Current Trends in Natural Sciences Vol. 5, Issue 10, pp. 102-106, 2016

Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521

Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

INVESTIGATION ON THE HYPOGLYCEMIANT EFFECT OF SOME VEGETAL EXTRACTS IN EXPERIMENTAL INTOXICATIONS AT CD-1 MICE

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Abstract

Using plants in treating some diseases has become a tradition, 'the nature's pharmacy' being an important source of therapy. At present, the medicinal plants properties are being re-assessed due to progress made in chemical, pharmaceutical and clinical research of plants and due to forms obtained from vegetal products, but especially due to the advantages they offer. In the context of more frequent use of natural products with pharmaceutical and therapeutical aims, both at a national and at an international level, our research hints at highlighting and giving details about a series of effects produced by vegetal extracts of Aronia melanocarpa and Silybum marianum on certain physiological, biochemical and histopathological processes at CD1 mice. In this study, 2 hydro-alcoholic extracts obtained from 2 species of medicinal plants (Aronia melanocarpa and Silybum marianum), were tested in order to analyse the hypoglycemiant activity. The data accumulated in the specialty literature reveal that the phytotherapeutic use of extracts of Aronia melanocarpa and Silybum marianum is based only on the major pharmacologic effect while the intimate action mechanism of the two vegetal products, al cellular and subcellular level, is not known. Starting from these premises, we considered useful the initiation of a comparative study regarding the antidiabetic influence of fluid extracts of Aronia melanocarpa and Silybum marianum upon mice experimentally intoxicated with alloxan monohydrate solution. CDI mice were used as an experimental model in order to induce diabetes: alloxan monohydrate was injected intraperitoneal with concentration of 130 mg/kg body (Ahmed Saber Abu – zaiton, 2013), dissolved in physiological serum, during two weeks, at an interval of 3 days. Both extracts acted positively by lowering blood sugar and by returning to normal body weight in diabetic mice. Aronia extract has a pronounced effect compared to milk thistle extract for both parameters monitored in the experiments.

Keywords: vegetal extracts, alloxan monohydrate, concentration, diabetes, physiological index, biochemical indices.

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1. INTRODUCTION

A general look upon the results registered during the last years in the field of medicinal and aromatic plants and the analysis of the statistic data show an obvious increase of the customers' interest towards green pharmacy, while the economic units oriented towards the production of phytotherapeutic, cosmetic and nutraceutical products, report increasing turnovers each year. Market researches also confirm the fact that the population opts for natural products due to the unanimously-recognized benefits of phytotherapy: large accessibility, rare side effects, lack of dependency, the products are perfectly assimilable, they allow the association with other therapies and diets, the products have attractive prices. At present, more and more people resort to naturist treatments, diets with teas or other various products made out of plants; furthermore, cultivating medicinal plants can represent a good business opportunity.

The aim of this paper is to establish, through physiological and biochemical methods and techniques, the functional changes which appear at CD1 mice treated with vegetal extracts of aronia and milk thistle.

2. MATERIALS AND METHODS

There have been used CD1 mice acquired from Cantacuzino Institute, unit which breeds laboratory animals; in the experiment we worked with senescent animals that were over 6 months and had a medium weight of 15-25 g. In order to breed the mice, there were assured standardized zoo-hygienic conditions: light/dark interval of 12 hours, relative air humidity of 60%, constant temperature of 20 ± 2°C, balanced diet with proteins, glucides, lipids and vitamins, administered in concentrated form and water *ad libitum*. It was undertaken an acute-type experiment, which lasted for 12 days; there were followed the physiological indices (weight) and the biochemical ones (glycaemia).

Black chokeberry (Aronia melanocarpa) native to North America is called black chokeberry, aronia by common people. Aronia fruit contain the biggest quantity of antioxidants within the berries range, surpassing blueberries and cranberries. It is also known, from specialty literature, that aronia is one of the richest fruit in flavonoids: anthocyanins and proanthocyanidins responsible with the fight against free radicals. Scientific studies reveal high concentrations of polyphenols, flavonoids, anthocyanins and antioxidants in aronia fruit. Moreover, the fruit contains vitamins B2, B6, C, E, folic acid (Zoriță, 2012).

