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Researches Regarding the Influence of Apitherapy Diet on Leukocyte Formula in Wistar Rats with Experimentally CCl_4 Induced Liver Disease

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Abstract. The purpose of this experiment is to evaluate the influence of apitherapy diet on CCl_4 induced hepatopathy in laboratory animals, by the means of leukocyte formula. The experiment was unfolded on six groups of Wistar rats: 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). The animals were handled under general anesthesia with thiopental. Hepatic lesion was induced by intraperitoneal injection of CCl_4 (dissolved in paraffin oil, 10% solution). Hepatoprotection was achieved with apitherapy products (*Apiregya, ApiImunomod, ApiImunostim, ApiImunostim Forte*). Determination of the leukocyte formula was achieved by using an automatic analyzer and commercial kits. Administration of apitherapy diet and respectively of apitherapy diet and royal jelly in laboratory animals with CCl_4 induced hepatopathy improves the values of parameters from the leukocyte formula when compared with CCl_4 group.

Keywords: apitherapy leukocyte formula, hepatopathy.

INTRODUCTION

Liver damage may be provoked by various chemical substances, among which carbon tetrachloride (CCl_4) stands for a vey well known experimental model of chemically induced hepatotoxicity (Stancey and Prietsly, 1978; Brattin et al., 1985; Rechnagel and Glene, 1973; Rikans et al, 1999; Kodavanti et al., 1989).

Granulopoiesis takes place at the level of bone marrow and it is considered that neutrophilic, eosinophilic and basophilic granulocytes follow the same scheme of proliferation, differentiation, maturation and passage into bloodstream (Schubitz, 2004).

Leukocytes are divided into two main groups: granulocytes (neutrophils, eosinophils, basophils) and agranulocytes (lymphocytes and monocytes) (Carmel, 2004).

Monocytes are the largest cells in the blood; they are part of the mononuclear phagocyte system/reticuloendothelial system composed of monocytes, macrophages and their bone marrow forerunners. The monocytes are released into the blood and, after a short circulation time, they migrate to different tissues, at random or in a specific manner, as a response to the various chemotactic factors**.** Phylogenetically, the cells of the phagocyte

system are primitive cells, as no animal can live without them. They fulfill a large variety of functions, including the removal of foreign, senescent, dead or damaged cells, regulation of the function of other cells, processing of antigens in immune reactions, implication in different inflammatory processes, destruction of bacterial and tumor cells. Monocytes and macrophages produce numerous bioactive compounds: enzymes, complement factors, coagulation factors, oxygen reactive species, nitrogen, angiogenesis factors, binding proteins (transferrin, transcobalamin II, fibronectin, apolipoprotein E), bioactive lipids (derivatives of arachidonic acid), chemotactic factors, cytokines, growing factors (interferon - IFN α and γ, interleukins - IL 1,3,6,8,10,12, fibroblast growth factor - FGF, platelet-derived growth factor - PDGF**,** tumor necrosis factor - TNF, macrophage colony-stimulating factor – MCSF) (Perkins, 2004; Fischbach, 2004; Weinberg, 2004).

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 daily administered 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. The investigated parameters were: number of leukocytes, neutrophils, eosinophils, basophils, lymphocytes and monocytes. 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 leukocytes

In animals with CCl_4 induced hepatopathy (group IV) a statistically significant decrease of WBC can be noticed when compared to the following groups: i) control group apitherapy diet (group II) (10.78 ± 1.62 versus 4.86 ± 0.69, $p<0.0001$); ii) control group apitherapy diet + RJ (group III) (11.92 ± 1.07 versus 4.86 ± 0.69, $p<0.0001$) (fig. 1).

Administration of apitherapy diet to laboratory animals with CCl_4 induced hepatopathy (group V) determines the statistically significant increase of WBC when compared with: i)

control group standard food (group I) (5.87±1.62 versus 10.39±2.6, p=0.0004); ii) CCl_4 group (group IV) (4.86±0.69 versus 10.39±2.6, p<0.0001) (fig. 1).

