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EVOLUTION OF THE HAEMATOLOGICAL INDICES AT CD1 MICE WITH INDUCED DIABETES, TREATED WITH VEGETAL EXTRACTS OF *ARONIA MELANOCARPA* AND *SILYBUM MARIANUM*

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Abstract
The aim of this paper is to investigate the effects produced by the vegetal extracts of Aronia melanocarpa and Silybum marianum on the haematological index at CD1 (the rederived mice from a non-consanguineous strain) mice with artificially induced diabetes. We established the number of haematites and leucocytes, as well as their morphological dimension and aspect on the blood smear. The diabetisation of the mice was carried out by administering two doses of 0.2 ml solution of Alloxan (130mg/kg body, dissolved in physiological saline), following which, during two weeks, vegetal extract of aronia and milk thistle was administered (0,2 ml). The results obtained showed that there are no significant changes of the number of haematites and leucocytes in comparison with the witness lot. As for the aspect and the dimensions of the figurate elements on the blood smear, in the case of the lot injected with alloxan monohydrate, it was noticed an increase of the haematites' volume and the apparition of some atypical cells – megalocytes, as well as the presence of apoptosis nuclei. Following the administration of the vegetal extracts of aronia and milk thistle, we notice a comeback of the haematites' dimension, as well as the lack of apoptosis micronuclei, which demonstrates the beneficial effect of the two extracts.

Keywords: alloxan, aronia extract, diabetes, figurate elements, milk thistle extract.

1. INTRODUCTION

Diabetes mellitus is a heterogeneous syndrome, characterised by a complex disorder in regulating the energetic metabolism of the organism, which affects both the use of carbohydrates, lipids and proteins, as well as that of other metabolisms. These disorders flow from a defect of secreting insulin associated with a variable peripheral resistance to insulin. The biochemical changes that these two disorders carry along lead to functional cellular changes followed by irreversible anatomic lesions in numerous tissues and organs (Ionescu, 2004). In this study, we have used alloxan monohydrate in order to induce diabetes to the mice tested. Alloxan is a toxic analogous of glucose, which selectively destroys the cells in the pancreas producing insulin when it is administered to rodents and to other laboratory species. This leads to the apparition of insulin dependent diabetes mellitus (called "alloxan diabetes") with characteristics which are similar to type 1 diabetes mellitus in humans. Methods used to induce diabetes mellitus with alloxan have been described for mice, rats, rabbits and dogs (Nitz de Carvalho et al., 2003). Inducing non-insulin

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dependant diabetes mellitus has been carried out by administering two doses of 130 mg/ kg body alloxan, and 48 hours later, glycaemia has been established by means of an Accutrend GCT device. The blood was collected from the tail veins by puncture (Parasuraman et al., 2010). After the diabetes was installed, the vegetal extract was administered by gavage (Popescu, 2014), and the evolution of the parameters of interest continued to be monitored.

Aronia melanocarpa, commonly called black chokeberry or aronia is a medicinal plant which belongs to Rosaceae family. The aronia fruits have a high concentration of antioxidants, actually the highest concentration of antioxidants that can exist in a fruit according to the results of the studies carried out up to present and to the values certified by ORAC (oxygen radical absorbance capacity). Aronia fruits can be beneficial for people who wish to prevent diabetes and they can also help with the management of diabetes when it is associated with the treatment recommended by the doctor. An important benefit of consuming aronia is that it helps with weight regulation, and an additional benefit brought by introducing aronia fruits in daily diet is that it controls the level of cholesterol and the triglycerides in the blood (Oprea et al., 2014; Lupaşcu et al., 2016). Several studies have been conducted and they analyse the way in which aronia fruits can bring benefits to the people with diabetes mellitus (Simeonov et al., 2002; Valceva- Kuzmanova et al., 2007).