Milk thistle *(Silybum marianum*) is a plant belonging to Asteraceae family. Initially, native in South Europe and Asia, it is, nowadays, found in the entire world. The seeds (fruit) are used with medicinal aims due to their content of silymarin (liver-protector). According to some authors, in the plant there were identified saponosides; the phytomelan in the oil extracted from the plant: dodeca – 1,11 – dien – 3,5,7,9 – tetrain; the fruit contain phenolic derivatives: silymarin and other flavonoids, fumaric acid, etc (Drăgănescu, 2014) .

Extraction of active principles out of vegetal material

The simplest method with an appropriate degree of extraction of the active principles in different parts of the plants is maceration.

Aronia fruit and milk thistle seeds are weighted with the help of an analytic balance, are chopped in a grounding mill or in a chopper, are homogenized by hand and after this, it is initiated the extraction of bioactive compounds (Drăgănescu, 2014); the extractive hydro-alcoholic solution was prepared, using ethyl alcohol of 60° as solvent; during 14 days it was agitated and kept at temperatures between 5-10°C; it followed a filtration of the extract and its purification 7 days after filtration by removing ballast substances. (Păun et al., 2012).

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Experimental lots

After the acclimatization period, the mice were divided into experimental lots as follows: 1 witness lot, 1 trial lot treated with alloxan monohydrate and 4 lots for the investigation of the hypoglycemiant effect.

Lot		Intraperitoneal injected	Gavage
1.Witness- one lot (6 mice)			
2.Alloxan monohydrate- one lot		X	
3.Aronia	3.1. Aronia extract+ alloxan – one lot	X	x
	3.2.Aronia extract – one lot		x
4.Milk thistle	4.1.Milk thistle extract+ alloxan- one lot	X	x
	4.2.Milk thistle extract- one lot		x

The experimental model for inducing non insulin dependant diabetes mellitus was carried out by administrating a single dosis of 130 mg/ kg body aloxan, and, 48 hours after the administration, the glycaemia was determined with an Accutrend GCT device. Blood was collected from the tail veins by puncture (Negres et al., 2013). The animals were weighted at the beginning and also 48 hours after the administration of aloxan in order to be able to quantify the substance influence upon the body weight parameter, since the data in literature reveal the fact that aloxan decreases the body weight. Glycaemia was determined in the morning and the animals were left in the presence of food during the night. After diabetes was installed, the vegetal extract was gavaged (Popescu, 2014) and the evolution of the interest parameters continued to be followed.

The mice were selected according to glycaemia values. The lots of mice with diabetes were chosen according to the glycaemia value, respectively over 110 mg/dl.

The animals with induced diabetes treated with vegetal extracts of aronia and armurariu were followed from the following biomarkers point of view:

- Tracking the weight parameter during the entire testing period;
- determinations of glycemia.

3. RESULTS AND DISCUSSIONS

In the case of diabetes mellitus aims are, in particular, the evolution of the body weight and the blood sugar throughout the experiment.

Regarding glicemic index, it decreased in both extracts, but a strong hypoglycemic action was recorded for aronia extract by 1.07% from milk thistle extract in diabetic mice.

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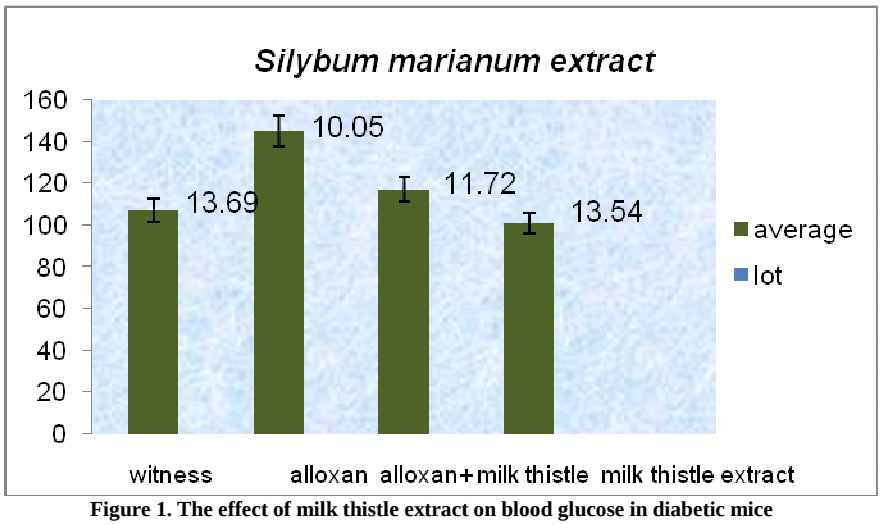

