

THE INFLUENCE OF APITHERAPY TREATMENT ON COAGULATION PARAMETERS IN EXPERIMENTALLY CARBON TETRACHLORIDE INDUCED HEPATOPATHY

INFLUENȚA APLICĂRII TRATAMENTULUI PRIN APIDIETĂ ASUPRA PARAMETRILOR DE COAGULARE ÎN HEPATOPATIA INDUSĂ EXPERIMENTAL CU TETRACLORURA DE CARBON

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Abstract. The present experiment evaluates the influence of apitherapy diet on carbon tetrachloride (CCl₄) induced hepatopathy in Wistar rats by the means of coagulation parameters (Quick's time, thrombin time and fibrinogen). The experimental model included 60 Wistar rats divided into 6 groups: control group standard food, control group apitherapy diet, control group apitherapy diet + royal jelly, CCl₄ group, CCl₄ group + apitherapy diet, CCl₄ group + apitherapy diet + royal jelly. The hepatopathy was experimentally induced by intraperitoneal injection of CCl₄ (dissolved in paraffin oil, 10% solution) in a dose of 2ml/100 g, once at two days, for 2 weeks. In order to reduce the factors that accelerate the progression of liver damage apitherapy products purchased from "Stupina" LLC have been administrated (Apiregya, Apilmunomod, Apilmunostim, Apilmunostim Forte). Administration of apitherapy diet led to the decrease of the investigated parameters when compared to the CCl₄ group: Quick's time (19.51±1.8 vs. 18.18±1.28), thrombin time (31.12±4.49 vs. 24.42±2.7), fibrinogen (395±48.95 vs. 267.1±56.83). Administration of apitherapy diet and royal jelly resulted in the decrease of the following parameters when compared to the CCl₄ group: Quick's time (19.51±1.8 vs. 17.68±0.87), thrombin time (31.12±4.49 vs. 24.16±1.31), fibrinogen (385.6±48.95 vs. 241.2±47.92).

Keywords: apitherapy, coagulation tests, liver disease.

Rezumat. Prezentul experiment evaluează influența apidietei asupra hepatopatiei induse experimental cu tetraclorură de carbon (CCl₄) la șobolani Wistar, urmărind parametrii de coagulare (timp Quick, timp de trombină și fibrinogen). Modelul experimental s-a desfășurat pe 6 loturi de șobolani Wistar: lot martor hrană standard, lot martor apidietă, lot martor apidietă și lăptișor de matcă, lot CCl₄, lot CCl₄ + apidietă, lot CCl₄ + apidietă + lăptișor de matcă. Afectarea hepatică a fost indusă prin injectarea i.p. a CCl₄ dizolvată în ulei de parafină, soluție 10%, în doză de 2 ml per 100 g, o dată la 2 zile, timp de 2 săptămâni. În scopul reducerii factorilor care accelerează progresia leziunilor hepatice s-au administrat produse apiterapice (Apiregya, Apilmunomod, Apilmunostim, Apilmunostim Forte), procurate de la firma S.C. Stupina S.R.L. Administrarea apidietei a condus la scăderea următorilor

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parametri, comparativ cu lotul CCl₄: timp Quick (19.51±1.8 vs. 18.18±1.28), timp de trombină (31.12±4.49 vs. 24.42.41±2.7), fibrinogen (395±48.95 vs. 267.1±56.83). Administrarea apidietei cu lăptișor de matcă a condus la scăderea următorilor parametri, comparativ cu lotul CCl₄: timp Quick (19.51±1.8 vs. 17.68±0.87), timp de trombină (31.12±4.49 vs. 24.16±1.31), fibrinogen (385.6±48.95 vs. 241.2±47.92).

Cuvinte cheie: apidietă, parametri de coagulare, hepatopatie.

INTRODUCTION

The liver plays a central role in the clotting process and acute and chronic liver diseases are invariably associated with coagulation disorders due to multiple causes: decreased synthesis of clotting and inhibitor factors, decreased clearance of activated factors, platelet defects, hyperfibrinolysis and accelerated intravascular coagulation (Amitrano et al., 2002). Liver damage is commonly associated with variable impairment of haemostasis. Many factors are responsible for maintaining the haemostatic balance, and, among them, hormones directly influence both primary and secondary haemostasis (Orwoll and Orwoll, 1987).

Fibrinogen is a protein produced by the liver. The increase of the fibrinogen synthesis after the acute period may be a sign of collagen affection (Dati and Wagner, 1998).

The prolongation of the thrombin time is met in severe liver damage (Fischbach, 2004).

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 (CCl₄ 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, the third group was given apitherapy diet and royal jelly (RJ). The next three groups of animals were intoxicated with CCl₄ and fed with normal food (group IV), apitherapy diet (group V) and apitherapy diet with royal jelly (group VI).

