IMPLICATIONS OF APITHERAPY REGARDING THE EVOLUTION OF MINERALS IN ACETAMINOPHEN INDUCED INTOXICATION

IMPLICAȚIILE APITERAPIEI PRIVIND EVOLUȚIA MINERALELOR ÎN INTOXICAȚIA MEDICAMENTOASĂ CU ACETAMINOFEN

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Abstract. *Acetaminophen is one of the most used analgesic and antipyretic substances in Europe and United States. Found in over 100 pharmaceutical products, it is one of the most common drugs with liver and kidney toxicity potential. The drug-induced affection was achieved by gavage administration of acetaminophen (100 mg/100 g), for 2 weeks. Administration of apitherapy preparations to the group with drug-induced toxicity, in comparison with the unprotected group, determined: decrease of iron (242±48.23 versus 73.6±6.29), decrease of potassium (6.24±0.43 versus 5.41±0.15), increase of serum calcium (7.96±0.4 versus 9.68±0.47), and increase of ionized calcium (3.86±0.24 versus 4.35±0.18). Supplementation of apitherapy diet with Royal Jelly determined a less important decrease of iron and a more significant increase of serum and ionized calcium compared to the group treated only with apitherapy diet. In conclusion, administration of apitherapy products proved to be efficient in improving the levels of iron, potassium, serum and ionized calcium.*
Key words: apitherapy, minerals, acetaminophen

Rezumat. *Acetaminofenul este unul dintre cei mai folosiți agenți analgezici şi antipiretici în Europa şi Statele Unite. Acesta se găseşte în peste 100 de produse farmaceutice, fiind unul dintre cele mai comune medicamente cu potențial toxic hepatic şi renal. Afectarea medicamentoasă a fost indusă prin administrarea prin gavaj a acetaminofenului în doză de 100 mg/100 g, timp de 2 săptămâni. Administrarea preparatelor apiterapice la lotul cu afectare medicamentoasă, comparativ cu lotul neprotejat, a determinat: scăderea sideremiei (242±48.23 versus 73.6±6.29), scăderea potasiului (6.24±0.43 versus 5.41±0.15), creşterea calciului seric (7.96±0.4 versus 9.68±0.47), creşterea calciului ionic (3.86±0.24 versus 4.35±0.18). Suplimentarea apidietei cu lăptişor de matcă a determinat reducerea mai puțin importantă a fierului şi creşterea mai semnificativă a calciului seric şi ionic, comparativ cu lotul tratat doar cu apidietă. In concluzie, administrarea apidietei şi lăptişorului de matcă loturilor cu afectare medicamentoasă s-a dovedit eficientă în normalizarea valorilor fierului, potasiului, calciului seric şi ionic.*
Cuvinte cheie: apiterapie, minerale, acetaminofen

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INTRODUCTION

Acetaminophen is one of the most used analgesic and antipyretic substances in Europe and United States, being included in over 100 pharmaceutical products. Therefore, acetaminophen is one of the most common drugs associated with accidental or voluntary intoxication. Acetaminophen may induce liver and kidney toxicity (Perry et al., 1998). Alcoholics develop acute hepatitis even with usual doses of acetaminophen (2.6-3.9 g/day) (Liaw *et al*., 1993). The prevalence of the drug induced liver lesions is not exactly known, as their diagnosisis is achieved with difficulty and frequently belays (Zimmerman et al., 1994).

Liver represents the main place for the metabolization of the foreign substances (Lotterer et al., 1999). The metabolism of acetaminophen implies several ways of biotransformation. The most important among these (almost 95%) is achieved by the conjugation with glucuronic acid and sulfate. Only 5% is metabolized by the hepatic microsomal system of oxidation with mixed function, mainly P_{450} 2E1 cytochrome (Brent et al., 1993; Hinson et al., 1994). The substances for these enzymatic systems include endogen compounds, such as steroids, fatty acids (including prostaglandins and leukotrienes), and compounds such as drugs, food additives or industrial products, that enter the organism at the same time with foods, injections, or by inhalation (Nelson et al., 1996; Vessey, 2003; Murray, 2000; Bohan et al., 2002; Vickers et al., 1999).