Figure 1. The effect of milk thistle extract on blood glucose in diabetic mice

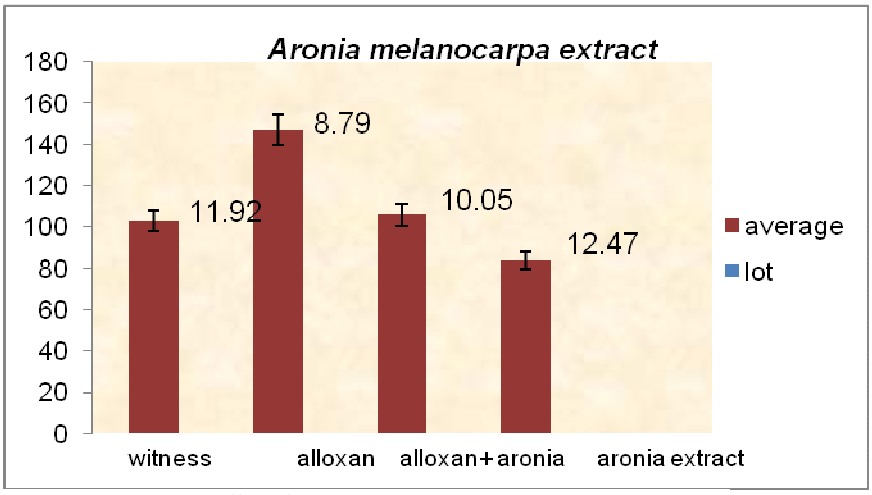

Figure 2. The effect of aronia extract on blood glucose in diabetic mice

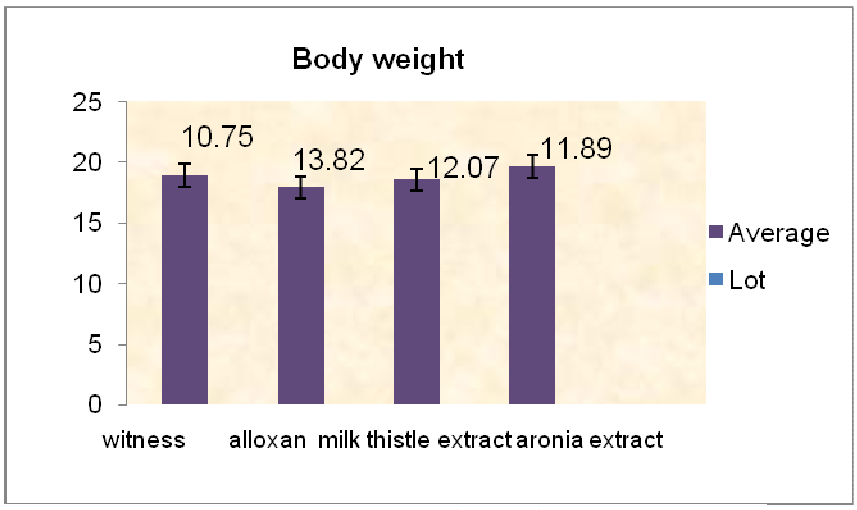

Figure 3. The body weight evolution under influence of extracts in diabetic mice

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The body weight parameter recorded oscillation of the during testing; a significant decline of this index has been observed in diabetic mice, about 1g compared to the witness and the other lots with milk thistle extract, respectively aronia extract.
There were not great differences between the two experimental models, aronia extract respectively milk thistle, but aronia juice decreased by 0.18% body weight in mice compared milk thistle extract.

5. CONCLUSIONS

1. The milk thistle extract reduce blood glucose in the diabetic mice, but not to the extent that operates the plant extract of aronia with a strongly hipoglicemiant effect.
2. Regarding the body weight, this parameter has declined to the diabetic mice. Both extracts have a beneficial effect on this physiological index.
3. Aronia extract has a pronounced effect compared to milk thistle extract for both parameters monitored in the experiments.

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