RESEARCH IN THE FIELD OF PHYTOPHARMACOLOGY

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KEYWORDS: Diabetes, Streptozotocin, Physiological serum, Polygonum persicaria, Polygonum hidropiper, Hesperis matronalis, Agrimonia pilosa, Calendula arvensis, Vaccinium myrtillus
1. Introduction

Diabetes mellitus is a metabolic syndrome known since Antiquity, today defined as a complex chronic disease with high blood glucose, hyperglycemia, which occurs through a deficiency of insulin secretion, its action or both [1]. In recent years, diabetes has become a major health problem worldwide, affecting people, regardless of their age, gender, ethnicity, race, and its prevalence has increased to alarming levels [2] that some publications speak of a real pandemic.

Although ethnopharmacology recognizes over 1200 medicinal plants [3] with hypoglycemic activity, the research for new herbal antidiabetic drugs is still attractive because the plants contain substances that prove alternative and safe therapeutic effects for diabetes [4]. Many plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc. that frequently have antidiabetic effects [5].

Starting from the complex picture of diabetes and the disastrous consequences of untreated or incorrectly treated diabetes in the doctoral thesis, we present the preclinical results of in vitro and in vivo research of extracts from some native plants in order to assess their hypoglycemic potential and antidiabetic effect.

The thesis is structured into two main parts. In the general part, representing 1/3 of the thesis, is presented the current state of knowledge in the field of diabetes and the place of phytotherapy in the management of this disease, with the assessment of the mechanisms involved both in the disease itself and in its complications.

In the part of personal research, in a preclinical experiment on a group of Swiss bee mice induced by streptozotocin diabetes mellitus, was studied the possible antidiabetic action of five pharmaceuticals (hydroalcoholic tinctures) from native plants Polygonum persicaria, Polygonum hidropiper, Hesperis matronalis, Agrimonia pilosa and Calendula Arvensis comparing a batch of control animals treated with saline and a batch of positive control (with streptozotocin diabetes treated with tincture of Vaccinium Myrtillus, a plant known to have antidiabetic effects).

2. The current state of knowledge

The current state of knowledge presented in the general part of this paper is a synthesis of the latest notions related to diabetes and plants with antidiabetic action. Thus, the concept of diabetes is defined starting from the definition and etymology to the short history and its current classification [6 - 8]. The pharmacological aspects and a short history of the development of antidiabetic medication are thereafter exposed [9]. In the epidemiological data we have assessed the incidence and prevalence of diabetes in general, the prevalence of diabetic ketoacidosis both in the diagnostic phase and in the evolution of type 1 and 2 diabetes, the prevalence of death from diabetes [10,11]. The etiological factors and risk factors for prediabetes and type 2 diabetes are presented. Genetic factors, autophagy dysfunction in diabetes, environmental factors are detailed. The pathophysiology of diabetes includes the mechanism of decreased insulin secretion, the induction mechanism of insulin resistance [12 - 14]. The natural evolution of diabetes and its prognosis ends the chapter of "Etiology and pathophysiology" [15].

The chapter "Diagnosis of diabetes" includes recommendations for the diagnosis of type 1 and 2 diabetes, prediabetic status, criteria for diagnosing type 2 diabetes in children and teenagers, monogenic diagnosis, diagnosis of metabolic syndrome [16 - 18].

The chapter "Complications of diabetes" presents their classification, the mechanisms of nephropathy, neuropathy, retinopathy, cardiovascular disease,
complications in the evolution of diabetes [19 - 23]. In "Involvement of oxidative stress in diabetes" are mentioned some plants with hypoglycemic, antidiabetic effects [24].