In the present experiment, the influence of apitherapy diet, single or in combination with Royal Jelly (RJ), in acetaminophen induced hepatotoxicity was studied by evaluating some parameters of the mineral profile such as iron (Fe), potassium (K), ionized and serum calcium.

MATERIAL AND METHOD

The experimental model included 6 groups of Wistar rats: control group standard food (group I - normal status, standard food), control group apitherapy diet (group II - normal status, apitherapy diet), control group apitherapy diet + RJ (group III - normal status, apitherapy diet and RJ), acetaminophen group (group IV – acetaminophen-induced toxicity, standard food), acetaminophen group + apitherapy diet (group V - acetaminophen-induced toxicity, treated with apitherapy diet), acetaminophen group + apitherapy diet + RJ (group VI - acetaminophen-induced toxicity, protected with apitherapy diet and RJ).

The drug-induced hepatotoxicity was achieved by gavage administration of acetaminophen (100 mg/100 g), for 2 weeks. The treatment consisted of apitherapy diet, single or in combination with RJ.

After 3 weeks of experiment, the laboratory animals were anesthesiated with thiopental (dose of 1 ml/100 g from a 0.01% thiopental solution), blood samples were collected by the punction of the cord with a Vacuette® system and submitted to the investigation of the parameters of the mineral profile: iron, potassium, serum and ionized calcium.

The statistical interpretation of the results was performed with One-Way ANOVA test and Tukey's post-hoc test. The results were given as mean ± standard deviation. The value of $p<0.05$ was considered significant.

RESULTS AND DISCUSSIONS

In animals with acetaminophen induced toxicity (group IV) there can be noticed, in comparison to the other experimental groups:

a) the increase of the following parameters for: i) control group standard food (group I) - Fe (56.85 ± 4.45 versus 242.6 ± 48.23) (fig.1), K (5.1 ± 0.16 versus 6.24 ± 0.43) (fig.2); ii) control group apitherapy diet (group II) - Fe (70 ± 3.21 versus 242.6 ± 48.23) (fig.1), K (5.34 ± 0.19 versus 6.24 ± 0.43) (fig.2); iii) control group apitherapy diet + RJ (group III) - Fe (94.28 ± 1.6 versus 242.6 ± 48.23) (fig.1), K (5.37 ± 0.18 versus 6.24 ± 0.43) (fig.2);

b) the decrease of the following parameters for: i) control group standard food (group I) – ionized calcium (4.19±0.07 versus 3.861 ± 0.24) (fig.4), serum calcium (8.81 ± 0.5 versus 7.9625 ± 0.4) (fig.3); ii) control group apitherapy diet (group II) – ionized calcium (4.43 ± 0.14 versus 3.861 ± 0.24) (fig.4), serum calcium (9.84 ± 0.09 versus 7.9625 ± 0.4) (fig.3); iii) control group apitherapy diet + RJ (group III) – ionized calcium (4.58 ± 0.06 versus 3.861 ± 0.24) (fig.4), serum calcium (10.51 ± 0.3 versus 7.9625 ± 0.4) (fig.3).

Administration of apitherapy diet to laboratory animals with acetaminophen induced toxicity (group V) determines, in comparison with the acetaminophen group (group IV), the following modifications: i) decrease of Fe (242.6 ± 48.23 versus 73.6 ± 6.29) (fig.1) and K (6.24 ± 0.43 versus 5.41 ± 0.15) (fig.2); ii) increase of ionized calcium (3.861 ± 0.24 versus 4.358 ± 0.18) (fig.4) and serum calcium (7.9625 ± 0.4 versus 9.68 ± 0.47) (fig.3).

Administration of apitherapy diet in combination with RJ to laboratory animals with acetaminophen induced toxicity (group VI) determines, in comparison with the acetaminophen group (group IV), the following modifications: i) decrease of Fe (242.6 ± 48.23 versus 91.5 ± 13.25) (fig.1); ii) decrease of K (6.24 ± 0.43 versus 5.33 ± 0.2), the values being comparable with the values of the other experimental groups (group I - 5.1 ± 0.16; group II – 5.34 ± 0.19; group III - 5.37 ± 0.18) (fig.2); iii) increase of ionized calcium (3.861 ± 0.24 versus 4.426 ± 0.14), and serum calcium (7.9625 ± 0.4 versus 10.25±0.58) (figs. 3 and 4).

