

THE EFFICIENCY OF APITHERAPY TREATMENT UPON THE EVOLUTION OF MINERALS IN CARBON TETRACHLORIDE INDUCED HEPATOPATHY

EFICIENȚA TRATAMENTULUI APITERAPIC ASUPRA EVOLUȚIEI MINERALELOR ÎN HEPATOPATIA INDUSA CU TETRACLORURĂ DE CARBON

ANDRIȚOIU C.V.¹, ANDRIȚOIU V.², PRISĂCARU Anca Irina¹
e-mail: calin.apitherapy@yahoo.com

Abstract. *The objective of the present experiment is to evaluate the influence of apitherapy diet on minerals' levels (iron, potassium, serum and ionized calcium) in carbon tetrachloride induced hepatopathy in Wistar rats. In order to reduce the factors that produce the modification of minerals' values, apitherapy diet and apitherapy diet in combination with royal jelly has been administered. The apitherapy diet consisted of 4 products produced by Stupina LLC. The hepatoprotective effects of apitherapy diet have been evaluated, thus leading to new perspectives of treatment. The administration of carbon tetrachloride to laboratory animals (group IV) results in the increase of iron and decrease of serum and ionized calcium. Administration of apitherapy diet, in comparison with the untreated group, leads to the decrease of iron (277.8 ± 77.8 vs. 173.1 ± 69.27), increase of serum calcium (7.83 ± 0.4 vs. 9.58 ± 0.49) and ionized calcium (3.54 ± 0.49 vs. 4.2 ± 0.1). Administration of apitherapy diet in combination with royal jelly produces, in comparison with the untreated group, the decrease of iron (277.8 ± 77.8 vs. 124.5 ± 32.19) and the increase of serum calcium (7.83 ± 0.4 vs. 10.15 ± 0.6) and ionized calcium (3.54 ± 0.49 vs. 4.38 ± 0.15).*

Key words: iron, potassium, calcium, hepatopathy, apitherapy

Rezumat. *Scopul acestui experiment a constat în studiul influenței apidietei asupra mineralelor (fier, potasiu, calciu seric și calciu ionic) în afectarea hepatică indusă de tetraclorura de carbon la șobolani rasa Wistar. În scopul reducerii factorilor care produc modificărilor mineralelor am administrat apidietă și apidietă în combinație cu lăptișor de matcă. Apidietă a constat din 4 preparate apiterapice procurate de la firma Stupina. Am urmărit efectele hepatoprotectoare ale administrării apidietei, deschizând astfel noi perspective de tratament. Administrarea tetraclorurii de carbon animalelor de experiență produce creșterea fierului și scăderea calciului seric și ionic. Administrarea apidietei, comparativ cu lotul netratat, conduce la scăderea fierului (277.8 ± 77.8 vs. 173.1 ± 69.27) și creșterea calciului seric (7.83 ± 0.4 vs. 9.58 ± 0.49) și ionic (3.54 ± 0.49 vs. 4.2 ± 0.1). Administrarea apidietei în combinație cu lăptișor de matcă, comparativ cu lotul netratat, conduce la scăderea fierului*

¹ "Gheorghe Asachi" Technical University of Iași, Romania

² Apitherapy Medical Center, Bălănești, Gorj, Romania

(277.8 ± 77.8 vs. 124.5 ± 32.19) și creșterea calciului seric (7.83 ± 0.4 vs. 10.15 ± 0.6) și ionic (3.54 ± 0.49 vs. 4.38 ± 0.15).
Cuvinte cheie: fier, potasiu, calciu, hepatopatie, apiterapie.

INTRODUCTION

A great interest has been shown lately on the protective role and mechanism of action of certain compounds that are naturally present in the biological systems (Bhadauria et al., 2008).

Propolis is considered to interfere positively in the absorption and use of different minerals due to the presence of organic acids derivatives that improve the physiological functions by modulating the enzymatically dependent ionic activity. Haro and colleagues demonstrate the benefic effects of pollen and/or propolis upon the metabolism of iron, calcium, phosphorus and magnesium in nutritional iron deficiency anemia in rats (Haro et al., 2000).

Numerous plasma proteins, including albumin, alpha- and betaglobulins, coagulation factors and transport proteins, are synthesized by the liver. These factors influence the homeostasia (for example, by binding the proteins, these factors modulate the total concentration of circulating Ca^{2+} and Mg^{2+} and also the concentration of other drugs). At the same time, the serum albumin levels regulate the colloid osmotic pressure of plasma, thus influencing the dynamics of fluids between blood and tissues (Jerca et al., 2004).