A significant chapter of the general part is "Phytotherapy in the management of diabetes" which focuses on the mechanisms by which various preparations of plants with antidiabetic effects have beneficial actions [25]:

- Phytotherapy in glycemic control of diabetes [26];
- Structural constituents from antidiabetic medicinal plants - pharmacological actions [27 - 29];
- Phytotherapy - role and mechanism in protection / regeneration of beta-pancreatic cells [30.31];
- Medicinal plants - the mechanism of action on oxidative stress in diabetes [32,33];
- Antidiabetic plants - mechanism of inhibition of digestion and absorption of carbohydrates [34,35];
- Antidiabetic plants - mechanism of up-regulation of glucose transporters and increase of glucose uptake [36,37];
- Antidiabetic plants - mechanism of activation of the nuclear factor PPARγ [38,39];
- Antidetict plants - insulinomimetic and insulinotropic effect [40];
- Medicinal plants - incretinomimetic and blocking effect of DPP-4 [41];
- Medicinal plants - inhibition or activation of enzymes involved in carbohydrate metabolism [42];
- Antidiabetic plants - increased adiponectin release [43];
- Antidiabetic plants - the mechanism of the protective effect on the evolution of chronic complications in diabetes [44 - 47].

3. Personal research

3.1. Research motivation

The preclinical research is reasoned by the possible antidiabetic action of five pharmaceuticals (hydroalcoholic tinctures) from native plants Polygonum persicaria, Polygonum hidropiper, Hesperis matronalis, Agrimonia pilosa and Calendula Arvensis [48].

3.2. Materials and general methodology

In order to obtain a physical-chemical characterization of the products from the plants studied, the vegetal material was obtained from species from “Alexandru Buia” University Botanical Garden from Craiova. The control samples, collected during various periods, between 2015–2018, are stored in the Collection of the Pharmacognosy Laboratory of the Faculty of Pharmacy, U.M.F Craiova.

In the preclinical experiment, in vitro research was performed on obtaining a physical-chemical characterization of tinctures from plant products Agrimonia pilosa herba (APH), Calendula arvensis herba (CAH), Hesperis matronalis herba (HMH) Polygonum hydropiper herba (PHH), Polygonum persicaria herba (PPH).

50 white mice were used to determine the acute and subacute toxicity of tinctures from APH, CAH, HMH, PHH, PPH. Under acute toxicity, water and food consumption, body weight were monitored. At the end of the subacute toxicity study, the animals were sacrificed and prepared for histopathological studies of the heart, liver, lung, kidney and pancreas.
The in vivo preclinical experiment was performed on a group of 90 white Swiss bee mice, male, from the Biobase of the University of Medicine and Pharmacy Craiova. For the study of the effect of tinctures from the respective plants in the experimental diabetes mellitus, the average 6-week-old male mice were divided into 8 sublots of 5 animals each, of which one sublot taken as witness or control was treated with saline 0, 9% (physiological serum), and in the other 7 sublots type 1 streptozotocin diabetes was induced by administering a single dose of the substance 180 mg / kgc, intraperitoneally, not exceeding 1 ml / kgc as a solution. The 2nd sublot with streptozotocin diabetes remained untreated, the 3rd sublot, control or positive control, was treated daily by gavage for 35 days with hydroalcoholic extract from the leaves of Vaccinium myrtillus, a plant known to have hypoglycemic, antidiabetic effects; the other 5 sublots were treated daily orally with effective doses of hydroalcoholic extracts (tinctures) of the plants studied: APH, CAH, HMH, PHH, PPH.

The animals were kept under standard conditions, they received standard food, fodder from the National Institute for Medical-Military Research Cantacuzino, Băneasa Resort, Bucharest.

The animals had an acclimatization period of 7 days. The experiment lasted 35 days (7 weeks).

The body weight was monitored on the empty stomach in the morning, between 9 am and 10 am, initially every 3 days, then 7 days and then once a week until the end of the experiment.

The consumption of liquids and food was monitored by measuring this consumption for each of the 8 groups of animals during a week, establishing the weekly average consumption per animal.

In order to determine the effect of the 6 herbal tinctures on blood glucose, total cholesterol and triglycerides in streptozotocin diabetes, these biochemical parameters were measured by using the GCT ACCUTREND Analyzer after collecting a drop of blood from the animal's tail. The determination of these parameters was performed during the morning between 9 and 10 at the beginning of the study, at 3 days, 7 days and then every 7 days up to 35 days.