Silybum marianum, commonly known as milk thistle is an invasive weed, which spreads rapidly in dry, deserted areas. Its seeds contain silymarin (silybin, silychristin and silydianin), bioflavonoids, amino acids (glycerin, leucine, cysteine, tyramine), glutamic acid, fumaric acid, saponin, sterols, tocopherols (Ciulei, 1993). Studies undertaken in Germany, United States, Italy, Austria have shown that milk thistle is one of the strongest remedies against hepatic diseases, which has boomed over the last decade. More than that, milk thistle is a powerful anti-toxic, which helps the body to resist against the most powerful poison, from poisonous mushrooms to heavy metals (Ionescu et al., 2014).

2. MATERIALS AND METHODS

CD1 mice were used for the testing and they were acquired from "Cantacuzino" National Research Institute in Bucharest. The animals were acclimatised for a week: they were kept in aquaterrariums, with natural light, respecting the diurnal/nocturne cycles, at adequate temperatures (19 - 25 °C), optimal conditions of noise and humidity (60%), they were properly fed, with free access to food and water while the experiments were carried out (Ciudin, 1996). The experimental lots also contain a control group for each experiment, which was formed by a number of minimum 5 animals. Vegetal extracts of aronia and milk thistle were obtained through a series of primary processing steps as well as advanced processing steps, by which alcoholic and hydraalcoholic extractive solutions were obtained from the vegetal material by using ethyl alcohol of different concentrations as solvent (Drăgănescu, 2014).

The experiments were carried out on 4 experimental lots, each having 5 mice/lot, as follows:

- a witness lot kept in conditions identical to the acclimatization ones;
- two lots of mice, each injected with 0.2 ml 130 mg/kg body alloxan, which received vegetal extracts after the diabetes was installed;
- a lot of mice with diabetes, which received by gavage 0.2 ml extract of vegetal aronia for two weeks;
- a lot of mice which received by gavage 0.2 ml extract of vegetal milk thistle for 2 weeks.

At the end of the testing, blood was collected by venous puncture from the tail and the two types of figurate elements, haematites and leucocytes, were numbered with the help of a Thomas-Zeiss counting chamber, at the optical microscope, based on some common techniques of different coloration; consequently, in the erythrocytes only the nuclei got slightly coloured, whereas the

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leucocytes got coloured in purple red (Popescu, 2003). The execution of the blood smear – the morphological examination of blood involves carrying out a smear in two steps: smearing the blood and May-Grunwald- Giemsa staining. Therefore, the blood smear is covered with May-Grunwald solution and it is left for 2-3 minutes (blood smear fixation), then an equal number of buffered water drops is added over the solution, they are homogenized and left for two minutes (diluted, May-Grunwald solution acts as a coloring agent), the coloring agent is removed without washing, and it is covered with Giemsa solution diluted with buffered water in proportion of 1/1 (1 drop of Giemsa solution for one ml of water) and it is left for 15-20 minutes. Then, it is washed under a flow of water, the surplus is absorbed and it is placed on a drying stand (Lotreanu, 2000).
All values were expressed as mean ± SD. Statistical analysis was carried out by one-way ANOVA, LSD test.

3. RESULTS AND DISCUSSIONS

Following the analysis of the blood plasma, at the optic microscope, and the counting of the figurate elements, erythrocytes and leucocytes, the following average values have resulted and they are presented in the table below.

Table.1. Average values of haematites and leucocytes at the lots subject to testing

S. no	Treatment	Erythrocyte number	Statistical significance	Leucocyte number	Statistical significance
1.	Control	8.63 ± 0.465	1:2 P >0.05	6.05 ± 0.763	1:2 P >0.05
2.	Diabetic	9.52 ± 0.861	2:4 P >0.05	6.62 ± 1.434	2:4 P >0.05
3.	Treated with extract	9.24 ± 1.071	2:3 P >0.05	5.83 ± 0.895	2:3 P >0.05
4.	Vegetable extract	8.5 ± 0.899	1:4 P >0.05	6.42 ± 1.125	1:4 P >0.05