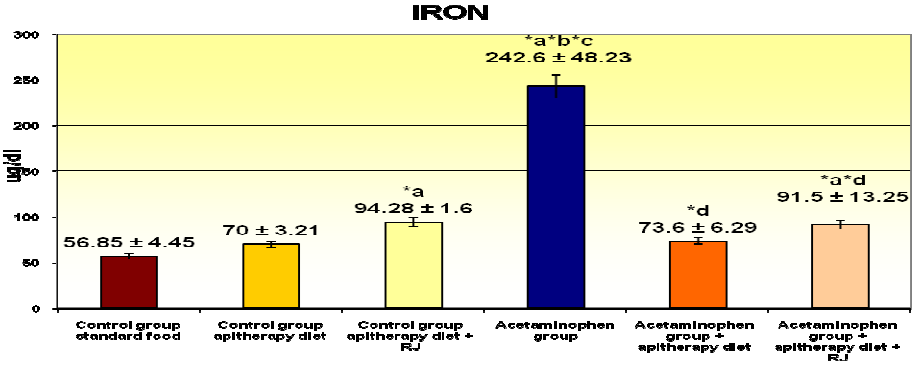

Fig. 1 - Mean values of iron levels and standard deviation (* a p<0.05 vs. control group standard food; * b p<0.0001 vs. control group apitherapy diet; * c p<0.0001 vs. control group apitherapy diet + RJ; * d p<0.0001 vs. acetaminophen group)

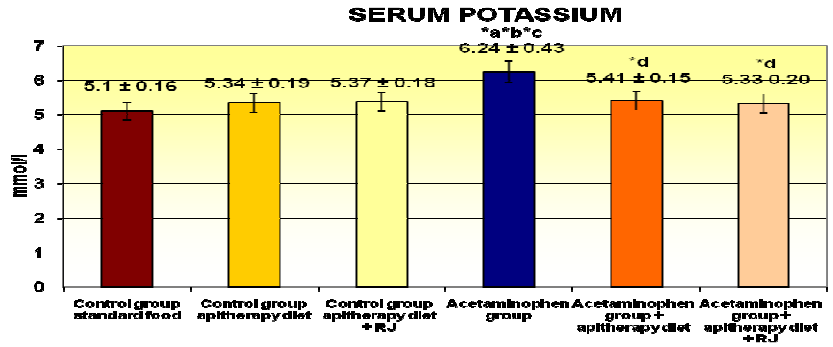

Fig. 2 - Mean values of serum potassium levels and standard deviation (* a p<0.0001 vs. control group standard food; * b p<0.0001 vs. control group apitherapy diet; * c p<0.0001 vs. control group apitherapy diet + RJ; * d p<0.0001 vs. acetaminophen group).

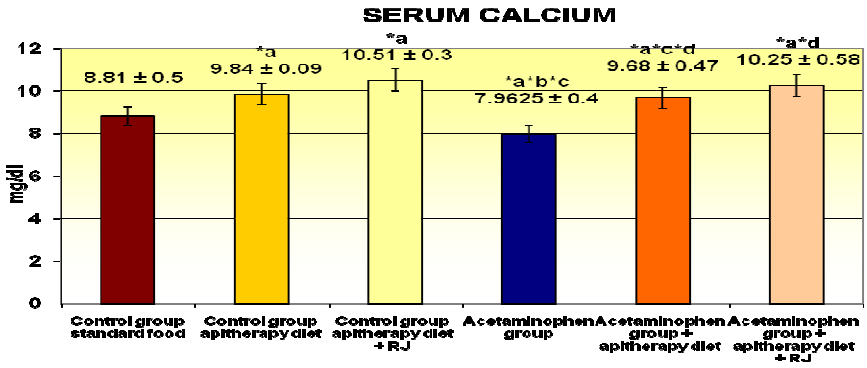

Fig. 3 - Mean values of serum calcium levels and standard deviation (* a p<0.05 vs. control group standard food; * b p<0.0001 vs. control group apitherapy diet; * c p<0.05 vs. control group apitherapy diet + RJ; * d p<0.0001 vs. acetaminophen group)