In order to determine the action of the 5 tinctures in oxidative stress in streptozotocin diabetes, their effects were compared at the end of the study on MDA, SOD, GPx, CAT in the study groups compared to the group with untreated diabetes, and the group without diabetes. The dosing of the oxidative stress parameters was performed in the Biochemistry laboratory of the Faculty of Pharmacy UMF Craiova.

Also, at the end of the study, the action of the 6 tinctures on renal function in chronic experimental diabetes was determined by dosing creatinine and serum albumin.

The animals were sacrificed after the 35 days of study according to the animal protection rules, collecting histopathological samples from the pancreas, lung, liver, kidneys, spleen. The histopathological analysis was performed in the histology laboratory of the Faculty of Medicine, UMF Craiova.

The experiment was carried out in accordance with the rules of the Commission for Animal Welfare and the opinion of the University and Scientific Ethics and Deontology Commission of the University of Medicine and Pharmacy Craiova (09/28.03.2018). In conducting the research, the general rules of conduct regarding ethical issues and needs in research were observed, observing the ethical principles underlying the Helsinki Declaration as well as the principles at institutional level (UMF Craiova University Code of Ethics) and national (Code of Medical Deontology from 06.06.1997; Law no.319 / 08.06.2003; Law no.206 / 27.05.2004). The following were also observed: Council Directive no. 86/609 / EEC
on the protection of laboratory animals used for experimental or other scientific purposes; Directive 2010/63 / EU amending Directive 86/609 / EEC on the protection of animals The Directive is firmly based on the principle of the three Rs (replace, reduce and refine the use of animals used for scientific purposes).

3.3. Statistic analysis

The statistic analysis was performed by using a dedicated program - IBM SPSS verse 23.

For the descriptive analysis of the batches, the mean, minimum and maximum value, standard deviation were used.

In order to compare the data, the Z score with a specificity threshold of 95% (p <0.05) was used, and this was calculated by the following nonparametric tests: the Mann-Whitney U test when independent data series were compared (e.g. blood glucose of group 1 compared to blood glucose of group 2) and the Wilcoxon test for the situation where comparisons were made between measurements from the same subjects (e.g: blood glucose day 1 compared to blood sugar day 7). In the study of fluid and food consumption, the Anova test was used to highlight differences between groups and for multiple comparisons between groups.

4. RESULTS

4.1. Research on obtaining and physical-chemical characterization of tinctures from vegetable products with hypoglycemic properties

Tinctures of the plants studied: APH, CAH, HMH, PHH, PPH, VMF. were obtained by simple percolation and then characterized from the physical-chemical point of view, according to F.R. X.

![Figure 1 – Chromatogram of polyphenols in tinctures (UV λ 254 nm, without derivatization). From left to right: first band, without application (free zone / background - B); the following three applications (2 μl) - APH, CAH and HMH tinctures; bands 5–8, four applications for reference substances (2 μl) - caffeic acid (Caf.), chlorogenic acid (Chl.), evercetol (Que.) and rutoside (Rut.); bands 9–11, respectively the last three applications (2 μl) - PHH, PPH and VMF tinctures. APH: Agrimonieae pilosae herba; CAH: Calendulae arvensis herba; HMH: Hesperis matronalis herba; PHH: Polygoni hydropiperis herba; PPH: Polygoni persicariae herba; VMF: Myrtilli folium.](image)
From the analysis of the experimental results, it is found that all tinctures contain flavonoids and polyphenolcarboxylic acids, certain components being identified by characteristic bands:

- in the analysis of flavonoids:
  - only rutoside was identified in the PPH tincture (549 μg / mL);
  - the other tinctures also contain flavonoids, but the lack of standards did not allow the accurate identification of these components.