Following the analysis of the blood plasma, at the optic microscope, and the counting of the figurate elements, erythrocytes and leucocytes, the following average values have resulted and they are presented in the table below.
Interpreting, from a statistical point of view, the values obtained for the number of haematites and leucocytes in the blood, there were not registered significant changes with any of the lots subject to testing, the values being within the normal limits considered by Fox et al. (2007) (between 7-11x 10^6 for haematites and between 5-9 x10^3 for leucocytes). Similar results on the number of erythrocytes and leukocytes were also observed by Pribac (2012), fallowing administration by *Trigonella* in diabetic mice. Significant changes were observate by Bacalov (2014) with a tendency to normalize, for erythrocytes with 6.06 *10^{12} e/l and for leucocytes with 6,8 • 10^9 ^{1}/l fallowing administration extract of herbs (FASSC-V). Other significant changes were recorded by Pankaj et al. (2013) after administration *Spirulina platensis*, this study indicates that SP might attenuate some disturbed hematological parameters of diabetic mice (when treated with SP powder showed leucocytes count decreased from 6.91±0.06 to 6.23±0.14 thousand cells/mm3 and insignificant change in erythrocytes count with 5.88±0.22 to 5.89±0.334 million RBC per cubic millimetre).
The morphological aspect of the figurate elements in the blood after administration the two doses of 0.2 ml solution of Alloxan (130mg/kg body, dissolved in physiological saline), and after treatment with vegetable extract of aronia and milk thistle (2 ml) for two weeks, is displayed in the following pictures:
Figure 1, the blood smear is shown for the mice in the witness lot, so the dimension and the morphological aspect of the figurate elements in the blood comes within normal limits of 7 µm diameter and 2,10- 2,13 µm thickness.

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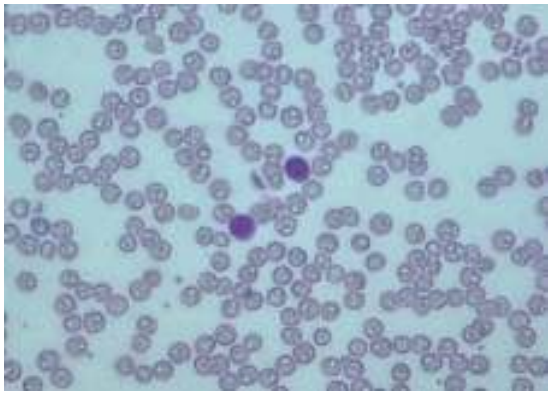

Figure 1. Morphological aspect and the dimension of the blood elements at the witness lot

By administering the toxic alloxan monohydrate, we notice the presence on the blood smear of the apoptosis micronuclei, as well as an increase of the volume of haematites and the emergence of some atypical cells, called megalocytes. The emergence of such apoptosis micronuclei is preliminary to the self-destruction of blood cells in response to the presence of the toxic alloxan (figure 2.). Similar results regarding the apoptosis of blood elements were obtained by Happi et al. (2012) in the case of injecting mice with *Trypanosoma brucei.*

In case of injecting the toxic alloxan monohydrate, the body eliminates the damaged or abnormal cells which might interfere with the good functioning of an organ or might become a malign element. The cells fragmentation takes place without the drainage of the cellular content into the extracelular space, and the removal of apoptotic cells does not determine an inflammatory response. The absence of the inflammation represents a crucial feature which allows, therefore, the death of the cells without damaging adjacent cells (Kerr et al., 1972).

