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Researches Regarding the Influence of Apitherapy Diet on Number of Thrombocytes, Mean Platelet Volume and Platelet Distribution Width in Wistar Rats with CCl_4 Experimentally Induced Liver Disease

Călin V. ANDRIŢOIU[1)], Vasile ANDRIŢOIU[2)], Anca I. PRISĂCARU[3)], Carmen E. COTRUTZ[2)], Tudor PETREUŞ[2)], Simona PARTENE VICOLEANU[4)] Ionel M. POPA[1)]

[1)]Department of Physical Chemistry, "Gheorghe Asachi" Technical University, Prof. dr. docent Dimitrie Mangeron 73 Street, Iaşi, România, dr_calin_andritoiu@yahoo.com;
[2)]Department of Cellular and Molecular Biology, University of Medicine and Pharmacy ,,Gr. T. Popa", Universitatii 16 Street, Iaşi, Romania;
[3)]Department of Natural and Synthetic Polymers, "Gheorghe Asachi" Technical University, Prof. dr. docent Dimitrie Mangeron 73 Street, Iaşi, România
[4)]Department of Immunology, University of Medicine and Pharmacy "Gr.T. Popa", Universitatii 16 Street, Iaşi, Romania

Abstract. The present experiment evaluates the influence of apitherapy diet on CCl_4 experimentally induced liver disease in Wistar rats, by the means of number of thrombocytes, mean platelet volume and platelet distribution width. Protection was achieved by the administration of apitherapy products for three weeks. Hepatic lesion was induced by intraperitoneal injection of CCl_4 (dissolved in paraffin oil, 10% solution). The experiment was unfolded on 60 Wistar rats, divided into 6 groups: control group standard food (group I), control group apitherapy diet (group II), control group apitherapy diet + royal jelly (group III), CCl_4 group (group IV), group CCl_4 + apitherapy diet (group V), group CCl_4 + apitherapy diet + royal jelly (group VI). In comparison with group IV, the administration of apitherapy diet (group V) reveals the increase of number of thrombocytes (765.7 ± 61.07 versus 918.3 ± 63.59), the decrease of the mean platelet volume (8.32 ± 0.69 versus 7.1 ± 0.65) and the decrease of platelet distribution width (9.31 ± 1.13 versus 7.14 ± 0.7). The administration of apitherapy diet and royal jelly (group VI) determines, when compared to CCl_4 group: the increase of number of thrombocytes (765.7 ± 61.07 versus 930.4 ± 7.16), the decrease of the mean platelet volume (8.32 ± 0.69 versus 6.74 ± 0.12) and the decrease of platelet distribution width (9.31 ± 1.13 versus 6.71 ± 0.15). The administration of apitherapy diet is indicated in chronic liver affection with the improvement of platelet parameters.

Keywords: apitherapy, thrombocyte, liver disease.

INTRODUCTION

Thrombocytes are anucleate cytoplasmic fragments, round or oval, biconvex discs, rich in granules, with a diameter of 2-4 μm. Thrombopoiesis takes place in bone marrow. Thrombocytes are involved in hemostasis and in the initiation of tissue repair and vascular constriction consequently to the vascular injury and during inflammatory processes (Wallach, 1996; Lancy, 2004, Levine 2004, Means, 2004, Thomas and Baril, 1998). The mean platelet

volume (MPV) gives information about the uniformity of the thrombocyte size (Fischbach, 2004). The platelet distribution width (PDW) can be used, along with the mean platelet volume, to distinguish the conditions associated with reduced production of thrombocytes from those associated with increased distruction of platelets (Synevo Laboratory, 2010).

The liver is also a hematopoietic organ (Steiff et al, 2003). The liver produces definitive erythrocytes (anucleate), granulocytes, and megakaryocytes (Peaul et al, 2003). In the conditions of liver affection hematological changes appear.

MATERIALS AND METHODS

Hepatic lesion was induced by intraperitoneal injection of 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, the third group was given apitherapy diet and royal jelly. The next three groups of animals were intoxicated with CCl_4 and fed with normal food (group IV), apitherapy diet (group V) and apitherapy diet with royal jelly (group VI).