- for the analysis of polyphenolcarboxylic acids:
  - chlorogenic acid was identified in VMF tinctures (2152 μg / mL) > APH (785 μg / mL) > PHH (512.5 μg / mL) > PPH (359.5 μg / mL) > CAH (276 μg / mL);
  - caffeic acid was identified in VMF tinctures (539 μg / mL) > HMH (326 μg / mL) > PHH (160 μg / mL) > PPH (167 μg / mL);

Table 1 summarizes the results on the in vitro antioxidant activity of the six tinctures, previously diluted 1: 5 with distilled water, in terms of the total content of polyphenols and flavonoids, respectively.

**Table 1 – In vitro antioxidant activity of hypoglycemic tinctures**

<table>
<thead>
<tr>
<th>Tincture (diluted 1: 5 with distilled water)</th>
<th>In vitro antioxidant activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total content of polyphenols [mg/l EAG]</td>
</tr>
<tr>
<td>Agrimonieae pilosae herba (APH)</td>
<td>280.58±5.57</td>
</tr>
<tr>
<td>Calendulae arvensis herba (CAH)</td>
<td>97.05±1.94</td>
</tr>
<tr>
<td>Hesperis matronalis herba (HMH)</td>
<td>237.72±4.71</td>
</tr>
<tr>
<td>Polygoni hydropiperis herba (PHH)</td>
<td>328.35±6.08</td>
</tr>
<tr>
<td>Polygoni persicariae herba (PPH)</td>
<td>269.28±5.25</td>
</tr>
<tr>
<td>Myrtilli folium (VMF)</td>
<td>433.89±8.67</td>
</tr>
</tbody>
</table>

EAG: gallic acid equivalents; EQ: cvercetol equivalents.

The total content of polyphenols [mg / 1 EAG] of the tinctures is directly correlated with the level of antioxidant activity in vitro, as it follows: VMF (433.89 ±
8.67) > PHH (328.35 ± 6.08) > APH (280.58 ± 5.57) > PPH (269.28 ± 5.25) > HMH (237.72 ± 4.71) > CAH (97.05 ± 1.94).

The same type of correlation is observed for the total flavonoid content [mg / l EQ]: VMF (154.38 ± 3.08) > PHH (151.65 ± 3.03) > APH (138.29 ± 2.76) > PPH (132.75 ± 2.65) > HMH (129.34 ± 2.58) > CAH (113.52 ± 2.27).

The results of the chemical analysis justify the need for pharmacodynamic and biochemical research in order to confirm the therapeutic value of the tinctures, in terms of hypoglycemic and antioxidant capacity.

**Partial conclusions**

1. Obtaining and physical-chemical characterization of tinctures from products with hypoglycemic properties takes into account the plant species studied, their medicinal uses and, in some cases, the cumulative toxicity during long-term administration.

2. The tinctures were obtained by simple percolation, according to F.R. X and then characterized from the physical-chemical point of view (color, taste, odor, relative density, refractive index, quality conditions - iron content, heavy metals, evaporation residue, alcohol concentration).

3. Qualitative and quantitative analysis of the tinctures was performed by CSS, for flavonosides (rutoside) and polyphenolcarboxylic acids (caffeic acid, chlorogenic acid). The separation and identification of the active components was performed on the basis of appropriate reference substances (standards). Quantitative analysis was also performed through the spectrophotometric method for phenylpropane compounds.

4. The in vitro antioxidant capacity of the tinctures was highlighted by the analysis of the total polyphenolic and the total flavonoid. The highest polyphenol content is included in the VMF tincture, followed, in order, by the tinctures PHH, APH, PPH, HMH and CAH.

5. Due to the content of flavonoids and polyphenolcarboxylic acids, which give the plant products hypoglycemic and antioxidant properties, tinctures could be recommended as natural sources of polyphenols with an adjuvant role in the prophylaxis and treatment of diseases caused by the presence of reactive oxygen species such as diabetes mellitus.