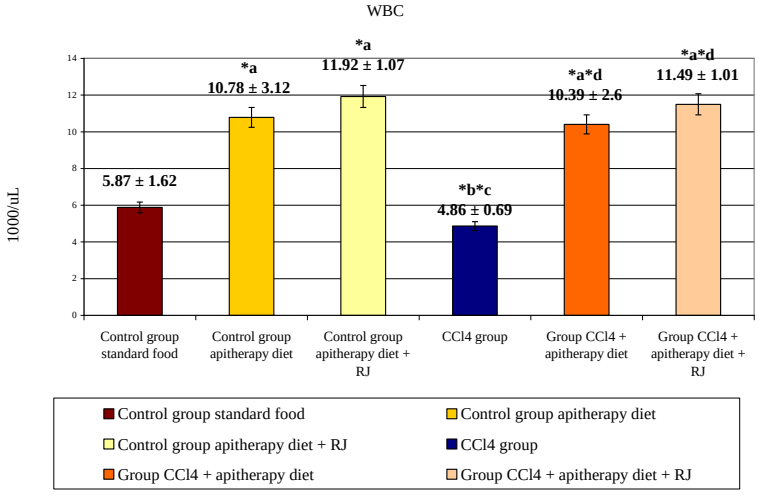

Fig. 1. Mean values of WBC ($10^3/\mu L$) and standard deviation (* a p<0.05 vs. control group standard food; * b p<0.0001 vs. control group apitherapy diet; * c p<0.0001 control group apitherapy diet+RJ, * d p<0.0001 vs. CCl_4 group).

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 WBC in comparison with: i) control group standard food (group I) (5.87±1.62 versus 11.49±1.01, p<0.0001); ii) CCl_4 group (group IV) (4.86±0.69 versus 11.49±11.49, 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 WBC could be noticed (fig. 1).

Number of neutrophils

In animals with CCl_4 induced hepatopathy (group IV) a statistically significant decrease of the number of neutrophils ($10^3/\mu L$) can be noticed when compared with the control group standard food (group I) (1.79±0.25 versus 1,009±0.13, p<0.0001). Administration of CCl_4 (group IV) determines a statistically significant increase of the number of neutrophils ($10^3/\mu L$) in comparison with control group apitherapy diet (group II) (0.791±0,13 versus 1,009±0.13, p<0.0018) (fig. 2).

Administration of apitherapy diet to laboratory animals with CCl_4 induced hepatopathy (group V) determines a statistically significant decrease of the number of neutrophils ($10^3/\mu L$) when compared with: i) control group standard food (group I) (1.791±0.25 versus 1.084±0.36, p<0.0001); ii) control group apitherapy diet + RJ (group III) (1.49±0.03 versus 1.084±0.36, p=0.0123) (fig. 2).

Administration of apitherapy diet and RJ to laboratory animals with CCl_4 induced hepatopathy (group VI) determines a statistically significant increase of the number of neutrophils ($10^3/\mu L$) in comparison with: i) control group apitherapy diet (group II) (0.791±0.13 versus 1.343±0.25, p=0.0003); ii) CCl_4 group (group IV) (1.009±0.13 versus 1.343±0.25, p<0.0001) (fig. 2).

No statistically significant differences regarding the number of neutrophils ($10^3/\mu L$) could be noticed between groups V (group CCl_4 + apitherapy diet) and VI (group CCl_4 + apitherapy diet + RJ) (fig. 2).

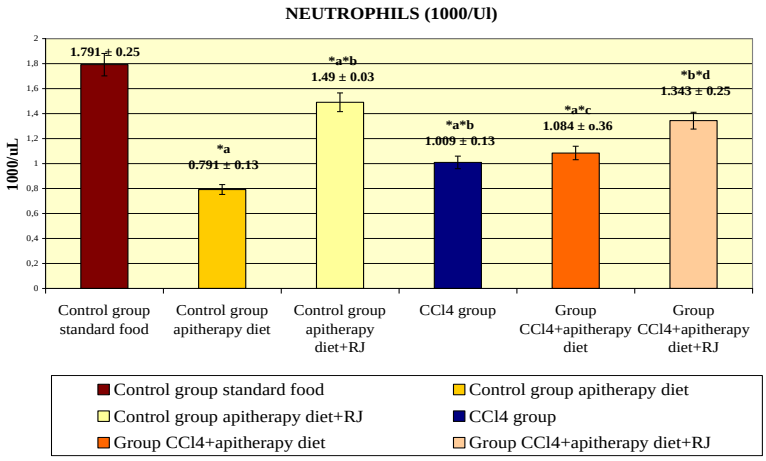

Fig. 2. Mean values of neutrophils ($10^3/\mu L$) and standard deviation (* a $p<0.0001$ vs. control group standard food; * b $p<0.05$ vs. control group apitherapy diet; * c $p=0.0123$ vs. control group apitherapy diet+RJ; * d $p=0.0296$ vs. CCl_4 group).