The laboratory animals were given food supplements produced by *S.C. STUPINA S.R.L*, Bălăneşti, Gorj, Romania, supplements represented by *Apiregya*, *ApiImunomod*, *ApiImunostim, ApiImunostim Forte*. The apitherapy diet was administered for a period of three weeks. The daily doses were 2g *Apiregya*, 1g *ApiImunomod*, 1g *ApiImunostim*, 1g *ApiImunomod Forte*. These preparates included in their composition: honey, royal jelly, propolis, and pollen. The preparates were registered to OSIM with number AO 1242.

The animals were sacrificed by administration of thiopental, after three weeks of apitherapy treatment. After 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 biochemical analysis. The investigated parameters were: the number of thrombocytes (PLT), the mean platelet volume (MPV) and platelet distribution width (PDW). The determination of the values of the investigated parameters were achieved with an automated analyzer (Aeroset, Abbott) and commercial kits (Abbott, USA).

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

Number of thrombocytes (PLT)

Administration of apitherapy diet and royal jelly (RJ) to laboratory animals with CCl_4 induced hepatopathy (group VI) determines the statistically significant increase of the number of thrombocytes in comparison with: i) control group standard food (group I) (799.28±16.47 versus 930.4±7.16, $p<0.0001$); ii) CCl_4 group (group IV) (765.7±61.07 versus 930.4±7.16, $p<0.0001$) (fig. 1).

Between groups V (group CCl_4 + apitherapy diet) and VI (group CCl_4 + apitherapy diet + RJ) no statistically significant differences regarding the number of thrombocytes could be noticed (fig. 1).

In conclusion: i) administration of standard food leads to a decrease of the number of thrombocytes; ii) administration of apitherapy diet and apitherapy diet + RJ to the groups that

had been previously given CCl_4 leads to the improvement of the number of thrombocytes, comparable to the values of the control groups (fig. 1).

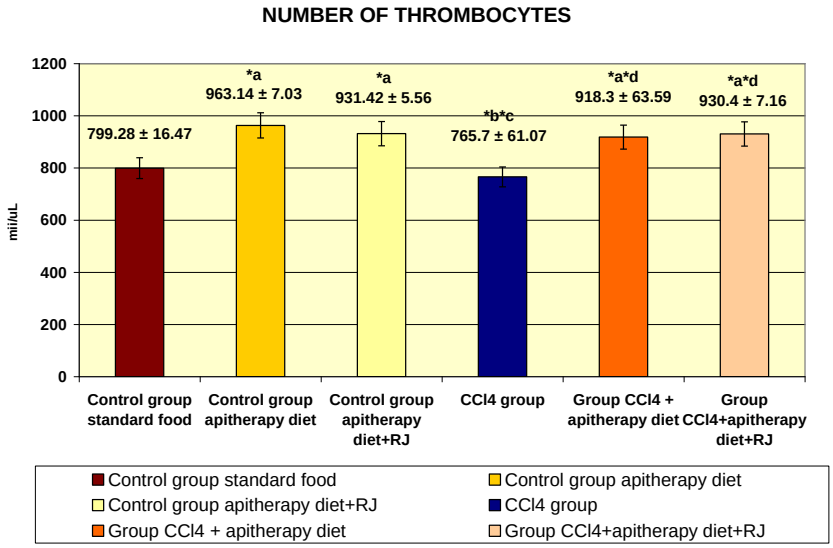

Fig. 1. Mean values of the number of thrombocytes and standard deviation (* a $p<0.05$ vs. control group standard food; * b $p<0.0001$ vs. control group apitherapy diet; * c $p<0.0001$ vs. control group apitherapy diet+RJ; * d $p<0.0001$ vs. CCl_4 group).

Mean platelet volume (MPV)

In animals with CCl_4 induced hepatopathy (group IV) a statistically significant increase of MPV can be noticed when compared to the following groups: i) control group apitherapy diet (group II) (7.2±0.57 versus 8.32±0.69, p=0.0007); ii) control group apitherapy diet + RJ (group III) (6.92±0.44 versus 8.32±0.69, p<0.0001) (fig. 2).