4.2. Acute and subacute toxicity testing in mice for tinctures in the plants studied.

4.2.1. Determination of the acute toxicity on mice

1. The animals used were male Swiss bee mice, 6-8 weeks old, with a weight that did not differ between subjects by more than +/- 20 percent (20-30 g).

2. In the acute toxicity testing experiment, we performed 5 batches of 10 male mice each. Each batch was subdivided into 5 sublots with 2 animals each which we administered by gavage, a single dose of the tinctures studied (1, 2, 3, 4, 5 g / kg body weight). We also had a control group who was given saline by gavage. In mice, the dose used was administered orally by gavage with a maximum volume of 50 ml / kg body weight. We monitored the parameters that followed the phenomenon of death (convulsions, apnea, coma) and change in behavior (gait, eyelid position, sedation or agitation, excessive salivation, sleep duration, appearance of fur and mucous membranes, tremor of extremities, photophobia,
frequency of respiratory movements) within the next 24 hours. We then used most of the mice from the 5 batches in each batch, keeping 8 mice from the 10. We excluded mice that received 5g / kg of tincture 72 hours ago. We divided each batch of 8 mice into sublots of 2 animals which were given doses of 6g, 7g, 8g and 9g / kg of tincture by gavage. On the 4th day after the first experiment, the animals were kept on the position from hour 20:00 until the next day. Between 9 and 10 o'clock the animals were weighed, a maneuver that was repeated after 24 hours. Blood glucose was collected before the tincture administration and after 24 hours.

3. Following the doses used for the tinctures studied for acute toxicity, we consider that the 5 tinctures fall into the category 5 of acute toxicity according to the United Nations Globally Harmonized Classification and Labeling of Chemicals 2011, practically do not show acute toxicity under therapeutic doses.

4.2.2. Determination of subacute toxicity for the tinctures of the plants studies

4. We made 6 batches of 4 male Swiss bee mice, weighing between 20 and 30 g, aged 4-6 weeks, 1 batch for each tincture and one batch that received saline as the control batch. The administration was performed by gavage; 400 mg dose for each tincture was given once a day, between 9 am and 10 am, for 14 days. After the tincture administration, the animals were monitored daily during the study for possible toxic or lethal effects. Food and water consumption, animal weight, mortality, signs of general toxicity were assessed daily. After 14 days the animals were slaughtered according to the animal protection regulations. Histopathological determinations were then made on the liver, lung, kidney, heart, pancreas, spleen.

5. Partial conclusions

1. According to the parameters followed, Hesperis matronalis and Calendula arvensis at a dose of 400 mg / kg / day tincture did not show subacute toxicity.

2. Polygonum hydropiper tincture has a moderate hepatotoxic action, without influencing the consumption of food, water or body weight. Histopathological examination revealed hepatocyte damage, with hepatotoxic lesions, without fibrosis or inflammatory phenomena.

3. Polygonum persicaria tincture has a high hepatotoxicity that did not influence the consumption of water, food and average body weight. Histopathological lesions are emphasized on the liver, with high granular-vacuolar degeneration, phenomena of hepatocellular cytolysis.

4. Agrimonia pilosa tincture showed hepatotoxic and renal histopathological lesions, without affecting the water consumption, with low food consumption compared to the statistically significant control during the 14 days. The pancreas showed a structural disorganization. The mean body weight was insignificantly altered from examination during this time, except on days 3, 7, 11, and 12 when the average weight of the mice of the group was lower.
4.2.3. Determination of the effective dose for the tinctures studied by using the oral glucose tolerance test in mice with normal pancreatic function.

