RESEARCHES REGARDING THE INFLUENCE OF APITHERAPY DIET ON ERYTHROCYTES IN WISTAR RATS WITH EXPERIMENTALLY CCl₄ INDUCED HEPATOTOXICITY

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Summary
The purpose of this experiment was to evaluate the influence of apitherapy diet in Wistar rats with experimentally carbon tetrachloride (CCl₄) induced hepatopathy by determining the evolution of hematological parameters (number of erythrocytes – RBC; hemoglobin – HGB; hematocrit – HCT; mean corpuscular volume – MCV, VEM; mean corpuscular hemoglobin - MCH, HEM; mean corpuscular hemoglobin concentration – MCHC, CHEM; red cell distribution width – RDW), under the conditions of chronic liver disease. In order to reduce the factors that accelerate the progression of liver damage apitherapy products (Apiregya, Apilmunomod, Apilmunostim, Apilmunostim Forte) have been administrated. The apitherapy products were purchased from ,,Stupina''. The animals were handled under general anesthesia with thiopental. The determination of investigated parameters was achieved using an automatic analyzer and commercial kits. The experiment was unfold on a total of 60 white rats, Wistar breeding, divided into 6 groups: standard food group (group I), apitherapy diet group (group II), apitherapy diet - royal jelly group (group III), CCl₄ group (group IV), CCl₄ – apitherapy diet group (group V), CCl₄ – apitherapy diet – royal jelly group (group VI). The hepatic injury was chemically induced by the intraperitoneal administration of CCl₄ (dissolved in paraffin oil, 10% solution). Two ml per 100 g were administered, once at 2 days, for 2 weeks. The administration of apitherapy diet, respectively of apitherapy diet and royal jelly in laboratory animals with CCl₄ induced hepatopathy resulted in the improvement of hematological parameters values to normal levels.

Keywords: apitherapy, erythrocytes, hepatotoxicity.

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Introduction
The liver functions as a hematopoietic organ (Steiff et al, 2003). The human stem cells for definitive hematopoiesis result from the endothelial cells of the hepatic vessels from the embryonic and fetal period (Peaul et al, 2003). In the conditions of hepatic injuries, hematological modifications clearly take place.

Material and methods
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 intraperitoneal injection of CCl₄ (dissolved in paraffin oil, 10% solution). Two ml per 100 g were administered, once at 2 days, for 2 weeks. The experiment included 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 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 food supplements produced by S.C. STUPINA S.R.L, Bălănești, Gorj, Romania, supplements represented by Apiregya, Apilmunomod, Apilmunostim, Apilmunostim Forte. The apitherapy products were administered for three weeks. The daily administered doses were 2g Apiregya, 1g Apilmunomod, 1g Apilmunostim, 1g Apilmunomod Forte. These preparates included in their composition: honey, royal jelly, propolis, and pollen. The preparates were registered to OSIM with number AO 1242.

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 puncture of the cord with a Vacuette® system and submitted to biochemical analysis. The investigated parameters were: number of erythrocytes – RBC; hemoglobin – HGB; hematocrit – HCT; mean corpuscular volume – MCV, VEM; mean corpuscular hemoglobin - MCH, HEM; mean corpuscular hemoglobin concentration – MCHC,CHEM; red cell distribution width – RDW. The determination of the values for 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 erythrocytes – RBC

For the animals with CCl₄ induced hepatopathy (group IV), there can be observed a significant decrease of RBC when compared to the control group standard food (group I) (8.51±0.04 versus 7.22±1.21, p<0.0033) (fig. 1).

In conclusion: i) administration of CCl₄ determines the decrease of HGB; ii) HGB values didn't register any significant differences for the animals with CCl₄ induced hepatopathy that were given the apitherapy diet and apitherapy diet + RJ when compared to the control groups (fig. 2).
Hematocrit % - HCT

For the animals with CCl₄ induced hepatopathy (group IV) there can be observed a statistically significant decrease of the HCT values when compared to all the experimental groups: i) compared to the control group standard food (group I) (44.65±2.05 versus 39.9±±4.3, p<0.0201); ii) compared to the control group apitherapy diet (group II) (44.25±3.24 versus 39.9±4.3, p<0.0413); iii) compared to the control group apitherapy diet + RJ (group III) (45.71±1.28 versus 39.9±±4.3, p<0.0001) (fig. 3).

![Fig. 3. Mean values of the hematocrit and standard deviation (* a  p=0.0201 vs. lotul martor hrană standard; * b  p=0.0413 vs. lotul cu apidietă; * c  p=0.0024 vs. lotul cu apidietă+LM; * d  p=0.05 vs. lot CCl₄).](image)

Administration of apitherapy diet to animals with CCl₄ induced hepatopathy (group V) causes a statistically significant increase of the HCT values when compared to the CCl₄ group (group IV) (39.9±±4.3 versus 44.01±2.44, p<0.0306).

Administration of apitherapy diet and RJ to animals with CCl₄ induced hepatopathy (group VI) causes a statistically significant increase of the HCT values when compared to the CCl₄ group (group IV) (39.9±4.3 versus 44.16±2.54, p<0.0226).

No statistically significant differences between groups V and VI could be observed regarding the HCT values.

In conclusion: i) administration of CCl₄ causes the decrease of HCT values; ii) administration of apitherapy diet and apitherapy diet + RJ, respectively, leads to an increase of the HCT values for the groups that were previously given CCl₄, compared to the group that received only CCl₄. The values obtained following the apitherapy treatment are comparable to the values obtained in the case of the control groups (healthy animals that received the same apitherapy treatment).