Number of lymphocytes

In animals with CCl_4 induced hepatopathy (group IV) a statistically significant decrease of the number of lymphocytes ($10^3/\mu L$) can be noticed when compared with the control group apitherapy diet+RJ (group III) (8.74±0.25 versus 3.64±0.55, $p<0.0001$) (fig. 3).

Administration of apitherapy diet to laboratory animals with CCl_4 induced hepatopathy (group V) determines a statistically significant increase of the number of lymphocytes ($10^3/\mu L$) when compared with: i) control group standard food (group I) (3.49±1.09 versus 6.52±1.92, $p<0.0001$); ii) CCl4 group (group IV) (3.64±0.55 versus 6.52±1.92, $p<0.0001$) (fig. 3). Co-administration of CCl_4 and apitherapy diet (group V) results in statistically significant decrease of the number of lymphocytes ($10^3/\mu L$) in comparison with control group apitherapy diet+RJ (group III) (8.74±0.25 versus 6.52±1.92, $p=0.004$) (fig. 3).

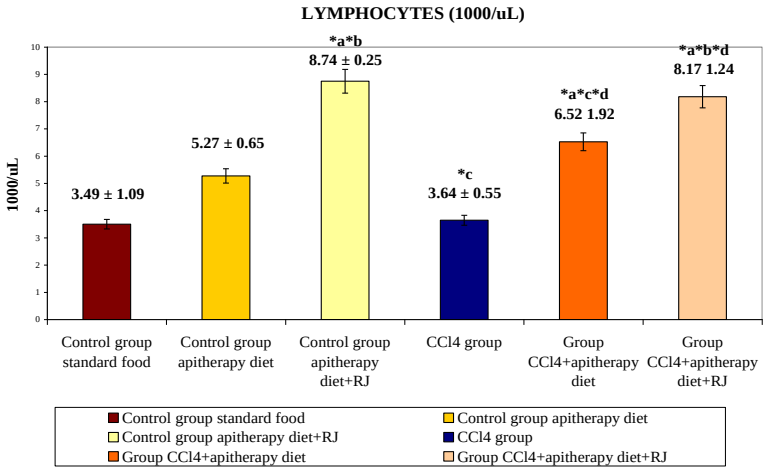

Fig. 3. Mean values of lymphocytes ($10^3/\mu L$) and standard deviation (* a $p<0.0001$ vs. control group standard food;; * b $p<0.0001$ vs. control group apitherapy diet; * c $p<0.05$ vs. control group apitherapy diet+RJ; * d $p<0.0001$ vs. CCl_4 group).

Administration of apitherapy diet and RJ to laboratory animals with CCl_4 induced hepatopathy (group VI) determines a statistically significant increase of the number of lymphocytes ($10^3/\mu L$) when compared with: i) control group standard food (group I) (3.49±1.09 versus 8.17±1.24, $p<0.0001$); ii) control group apitherapy diet (group II) (5.27±0.65 versus 8.17±1.24, $p<0.0001$); iii) CCl_4 group (group IV) (3.64±0.55 versus 8.17±1.24, $p<0.0001$) (fig. 3).

No statistically significant differences regarding the number of lymphocytes ($10^3/\mu L$) could be noticed between groups V (group CCl_4 + apitherapy diet) and VI (group CCl_4 + apitherapy diet + RJ) (fig. 3).

Number of monocytes

No statistically significant differences regarding the number of monocytes could be noticed for the experimental groups (fig. 4).

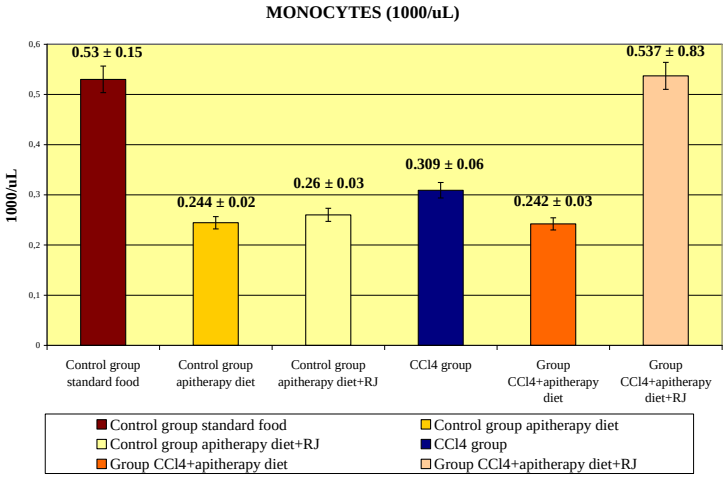

Fig. 4. Mean values of the number of monocytes and standard deviation.