There are also studies regarding the hepatoprotective effect of other natural products upon experimentally CCl_4 induced hepatopathy: lycopene from tomatoes (Sakin et al., 2011); the diterpenes kahweol and cafestol from coffee (Lee et al., 2007); the flavone luteolin (3',4',5,7-tetrahydroxyflavone) (Domitrović et al., 2009); olive oil (Fang et al., 2008); potato peel extract (Singht. et al., 2008); resveratrol (Fan et al., 2009).

This study continues the direction of research regarding the protective and therapeutical effect of different preparations containing natural compounds.

MATERIAL AND METHOD

All the experimental proceedings achieved on laboratory animals (Wistar rats) in this study were in agreement with the international ethics regulations. Hepatic lesion was induced by i.p. injection of carbon tetrachloride (CCl_4) dissolved in paraffin oil, 10% solution. Two ml per 100 g were administered, once at 2 days, for 2 weeks.

The experiment was unfolded on six groups of Wistar rats. The first group served as control, the second one was fed with apitherapy diet, and the third group was given apitherapy diet and royal jelly (RJ). The next three groups of animals were administered CCl_4 and fed with normal food (group IV), apitherapy diet (group V) and apitherapy diet with RJ (group VI). The apitherapy diet consists of four apitherapy products purchased from STUPINA LLC, Bălănești, Gorj, Romania (*Apiregya*, *Apilmunomod*, *Apilmunostim*, *Apilmunostim Forte*). These products included in their composition honey, royal jelly, propolis, and pollen and were registered to OSIM with number AO 1242. After three weeks of apitherapy treatment, the laboratory animals were anesthetized with thiopental (dose of 1 ml/100 g from a 0.01% thiopental solution), and blood samples were collected

by the puncture of the cord with a Vacuette® system and submitted to biochemical analysis regarding the levels of minerals.

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 CCl_4 induced hepatopathy (group IV) there can be noticed, when compared to: a) control group standard food (group I) - the increase of iron levels (56 ± 4.83 vs. 247.8 ± 77.48) (fig.1), the decrease of serum calcium (8.81 ± 0.96 vs. 7.83 ± 0.4) (fig.3), and the decrease of ionized calcium (4.19 ± 0.13 vs. 3.54 ± 0.49) (fig.4); b) control group apitherapy diet (group II) - the increase of iron levels (69.71 ± 9.01 vs. 247.8 ± 77.48) (fig.1), the decrease of serum calcium (9.78 ± 0.5 vs. 7.83 ± 0.4) (fig.3), and the decrease of ionized calcium (4.427 ± 0.21 vs. 3.54 ± 0.49) (fig.4); c) control group apitherapy diet and RJ (group III) - the increase of iron levels (94 ± 7.91 vs. 247.8 ± 77.48) (fig.1), the decrease of serum calcium (10.5 ± 0.39 vs. 7.83 ± 0.4) (fig.3), and the decrease of ionized calcium (4.58 ± 0.16 vs. 3.54 ± 0.49) (fig.4).

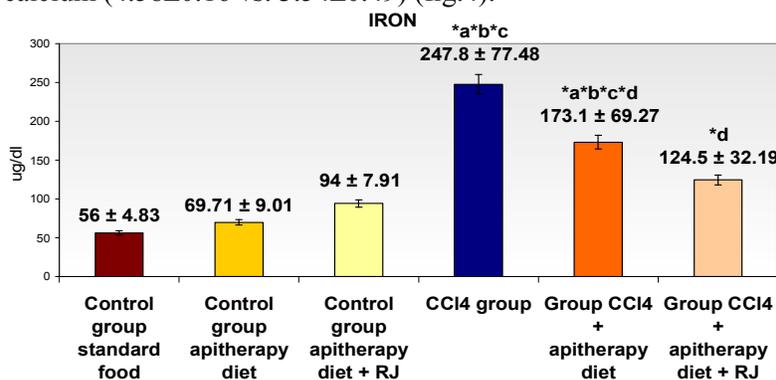


Fig. 1 - Mean values of iron levels and standard deviation

(* a $p < 0.05$ vs. control group standard food; * b $p < 0.05$ vs. control group apitherapy diet; * c $p < 0.05$ vs. control group apitherapy diet + RJ; * d $p < 0.05$ vs. CCl_4 group).

Regarding the potassium levels, no statistically significant differences have been obtained. A slight increase for the group that received CCl_4 has been noticed, but the values reached the normal range for the groups treated with apitherapy diet/apitherapy diet and RJ (fig. 2).