Mean corpuscular volume – MCV, VEM

Administration of apitherapy diet (control group apitherapy diet) and of apitherapy diet + RJ (control group apitherapy diet + RJ) determines a significant increase of MCV values when compared to the control group standard food: i) 53.11±0.83 versus 58.78 ± 2.01, p = 0.0148); ii) 53.11±0.83 versus 59.38±1.26, p=0.0053) (fig. 4).

Administration of apitherapy diet, respectively of apitherapy diet + RJ leads to an improvement of the MCV values for the groups of animals that were previously given CCl₄. The values are comparable to those obtained for the control groups (healthy animals that were given the same apitherapy treatment) (fig. 4).

![Fig. 4. Mean values of the mean corpuscular volume and standard deviation (* a  p<0.05 vs. lotul martor hrană standard).](image)

Mean corpuscular hemoglobin - MCH, HEM

Administration of apitherapy diet to animals with CCl₄ induced hepatopathy (group V) causes a statistically significant increase of the MCH values when compared to control group standard food (group I) (16.88±1.29 versus 18.79±0.77, p=0.0233) (fig. 5).

Administration of apitherapy diet and RJ to animals with CCl₄ induced
hepatopathy (group VI) causes a statistically significant increase of the MCH values compared to the control group standard food (group I) (16.88±1.29 versus 18.89±1.05, p=0.0147) (fig. 5).

No statistically significant differences regarding the MCH values could be observed between groups V and VI.

In conclusion: i) administration of standard food leads to a decrease of the MCH values; ii) administration of apitherapy diet and apitherapy diet + RJ improves the MCH values for the groups that had previously received CCl4, the values being comparable to those obtained for the control groups (fig. 5).

Mean corpuscular hemoglobin concentration – MCHC, CHEM

Administration of apitherapy diet and RJ to animals with CCl4 induced hepatopathy (group VI) causes a statistically significant increase of the MCH values compared to the CCl4 group (group IV) (31.94±0.77 versus 33.31±0.11, p<0.0108) (fig. 6).

No statistically significant differences between groups V and VI could be observed regarding the MCHC values (fig. 6).

Red cell distribution width – RDW

For the animals with CCl4 induced hepatopathy (group IV) there can be observed a statistically significant decrease of the RDW values when compared to all the experimental groups: i) compared to the control group standard food (group I) (16.38±1.04 versus 19.51±1.62, p<0.0001); ii) compared to the control group apitherapy diet (group II) (19.51±1.62 versus 16.38±1.04, p<0.0001) (fig. 7).

Administration of apitherapy diet to laboratory animals with CCl4 induced hepatopathy (group V) determines the decrease of RDW when compared to: i) the control group standard food (group I) (18.74±0.21 versus 15.55±1.16, p<0.0001); ii) the CCl4 group (group IV) (19.51±1.62 versus 15.55±1.16, p<0.0001) (fig. 7).
Administration of apitherapy diet + RJ to laboratory animals with CCl₄ induced hepatopathy (group VI) determines the decrease of RDW when compared to: i) the control group standard food (group I) (18.74±0.21 versus 15.44±0.9, p<0.0001); ii) the CCl₄ group (group IV) (19.51±1.62 versus 15.44±0.9, p<0.0001).

No statistically significant differences between groups V and VI could be observed regarding the RDW values.

In conclusion: i) administration of apitherapy diet, respectively of apitherapy diet and RJ maintains the RDW values obtained for the animals that had previously been given CCl₄ in a range comparable to those obtained for the control groups; ii) administration of CCl₄ leads to an increase of RDW levels; iii) administration of standard food also determines an increase of the RDW values, very close to those obtained for the CCl₄ group.

The hematocrit depends on the erythrocyte mass, mean corpuscular volume and plasmatic volume. Generally, when the erythrocytes have a normal size, the modifications of HCT follow the RDW changes (Wallach, 1996; Perkins, 2004). MCV may suggest the physiopathologic mechanism of erythrocyte disorder. MCV depends on the plasmatic osmolarity and number of the erythrocyte fractions. Clinical significance – in most cases of anemia, MCH correlates with MCV. Because of the similar behaviour of the MCV and MCH, MCHC remains constant in many hematopoietic disorders (Glader, 2004).

The administered apithrapy preparates demonstrate the protective effect at the hepatic level. There are also studies regarding other natural products with protective effect in CCl₄ hepatopathy: Saccharomyces cerevisiae (Lai et al., 2009); lycopene from tomatoes (Kim et al., 2004); dehydrocavidine, an active compound from Corydalis saxicola Bunting (Yanhuanglian) (Wang et al., 2008); the diterpenes kahweol şi cafestol from coffee (Kyung Jin Lee et al., 2007); electrolysed wáter (Tsai et al., 2009); the flavone luteolin (3’,4’,5,7-tetrahydroxyflavone) (Domitrović et al., 2009); hyaluronic acid and chondroitin-4-sulfate (Campos et al., 2004); olive oil (Fang et al., 2008); potato peel extract (Singht et al., 2008); resveratrolul (Fan et al., 2009).

Conclusions

The administration of apithrapy products Apiregya, ApImunomod, ApImunostim, ApImunostim Forte is recommended for their hepatoprotective role against chronic liver disorders and for their positive effect upon the hematological parameters.

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References