Number of eosinophils

In animals with CCl_4 induced hepatopathy (group IV) a statistically significant decrease of the number of eosinophils can be noticed when compared with control group standard food (group I) (0.431±0.11 versus 0.285±0.09, $p=0.01$). In animals with CCl_4 induced hepatopathy (group IV) a statistically significant increase of the number of eosinophils ($10^3/\mu L$) can be noticed when compared with control group apitherapy diet (group II) (0.158±0.02 versus 0.285±0.09, $p=0.037$) (fig. 5).

Administration of apitherapy diet to laboratory animals with CCl_4 induced hepatopathy (group V) determines a statistically significant decrease of the number of eosinophils ($10^3/\mu L$) when compared with control group standard food (group I) (0.431±0.11 versus 0.236±0.1, $p<0.0003$) (fig. 5).

Administration of apitherapy diet and RJ to laboratory animals with CCl_4 induced hepatopathy (group VI) determines a statistically significant decrease of the number of

eosinophils ($10^3/\mu L$) when compared with control group standard food (group I) (0.431 ±0.11 versus 0.255±0.07, p<0.0011) (fig. 5).

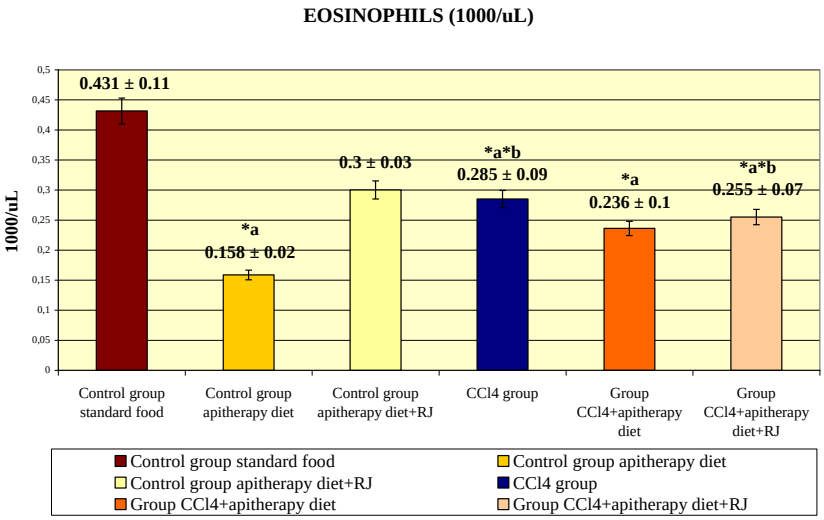

Fig. 5. Mean values of eosinophils ($10^3/\mu L$) and standard deviation (* a p<0.05 vs. control group standard food; * b p<0.05 vs. control group apitherapy diet).

Administration of apitherapy diet and RJ to laboratory animals with CCl_4 induced hepatopathy (group VI) results in a statistically significant increase of the number of eosinophils ($10^3/\mu L$) when compared with control group apitherapy diet (group II) (0.158± 0.02 versus 0.255±0.07, p<0.0289) (fig. 5).

No statistically significant differences regarding eosinophils could be noticed between groups V (group CCl_4 + apitherapy diet) and VI (group CCl_4 + apitherapy diet + RJ) (fig. 5).

Number of basophils

No statistically significant differences regarding the basophils can be noticed for the experimental groups, except for the group that received only CCl_4, where a marked decrease of the values can be observed (fig. 6).