The laboratory animals were given apitherapy products produced by STUPINA LLC, Bălănești, Gorj, Romania (*Apiregya*, *Apilmunomod*, *Apilmunostim*, *Apilmunostim Forte*). The daily administered doses were 2g *Apiregya*, 1g *Apilmunomod*, 1g *Apilmunostim*, 1g *Apilmunomod 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. The investigated parameters were: Quick's time (QT), thrombin time (TT) and fibrinogen.

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 DISCUSSION

Quick's time (QT)

In animals with CCl₄ induced hepatopathy (group IV) a significant increase of QT can be noticed when compared to all the experimental groups: i) control group apitherapy diet (group II) (16.5±0.36 versus 19.51±1.8, p<0.0001); ii) control group apitherapy diet + RJ (group III) (15.65±0.39 versus 19.51±1.8, p<0.0001) (fig. 1).

Administration of apitherapy diet to laboratory animals with CCl₄ induced hepatopathy (group V) determines the statistically significant increase of QT when compared to: i) control group apitherapy diet (group II) (16.5±0.36 vs. 18.18±1.28, p<0.0495); ii) control group apitherapy diet + RJ (group III) (15.65±0.39 vs. 18.18±1.28, p<0.0007) (fig. 1).

Administration of apitherapy diet and RJ to laboratory animals with CCl₄ induced hepatopathy (group VI) produces the statistically significant decrease of QT in comparison with CCl₄ group (group IV) (19.51±1.8 vs. 17.68±0.87, p<0.0103) (fig. 1).

No statistically significant differences regarding QT could be noticed between groups V (group CCl₄ + apitherapy diet) and VI (group CCl₄ + apitherapy diet + RJ) (fig. 1).

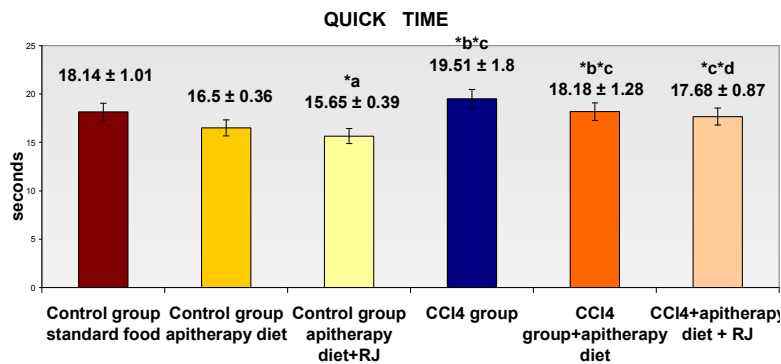


Fig. 1 - Mean values of the QT and standard deviation (* a p=0.0025 vs. control group standard food; * b p<0.05 vs. control group apitherapy diet; * c p<0.05 vs. control group apitherapy diet + RJ; * d p=0.0103 vs. CCl₄ group)

Thrombin time (TT)

In animals with CCl₄ induced hepatopathy (group IV) a significant increase of TT can be noticed when compared to all the other experimental groups: i) control group standard food (group I) (21.65±0.89 vs. 31.12±4.49, p<0.0001); ii) control group apitherapy diet (group II) (18.64±1.38 vs. 31.12±4.49, p<0.0001); iii) control group apitherapy diet + RJ (group III) (19.04±1.18 vs. 31.12±4.49, p<0.0001) (fig. 2).

Administration of apitherapy diet to laboratory animals with CCl₄ induced hepatopathy (group V) determines the statistically significant increase of TT when compared to: i) control group apitherapy diet (group II) (18.64±1.38 vs. 24.42±2.7, p<0.0004); ii) control group apitherapy diet + RJ (group III) (19.04±1.18 vs. 24.42±2.7, p<0.0012) (fig. 2).

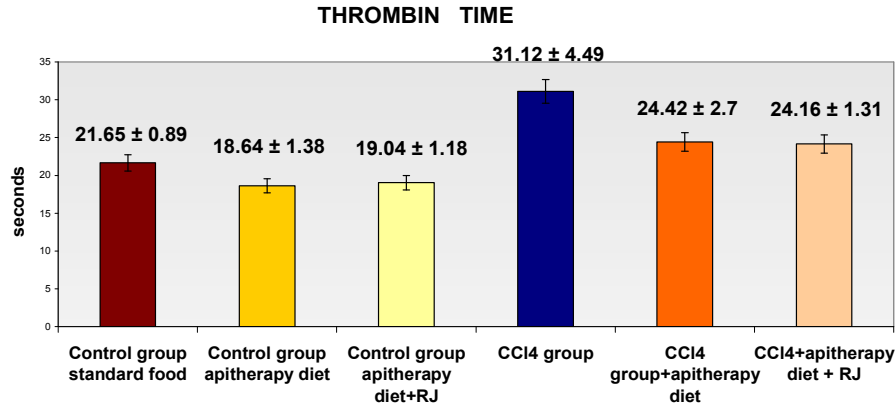


Fig. 2 - Mean values of TT and standard deviation (* a p<0.0001 vs. control group standard food; * b p<0.05 vs. control group apitherapy diet; * c p<0.05 vs. control group apitherapy diet+RJ; * d p<0.0001 vs. CCl₄ group)

Fibrinogen

In animals with CCl₄ induced hepatopathy (group IV) a significant increase of fibrinogen can be noticed when compared to all the other experimental groups: i) control group standard food (group I) (322±8.64 vs. 385±48.95, p<0.0295); ii) control group apitherapy diet (group II) (214.28±9.01 vs. 385±48.95, p<0.0001); iii) control group apitherapy diet + royal jelly (RJ) (group III) (208.4±5.79 vs. 385±48.95, p<0.0001) (fig. 3).