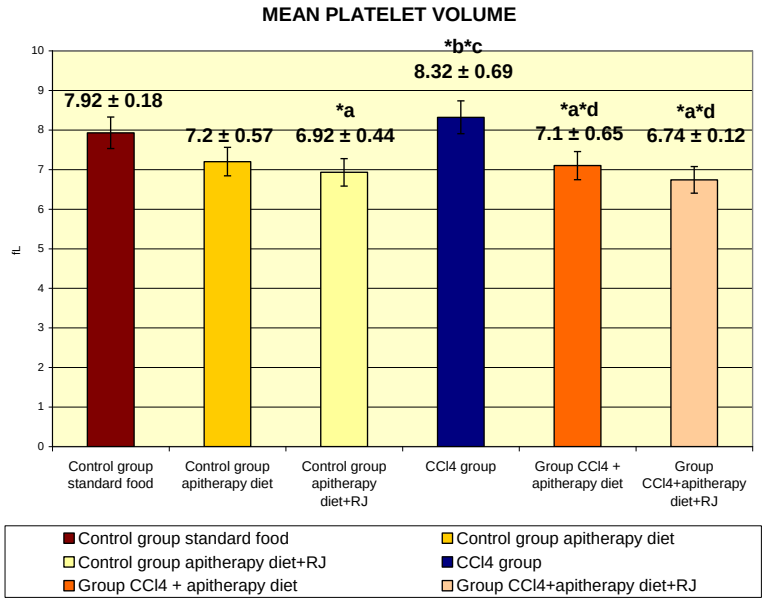

Fig. 2. Mean values of the mean platelet volume and standard deviation (* a $p<0.05$ vs. control group standard food; * b $p<0.0007$ vs. control group apitherapy diet; * c $p<0.0001$ vs. control group apitherapy diet+RJ; * d $p<0.0001$ vs. CCl_4 group).

Administration of apitherapy diet to laboratory animals with CCl_4 induced hepatopathy (group V) determines the statistically significant decrease of the mean platelet volume: i) compared to the control group standard food (group I) (7.92±0.18 versus 7.1±0.65, $p=0.0221$); ii) compared to the CCl_4 group (group IV) (8.32±0.69 versus 7.1±0.65, $p<0.0001$) (fig. 2).

Administration of apitherapy diet and RJ to laboratory animals with CCl_4 induced hepatopathy (group VI) determines the statistically significant decrease of the mean platelet volume: i) compared to the control group standard food (group I) (7.92±0.18 versus 6.74±0.12, $p=0.0003$); ii) compared to the CCl_4 group (group IV) (8.32±0.69 versus 6.74±0.12, $p<0.0001$) (fig. 2).

No statistically significant differences regarding MPV could be noticed between groups V (group CCl_4 + apitherapy diet) and VI (group CCl_4 + apitherapy diet + RJ) (fig. 2).

In conclusion: i) administration of CCl_4 leads to the increase of MPV; ii) administration of apitherapy diet and apitherapy diet + RJ leads to the improvement of MPV values, comparable to those obtained for the control groups (fig. 2).

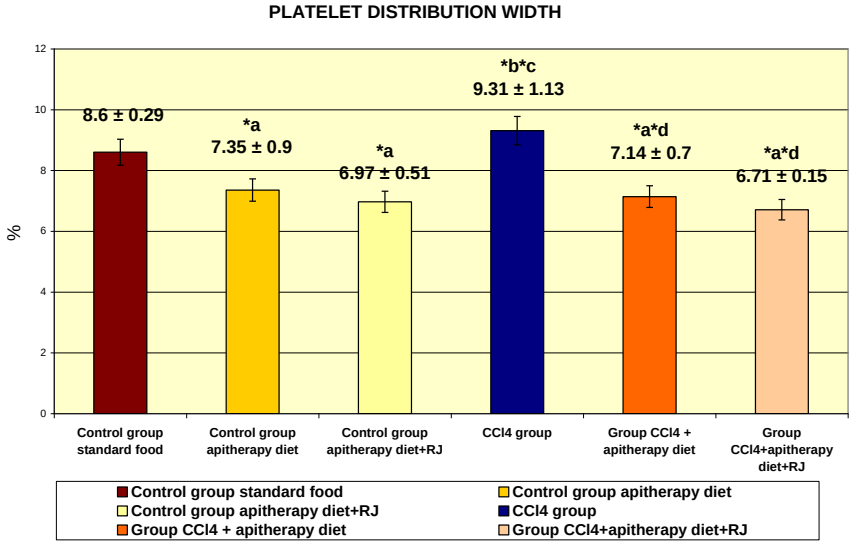

Fig. 3. Mean values of the platelet distribution width and standard deviation (* a $p<0.05$ vs. control group standard food; * b $p<0.0001$ vs. control group apitherapy diet; * c $p<0.0001$ vs. control group apitherapy diet+RJ; * d $p<0.0001$ vs. CCl_4 group).