Administration of apitherapy diet to animals with CCl_4 induced hepatopathy (group V) produces, in comparison with group IV: decrease of iron levels (247.8 ± 77.48 vs. 173.1 ± 69.27) (fig.1), increase of serum calcium (7.83 ± 0.4 vs. 9.58 ± 0.49) (fig.3), and increase of ionized calcium (3.54 ± 0.49 vs. 4.2 ± 0.1) (fig.4). Administration of apitherapy diet in combination with RJ to animals with CCl_4 induced hepatopathy (group VI), in comparison with group IV,

leads to: decrease of iron levels (247.8 ± 77.48 vs. 124.5 ± 32.19) (fig.1), increase of serum calcium values (7.83 ± 0.4 vs. 10.15 ± 0.6) (fig.3), and increase of ionized calcium levels (3.54 ± 0.49 vs. 4.38 ± 0.15) (fig.4).

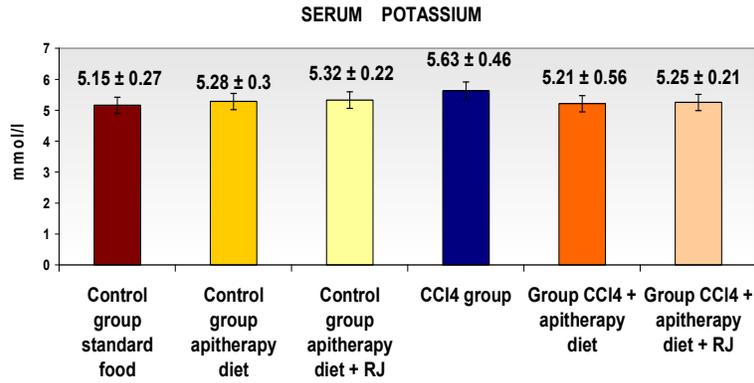


Fig. 2 - Mean values of serum potassium levels and standard deviation

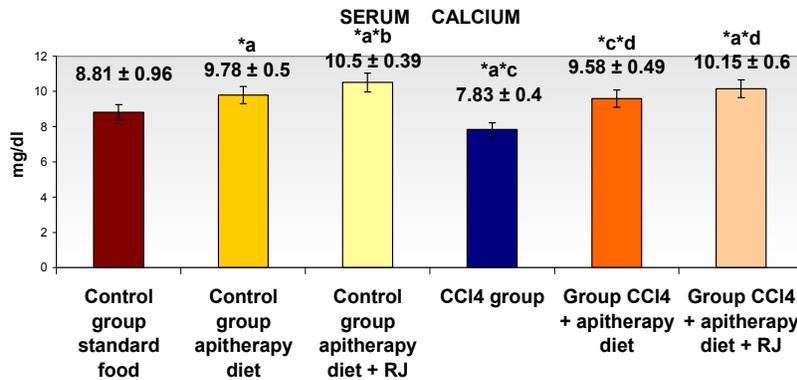


Fig. 3 - Mean values of serum calcium levels and standard deviation

(* 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. CCl₄ group).

Administration of apitherapy diet/apitherapy diet and RJ to laboratory animals with CCl₄ induced hepatopathy (group V) determines, when compared with the animals that were given CCl₄ without protection: decrease of iron, increase of serum and ionized calcium.

In normal conditions, the intestinal absorption of calcium takes place only in the presence of optimal concentrations of: vitamins A, C and D (in its active form, 1,25-dihydroxyvitamin D₃); minerals such as magnesium, iron, manganese; hormones: parathyroid hormone and calcitonin; proteins, lactose, lactic acid, citric acid, bile salts; acid gastric pH; phosphocalcic balance. The lack of the mentioned compounds, along with the unbalance in the homeostasis of different organs mark the assimilation and bioactive function exerting of this important mineral. This statement can be concluded also from the fact that

women, although they take high doses of calcium and vitamin D₃ for long periods of time, they can not surpass the hypocalcemia, finally reaching to osteoporosis, with all the disorders of the bone and neuroendocrin system (Andrițoiu and Andrițoiu, 2004).

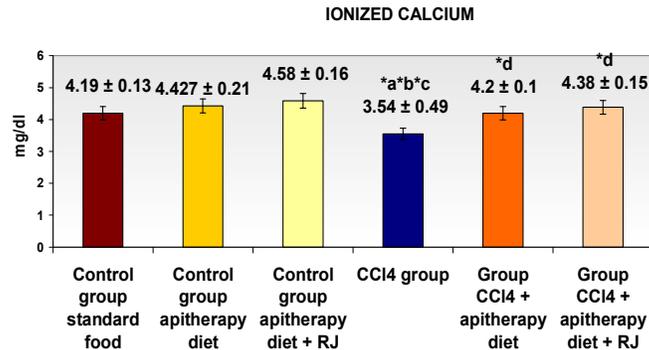


Fig. 4 - Mean values of ionized calcium levels and standard deviation (* a p=0.0001 vs. control group standard food; * b p<0.0001 vs. control group apitherapy diet; * c p<0.0001 vs. control group apitherapy diet + RJ; * d p<0.0001 vs. CCl₄ group).