Administration of apitherapy diet to laboratory animals with CCl₄ induced hepatopathy (group V) determines the statistically significant decrease of fibrinogen when compared to CCl₄ group (group IV) (385±48.95 vs. 267.1±56.83, p<0.0001) (fig. 3).

Administration of apitherapy diet and RJ to laboratory animals with CCl₄ induced hepatopathy (group VI) leads to the significant decrease of fibrinogen values in comparison with: i) control group standard food (group I) (322±8.64 vs. 241.2±47.92, p<0.0023); ii) CCl₄ group (group IV) (385±48.95 vs. 241.2±47.92, p<0.0001) (fig. 3).

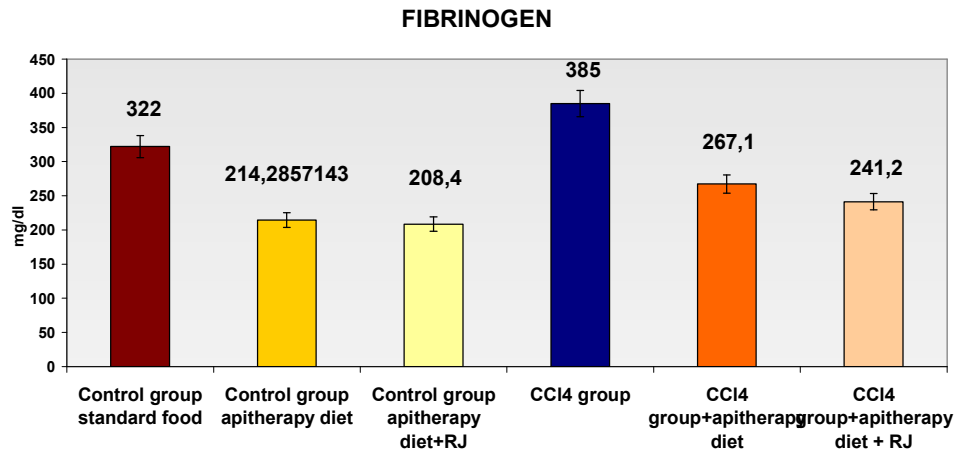


Fig. 3 - Mean values of fibrinogen and standard deviation (* a $p < 0.05$ vs. control group standard food; * b $p < 0.0001$ vs. control group apitherapy diet; * c $p < 0.0001$ vs. control group apitherapy diet + RJ; * d $p < 0.0001$ vs. CCl₄ group)

There can be noticed in the present study that administration of standard food produces an increase of the fibrinogen levels, modification also seen after the administration of CCl₄.

Administration of apitherapy diet and, respectively, of apitherapy diet + royal jelly to laboratory animals with CCl₄ induced hepatopathy led to values of fibrinogen comparable to the values obtained for the control healthy groups.

Regarding the results for QT, administration of standard food leads to a value that can be compared to the quick time level for the group with CCl₄ induced hepatopathy. Administration of apitherapy diet and, respectively, of apitherapy diet and RJ improves the QT levels towards the normal limits.

Administration of CCl₄ leads to an increase of TT, while administration of the apitherapy treatment lowers the values of TT towards the normal limits.

Vitamin K is necessary for gamma-carboxylation of factors II, VII, IX and X. The intestinal absorption of vitamin K, soluble fat, is conditioned by the presence of bile salts (Szántó P., 2004). The hepatic synthesis of these clotting factors requires the presence of vitamin K for the sake of adding the molecule of carboxylic acid in the position of gamma glutamic acid residues (Ansell J. *et al.*, 2008). The gamma-carboxylation is a post-translational process that enables those of coagulation factors to bind calcium ions (Buligescu L., Lupescu I., 1999). Vitamin K is found in bee products and preparations. It is absorbed in the small intestine and partially, at the level of the colon. After absorption, vitamin K incorporated into chylomicrons shall pass into the lymph and reach the liver, where it is incorporated into lipoproteins VLDL and LDL, the form under which it is transported to tissues (Andrițoiu V., 2006).

CONCLUSIONS

1. Administration of the toxic carbon tetrachloride to the laboratory produces a significant increase of Quick's time, Thrombin time and fibrinogen.
2. Administration of standard food leads to an increase of the fibrinogen levels, modification also seen after the administration of CCl₄.
3. The treatment with apitherapy products *Apiiregya*, *ApiImunomod*, *ApiImunostim*, *ApiImunostim Forte* improve the values for Quick's time, Thrombin time and fibrinogen.

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