Platelet distribution width (PDW)

For the animals with CCl_4 induced hepatopathy (group IV) a statistically significant increase of platelet distribution width (PDW) can be noticed when compared with all the experimental groups: i) control group apitherapy diet (group II) (7.35±0.9 versus 9.31±1.13, $p<0.0001$); ii) control group apitherapy diet + RJ (group III) (6.97±0.51 versus 9.31±1.13, $p<0.0001$) (fig. 3).

Administration of apitherapy diet to laboratory animals with CCl_4 induced hepatopathy (group V) determines the statistically significant decrease of the PDW values when compared

with: i) control group standard food (group I) (8.6±0.29 versus 7.14±0.7, p=0.0021); ii) CCl_4 group (group IV) (9.31±1.13 versus 7.14 ±0.7, p<0.0001) (fig. 3).

Administration of apitherapy diet and RJ to laboratory animals with CCl_4 induced hepatopathy (group VI) determines the statistically significant decrease of the PDW values when compared with: i) control group standard food (group I) (8.6±0.29 versus 6.71±0.15, p<0.0001); ii) CCl_4 group (group IV) (9.31±1.13 versus 6.71±0.15, p<0.0001) (fig. 3).

No statistically significant differences regarding PDW could be noticed between groups V (group CCl_4 + apitherapy diet) and VI (group CCl_4 + apitherapy diet + RJ) (fig. 3).

In conclusion: i) administration of standard food reveals values of PDW comparable to the CCl_4 group; ii) administration of apitherapy diet and apitherapy diet + RJ to the groups that had been previously given CCl_4 derermines the improvement of the PDW values, results comparable to those of the control groups (fig. 3).

Thrombocytopenia may take place through different mechanisms. Regarding the present experiment, the number of thrombocytes decreases as a consequence of CCl_4 administration. The accelerated destruction of thrombocytes is the most frequent cause of thrombocytopenia. This leads to the stimulation of thrombopoiesis, thus resulting in the increase of the number, size and maturation of medullary megakaryocytes (Rodgers, 2004).

In the case of CCl_4 administration, an increase of the MPV takes place. MPV varies inversely proportional to the number of thrombocytes, revealing higher values for the patients with thrombocytopenia due to the peripheral dysfunction and increased platelet turnover. In inefficient thrombocytopoiesis coupled with megaloblastic hematopoiesis due to the vitamin B_{12} and/or folic acid deficit, the circulating thrombocytes are abnormally big (Perkins, 2004; Fischbach 2004).

The administered apitherapy products demonstrate the protective effect at the hepatic level. There are also studies revealing the hepatoprotective effect of other natural products in CCl_4 induced hepatopathy. Among the natural products of vegetal origin, there can be named: extract of *Coriandrum sativum* (Sreelatha et al., 2009); *Dunaliella salina*, a carotenoids-rich alga **(**Yu-Wen et al., 2008); extract of *Passiflora alata* (Rudnicki et al., 2007); *Lygodium flexuosum* (L.) Sw. (Wills et Asha, 2006); ginsan, an acidic polysaccharide from *Panax ginseng* (Ji-Young S., et al., 2010); extract of *Aloe barbadensis* Mill. (Chandan et al., 2007); *Vernonia amygdalina* (Asteraceae) (Omolola et Ebenezer, 2008); *Laggera alata* (Yi-Hang Wu et al., 2009); root of *Taraxacum officinale* (Domitrović et al., 2010) and many others. In conclusion, there has been a considerable interest regarding the effect of natural products in liver affections and the present experiment follows this direction of research.

CONCLUSIONS

Administration of apitherapy products (*Apiregya, ApiImunomod, ApiImunostim, ApiImunostim Forte*) is recommended for the hepatoprotective role and for the positive impact upon some hematological parameters.

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