The alteration of the transmembrane ion transport takes place mainly due to the effects of reactive oxygen species on membranes. It is known that ischemia is associated with alterations of ion exchanges: loss of potassium and entry of calcium into the cells. Further more, the increase of the passive permeability of K⁺ seems to be correlated with the level of membrane peroxidation (Kehrer, 1993).

The apitherapy products used in this experiment (*Apiiregya*, *ApiImunomod*, *ApiImunostim*, *ApiImunostim Forte*), due to their complex chemical composition, improve the intestinal absorption of calcium and the transmembrane ion transport.

Honey raises the absorption of calcium *in vitro* and *in vivo* in laboratory animals. A benefic effect of honey has also been reported in patients with hepatitis A. A sustaining effect has been demonstrated in patients with cancer that, consequently to radiotherapy, show a decrease of mucositis due to the radiations (Bogdanov, 2007).

CONCLUSIONS

1. Administration of the hepatotoxic carbon tetrachloride to laboratory animals leads to the increase of iron levels and the decrease of serum and ionized calcium.
2. Regarding the potassium levels, a slight increase for the group that received the toxic has been noticed, but the values reached the normal range for the groups treated with apitherapy diet/apitherapy diet and RJ
3. Administration of apitherapy products used in this study (*Apiiregya*, *ApiImunomod*, *ApiImunostim*, *ApiImunostim Forte*) leads to the improvement of iron, serum and ionized calcium levels.

Acknowledgement. *This paper was supported by the project PERFORM-ERA "Postdoctoral Performance for Integration in the European Research Area" (ID-57649), financed by the European Social Fund and the Romanian Government.*

REFERENCES

1. **Andrițoiu V., Andrițoiu C.V., 2004** - *Compoziția biochimică a produselor apicole, suport inestimabil în terapia bolilor imunodepresive și autoimune*, Compendiul Conferinței Internaționale de Terapii Complementare, Arad, pp. 133-138.
2. **Bhadoria M., Nirala S.K., Shukla S., 2008** - *Multiple treatment of propolis extract ameliorates carbon tetrachloride*, Food Chem Toxicol, 46, pp. 2703-2712.
3. **Bogdanov S., 2007** - *Bee products and health: a short review*, in: Mărghitaș Al L, Dezmirean D (eds), *Apiculture-from science to agribusiness and apitherapy*; Editura AcademicPres, Cluj-Napoca, pp. 30-39.
4. **Domitrović R., Jakovac H., Tomac J., Šain I., 2009** - *Liver fibrosis in mice induced by carbon tetrachloride and its reversion by luteolin*, Toxicol Appl Pharmacol, 241, pp. 311–321.
5. **Fan G., Tang J.J., Bhadoria M., Nirala S.K., Dai F., Zhou B., Li Y., Liu Z.L., 2009** - *Resveratrol ameliorates carbon tetrachloride-induced acute liver injury in mice*, Environ Toxicol Pharmacol, 28, pp. 350–356.
6. **Fang H.L., Lai J.T., Lin W.C., 2008** - *Inhibitory effect of olive oil on fibrosis induced by carbon tetrachloride in rat liver*, Clin Nutr, 27, pp. 900-907.
7. **Haro A., Lopez-Aliaga I., Lisbona F., Barrionuevo M., Alferrez M.J.M., Campos M.S., 2000** - *Beneficial effect of pollen and/or propolis on the metabolism of iron, calcium, phosphorus, and magnesium in rats with nutritional ferropenic anemia*, J Agric Food Chem, 48, pp. 5715–5722.
8. **Jerca L., 2004** - *Specii radicalice ale oxigenului*. Biochimia NO, Editura Tehnică, Științifică și Didactică CERMI, pp. 59-93.
9. **Kehrer J.P., 1993** - *Free radicals mediators of tissue injury and disease*, Crit Rev Toxicol, 23, pp. 21-48.
10. **Lee K.J., Choi J.H., Jeong H.G., 2007** - *Hepatoprotective and antioxidant effects of the coffee diterpenes kahweol and cafestol on carbon tetrachloride-induced liver damage in mice*, Food Chem Toxicol., 45, pp. 2118–2125.
11. **Sakin F., Bulmuş F.G., Servi K., Popa L., 2011** - *Protective effect of lycopene on oxidative stress induced by different doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin in brain, liver, kidney, and heart tissue of rats*, Farmacia, 59(4), pp. 462-470.
12. **Singh N., Kamath V., Narasimhamurthy K., Rajini P.S., 2008** - *Protective effect of potato peel extract against carbon tetrachloride-induced liver injury in rats*, Environ Toxicol. Pharmacol, 26, pp. 241–246.