş, 2005): arginine 5.70 %, histidine 2.4%, isoleucine 4.50%, leucine 6.7%, lysine 5.7%, methionine 1.80%, phenylalanine 3.9%, threonine 4.0%, tryptophan 1.3%, valine 5.7% (Mărghiţaş, 2005). Azotated substances were identified, also: xanthine, hypoxanthine, geranine, trimethylamine (Mărghiţaş, 2005; Andriţoiu, 2005). Propolis is a resinous substance, with anti-mutagen, hepato-protective, anti-inflammatory, antioxidant, and anesthetic effects.

Acrylamide was obtained for the first time by Moureu in Germany, in 1893 (Eriksson, Acrylamide, 2005), and industrial production was inaugurated by American Cyanamid Inc., in 1954 (NICNAS, Acrylamide. Priority Existing Chemical. Assessment Report No. 23, 2002). Acrylamide is used nowadays in the drinking water and wastewater treatment, plastic fabrication and some packs for food products, paper and cellulose manufacture, sugar refining, and raw oil processing (Cuciureanu, Bulea, 2005, ***; CERHR, 2005).

The aim of the present study was represented by the assessment of parameters of the seric proteins electrophoresis, in conditions of toxic hepatic diseases induced by acrylamide administration. In order to reduce the influence of factors affecting progression of hepatic lesions, we have administrated apidiet products.

Material and methods
We have worked on 40 Wistar rats, equally distributed into 4 groups: control group standardized food (group I), group apidiet+royal jelly (group II), group treated with acrylamide (group III), group treated with acrylamide and apidiet+royal jelly (group IV).

The average weight of used rats was 250g. The animals were kept in corresponding light and temperature conditions, with access to food and water. From these subjects, we have kept 3 for further tests. We mention that, in the present study, the group receiving acrylamide did not survive without therapy, thus the results obtained in case of group IV (apidiet plus royal jelly) are highly significant.

We have compared the results obtained in acrylamide+apidiet+royal jelly group with the results of the two control groups (control group with standardised food and control group with apidiet and royal jelly).

Toxic hepatopathy was experimentally induced administrating of acrylamide (water solution, 50mg/L concentration) by gastric gavage. Animals were sacrificed by thiopental anaesthesia, 1mL/100g bw, using 0.01% concentration solution. After thiopental anaesthesia, we have punctured the cord with a Vacuette®-harvesting system, in order to obtain blood necessary for biochemical analysis of the seric proteins electrophoretic profile.

The apitherapy product (Apiregya, ApiImunomod, ApiImunostim) were obtained from „Stupina SRL“. Biochemical investigation was performed using an automatic analyser (Aeroset, Abbott) and recommended commercial kits (Abbott, USA).

Results
Albumins
Co-administration of acrylamide and apidiet (lot III) led to a significant statistic increase of albumin level,
comparatively to the control lot with standard food (lot I) (30.14±0.69 versus 41.7±1.34, p<0.0001) (Figure no. 1).

Administration of apidiet+RJ (lot II) led to a significant statistic increase of albumin level, in comparison to the control lot with standard food (lot I) (30.14±0.69 versus 40.71±0.95, p<0.0001) (Figure no. 1).

**Globulins**

Co-administration of acrylamide and apidiet (lot III) determined a significant statistic decrease of globulins level comparatively to the control lot with standard food (lot I) (69.85±0.69 versus 58.41±1.12, p<0.0001) (Figure no. 2). Administration of apidiet+RJ (lot II) led to a significant statistic decrease of globulins level, in comparison to the control lot with standard food (lot I) (69.85±0.69 versus 59.14±1.06, p<0.0001) (Figure no. 2).