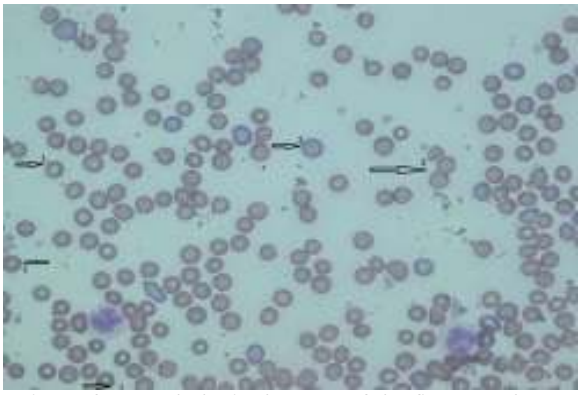

Figure 2. Morphological aspect of the figurate elements at the lot with diabetes induced by alloxan;1. Apoptosis micronuclei 2. Megalocytes

As for the aspect and the dimension of the blood plasma elements, after the weeks in which the vegetal extracts of aronia and milk thistle were administered to mice with diabetes induced by alloxan, we notice a comeback of the dimension of blood haematites, without registering the presence of apoptosis nuclei, which means that the two vegetal extracts have a beneficial effect on mice with diabetes induced by alloxan (figure 3, figure 4).

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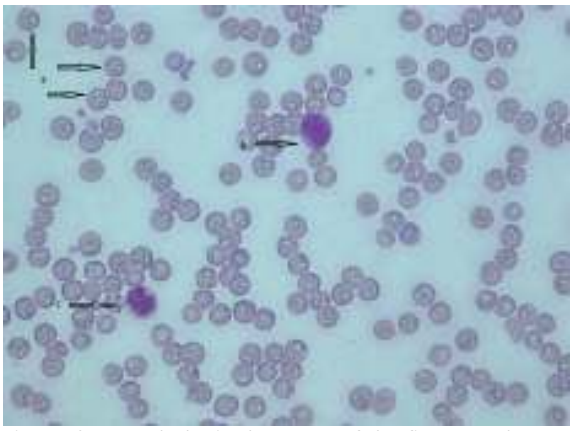

Figure 3. Morphological aspects of the figurate elements at the lot treated with aronia extract:a-erythrocytes; b- lymphocytes; c- monocyte

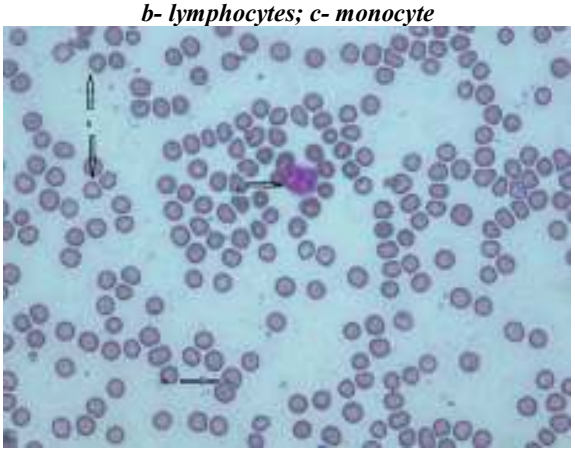

Figure 4. Morphological aspect of blood elements at the lot treated the with milk thistle extract: a- helmet cell (fragmental cell); b- teardrops cells; c- eosinophil

4. CONCLUSIONS

● The values of the haematological index obtained after administration the two doses of 0.2 ml solution of Alloxan (130mg/kg body, dissolved in physiological saline), and after treatment with vegetable extracts of aronia and milk thistle (2 ml) for two weeks, does not reveal significant changes of the number of erythrocytes and leucocytes in the blood plasma, in accordance with the statistical analysis out by ANOVA, LSD test.

● As for the morphological profile of the haematological index for the lot with induced diabetes, there can be noticed the presence of apoptosis micronuclei, as well as the increase of the volume of haematites with the apparition of some atypical cells called megalocytes, this being due to the toxic alloxan monohydrate.

● By analysing under the microscope the images for the lots of diabetic mice treated with the two vegetal extracts, we notice a comeback of the erythrocytes dimensions, as well as the lack of the aspects which belong to apoptosis.

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● As a general conclusion, we could state that the two vegetal extracts of *Aronia melanocarpa* and *Silybum marianum* have a beneficial and restructuring effect over the morphological aspect and the dimension of the figurate elements on the blood smear in the case of diabetic mice.

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