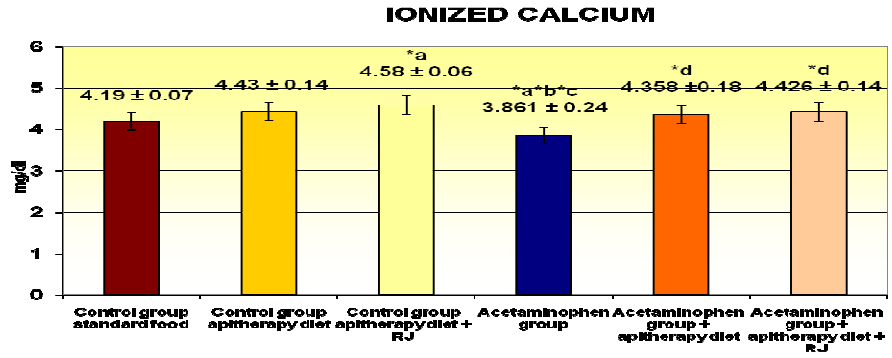

Fig. 4 - Mean values of ionized calcium levels and standard deviation (* a p<0.05 vs. control group standard food; * b p<0.0001 vs. control group apitherapy diet; * c p<0.0001 vs. control group apitherapy diet + RJ; * d p<0.0001 vs. acetaminophen group)

The increase of iron levels simultaneous to the presence of serum siderophilin, cellular protein that fixes the storing iron and which is normally absent in the blood, pleads for the cellular origin of iron. The factors responsible for the increase of iron levels are: the release of iron from the liver in conditions of hypoxia, excessive haemolysis, increased duodenal absorption of the food iron.

In subjects with chronic hepatopathies, it has been proven that the intestinal absorption of iron is 10 times higher than in healthy subjects. As the iron enters in higher amounts in the liver, it circulates under its unoxidized and free form through the portal system, favorizing its deposition as hemosiderin. Hemosiderin is a complex of heterogeneous iron achieved at the level of hepatocyte and reticulohistiocytic cells (Buligescu et al., 1999). At the level of liver, the deposition of iron as hemosiderin is preceded by the multiplication of lisosomes that elaborate a PAS-positive glycoprotein with a high affinity for iron. The lisosomes become siderosomes consequently to the incorporation of iron. By breaking their membrane, the iron is able to exert the negative impact upon the liver: interruption of oxidative phosphorylation, decrease of ATP and enzyme content, lactate accumulation, diminution of $NADH_2$ oxidation (Buligescu et al, 1999).

Acetaminophen is metabolically activated to produce reactive oxygen species that get fixed by a covalent bond to the macromolecules of the hepatocyte. The extension of these bonds is correlated to the incidence and severity of the liver necrosis. Accumulation of activated ionized calcium from the liver membrane decreases with 60-75% after a hepatotoxic dose of acetaminophen (Lewis et al., 1991).

In the case of therapeutical doses, 75% of the plasmatic acetaminophen undergoes sulfation or glucuronidation, but 5-10% is oxidized by the P_{450} cytochrome into toxic metabolites such as N-acetyl-p- benzoquinone imine (Jerca et al., 2007).

The modifications of iron levels in chronic hepatitis interfere with multiple mechanisms, respectively with factors that increase or decrease the iron values. The present study reveals that chronic drug administration (acetaminophen), interferes with the levels of iron, potassium, ionized and serum calcium. Furthermore, there can be concluded from this experiment that the apitherapy treatment has benefic results that lead to the improvement of the above mentioned parameters to normal levels.

CONCLUSIONS

1. Administration of apitherapy diet to the group with acetaminophen induced toxicity proved to be efficient by improving the iron levels to normal values.

2. Supplementation of the apitherapy diet with Royal Jelly for the group with acetaminophen induced toxicity led to a decrease of the iron levels, but not as important as in the case of the group treated only with apitherapy products.

3. Administration of apitherapy diet led to a significant decrease of serum potassium in comparison with the group with induced toxicity. Supplementation of

the apitherapy diet with Royal Jelly determined a more significant decrease of the potassium levels when compared to the group treated only with apitherapy diet.

4. Supplementation of the apitherapy diet with Royal Jelly determined a more significant increase of the ionized and serum calcium levels when compared to the group treated only with apitherapy products.

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