**Alpha-1 globulin**

Co-administration of acrylamide and apidiet (lot III) determined the significant statistic decrease of alpha-1 globulin, in comparison to the control lot with standard food (lot I) (28.64±0.22 versus 23.83±1.62, p<0.0001) (Figure no. 3). Administration of apidiet+RJ (lot II) determined a significant statistic decrease of alpha-1 globulin, comparatively to the control lot with standard food (lot I) (28.64±0.22 versus 25.12±0.24, p<0.0001) No significant statistic differences for alpha-2 globulins level between the studied lots were registered (Figure no. 3).

**Betta-globulina**

Co-administration of acrylamide and apidiet (lot III) led to the significant statistic decrease of beta-globulin, comparatively to the control lot with standard food (lot I) (26.47 ± 0.44 versus 19.27±1.32, p<0.0001) Administration of apidiet+RJ (lot II) led to the significant statistic decrease of beta-globulin in relation to the control lot standard food (lot I) (26.47±0.44 versus 18.32±0.26, p<0.0001) (Figure no. 4).

**Albumins/Globulins ration**

Co-administration of acrylamide and apidiet (lot III) determined the significant statistic increase of albumin/globulin ratio, in relation to the control lot with standard food (lot I) (0.43±0.01 versus 0.72±0.04, p<0.0001) (Figure no. 7). Administration of apidiet+RJ (lot II) led to the significant statistic increase of albumin/globulins ratio in comparison to the control lot receiving standard food (lot I) (0.43±0.01 versus 0.68±0.03, p<0.0001) (Figura nr.5).

No significant statistic differences between the studied lots for alpha-2 and gamma-globulin were registered.
Discussions Data regarding toxicokinetics in case of acrylamide are scarce. Anyway, symptoms observed in case of acrylamide intoxication indicate that this toxic is absorbed in the human body both in case of inhalation and ingestion (**IPCS.1985, **European risk assessment).

Acrylamide is metabolized in two ways: by conjugation with glutathione or by oxidation to glycidamide, a toxic epoxide (Fennel et al., 2003). Direct conjugation of acrylamide with glutathione leads to N-acetylS-(3-amino-3-oxopropil)cysteine and S-(3amino-3-oxopropil)cysteine, which are excreted in urine (Sumner et al., 2003, Friedman M.,2003). Glycidamide is excreted in urine also, and, after that, gets through the same way of conjugation with glutathione, leading to N-acetylS-(3-amino-2-hydroxi-3-oxopropyl)cysteine and N-acetyl-S-(1-carbamoil-2-hydroxi-etil)cysteine, which are excreted by renal way, also (Fennel et al., 2003). The main iso-enzyme involved in the acrylamide metabolism is cytochrome P450E1 (Park et al., 2002); the other cytochromes do not metabolize acrylamide in the absence of P450E1 (Sumner et al., 2003).

In April, 2002, researchers from the Swedish National Food Administration and Stockholm University announced the presence of acrylamide in various foods, alerting the whole world, because of the well-known toxic profile of this compound. Many countries were involved in an international program with the following objectives: to develop sensitive methods of quantitative analysis of acrylamide, to determine the toxic concentration in different foods, to discover its mechanism, and possibilities of reducing and preventing the presence of acrylamide in foods (Lindsay, 2002).

Cuciureanu et al. (2005) noted: beginning with the first day of experiment, the animals behavior was completely modified. Animals in the lot treated with acrylamide presented specific symptoms of neuro-toxic effects of it: weight loss, depression, trembling, weakness in extremities. These phenomena were absent in lots treated with bee products, and animal behaviour was almost similar with the control lot; exception, an apathy state of 30-40 minutes after the toxic administration.

Conclusions

Standardized food administration determined a decrease of albumin concentration and an increase in globuline serum proteins (alpha1, alpha2, beta and gamma), this might cause hepatic affecting. Administration of apid diet associated with royal jelly to groups which had previously received acrylamide maintains both albumine and globulines (alpha1, alpha2, beta and gamma) to normal values, comparable to those registered in case of healthy control animals receiving apiterapic products only.
References