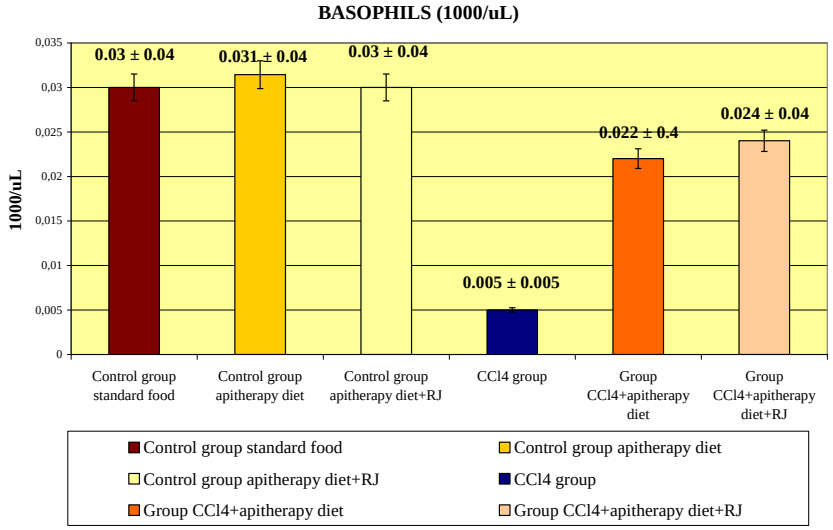

Fig. 6. Mean values of the number of basophils and standard deviation.

The most numerous type of leukocytes (neutrophils) plays a major role in primary anti-infectious defence of the organism by phagocytosis and digestion of microorganisms. Their inappropriate activation may lead to the lesion of normal tissues by releasing enzymes and pyogenic agents (Schubitz, 2004).

The increase of neutrophils takes place, among other causes, due to the toxic substances and certain drugs. Neutriphilia can be also caused by infections, inflammatory, metabolic and mieloproliferative disorders, tissue necrosis, acute hemorrhagia, malignant tumors (DeMott and Tilzer, 1994; Thomas and Bartl, 1998).

In the blood of healthy subjects, eosinophils are found in small amounts but become prevalent in blood and tissues in the conditions of different allergic, parasitary or malignant diseases (Fischbach, 2004; Skubitz K, 2004).

Few mastocyte populations answer to some neuropeptids, and the tight anatomical association between mastocytes and nerves represent the evidence of the neurogenic component dependent on the allergic reactions mastocytes (Befus et al, 2004).

Monocytosis appears in hepatic cirrhosis, parasitary and gastrointestinal diseases, collagen disorders, post-surgery and other conditions (Weinberg, 2004).

The decrease of the basophilic population can be remarked after the administration of CCl_4. The diminution of the basophils values are also met after administration of procainamide and thiopental (Fischbach, 2004).

Basophils and mastocytes are two types of leukocyte populations that have many similarities but also some differences. The presence of the mastocyte forerunners in blood is met in liver and kidney chronic diseases, asthma, anaphylactic shock, macroglobulinemia, lymphoma with medullary invasion, suprarenal insufficiency, osteoporosis (DeMott and Tilzer, 1994; Thomas and Bartl, 1998). In the present experiment, a marked decrease of basophils after the administration of CCl_4 can be noticed.

In this study, the protective role of apitherapy products on the leukocyte formula in experimentally CCl_4-induced hepatotoxicity in rats has been evaluated. The results of our study suggest that the pretreatment with apitherapy products plays a protective role in coagulation disorders associated with CCl_4 administration in rats.

Administration of standard food causes a significant increase of the number of neutrophils, while administration of CCl_4 determines the decrease of neutrophils.

Administration of standard food (group I) and CCl_4 (group IV) determines a significant decrease of the number of lymphocytes, while administration of apitherapy diet and RJ determines the increase of lymphocyte values, both in the control group and the group that was given CCl_4. Administration of apitherapy diet to the animals from the control group standard food and CCl_4 group leads to a mean value of lymphocytes. Administration of standard food leads to an increase of the eosinophilic values, increase that is more advanced when compared to increase produced by administration of CCl_4. Administration of apitherapy diet and respectively of apitherapy diet and RJ to the groups that had been previously given CCl_4 leads to a value of eosinophils comparable to the control groups. Administration of standard food produces the decrease of the leukocyte number, while the apitherapy diet and respectively, the apitherapy diet and RJ given to the control healthy groups results in the increase of the leukocyte values. Administration of apitherapy diet and respectively, of apitherapy diet and RJ leads to an increase of the leukocyte values for the groups that had been previously given CCl_4.

There are also studies regarding other natural products with hepatoprotective effect in experimentally CCl_4 induced 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 cafestol and kahweol from coffee (Lee et al., 2007); electrolyzed water (Tsai et al., 2009); the flavone luteolin (3′,4′,5,7-tetrahydroxyflavone) (Domitrović et al., 2009); hyaluronic acid and chondroitin-4-sulfate (Campo et al, 2004); olive oil (Fang et al., 2008); potato skin extract (Singht et al., 2008); resveratrol (Fan et al., 2009).

CONCLUSIONS

Administration of apitherapy products leads to the improvement of the leukocyte parameters in experimentally CCl_4 hepatopathy.

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