Tinctures decrease significantly (p <0.05) blood glucose in mice with gavage-induced hyperglycemia by gavage at subsequent doses:

- *Polygonum persicaria* at 200 mg/kgc after 90 minutes and 120 minutes from the glucose administration;
- *Polygonum hydropiper* at 100 mg/kgc and 150 mg/kg after 90 minutes;
- *Hesperis matronalis* at 200 mg/kgc after 90 minutes and 120 minutes
- *Calendula arvensis* at 150 mg/kgc after 120 minutes and 200 mg/kgc after 90 minutes
- *Agrimonia pilosa* at 200 mg/kgc after 30 minutes, 60 minutes, 90 minutes, 120 minutes
- *Vaccinium myrtillus* at 100 mg/kg and 150 mg/kgc after 120 minutes

The doses subsequently used by gavage in the chronic experiment with tinctures from the plants studies resulting from this study are for:

- *Polygonum persicaria* 200 mg/kgc
- *Polygonum hydropiper* 100 mg/kgc
- *Hesperis matronalis* 200 mg/kgc
- *Calendula arvensis* 150 mg/kgc
- *Agrimonia pilosa* 200 mg/kgc
- *Vaccinium myrtillus* 100 mg/kgc

4.3. Evolution of blood glucose in groups with streptozotocin-induced diabetes in mice treated with pharmaceutical preparations from the studied plants

1. The study was performed on a group of 40 male Swiss bee mice, with an average age of 4-6 weeks, male, divided into 8 sublots of 5 mice each, of which one sublot was taken as witness or control and treated with saline. 0.9% (saline), in the other 7 subgroups, was induced streptozotocin type 1 diabetes by administering a single dose of 180 mg / kg intraperitoneally. The division of the other 7 lots is presented in the chapter Materials and method.

2. Streptozotocin at a dose of 180 mg / kg administered i.p. on a batch of 5 Swiss male bees of the same age, male, caused hyperglycaemia from the 3rd day after substance administration at an average of 335 ± 27.98 mg / dl which was maintained and even increased to the average value of 358.60 ± 27.44 mg / dl on the 35th study day. The difference between the blood glucose value at the onset of diabetes and the mean blood glucose value at the end of the study is statistically significant (p <0.01) which shows us that the diabetes induced did not correct over time, but even worsened. Mean baseline blood glucose values before streptozotocin administration were 92.40 ± 10.36 mg / dl.

3. Partial conclusions

1. All plants studied showed an antihyperglycemic effect.
2. The antihyperglycemic effect was close to that obtained with Vaccinum Myrtillus (approximately 50%) and increased in the order of Hesperis matronalis <Polygonum hidropiper <Agrimonia pilosa.

4. However, 2 mice died from the Agrimonia pilosa group, which meant that the difference in blood glucose at the end of the study compared to day 7 (considered full onset of streptozotocin diabetes) was not statistically significant.

5. Polygonum persicaria and Calendula arvensis showed a lower antihyperglycemic effect (the average blood glucose value being 72.22%, respectively 73.91% of the initial value after diabetes induction).

6. Compared to the control group treated with saline 0.9% after administration of the plant extract all blood glucose values were significantly higher during the study in Vaccinum Myrtillus, Polygonum persicaria, except for day 35 in Polygonum hidropiper, Hesperis matronalis, Calendula arvensis.

7. Between days 3 and 7 after the induction of streptozotocin diabetes, there are 3 situations: a moderate decrease in blood glucose on day 7 compared to day 3 in Vaccinum myrtillus and Polygonum hydropiper, a stationary blood glucose value in Agrimonia pilosa and an increase in blood sugar in Polygonum persicaria, Hesperis matronalis and Calendula arvensis.

8. The order of increase in the concentration of polyphenols is as it follows: Calendula arvensis herba <Hesperis matronalis herba <Polygonum persicaria herba <Agrimonia pilosa herba <Polygonum hydropiper herba <Vaccinium myrtillus herba. The results are corroborated with the hypoglycemic effects of the studied tinctures.

9. The highest polyphenol content is found in plants with the strongest hypoglycemic effect Agrimoina pilosa, Polygonum hydropiper. A lower hypoglycemic effect was found in Polygonum persicaria and Calendula arvensis which have the lowest amount of polyphenols.

10. In Hesperis matronalis there is a discrepancy between the hypoglycaemic effect close to Polygonum hidropiper and the polyphenol content close to Polygonum persicaria.

11. Polygonum hydropiper and Agrimonia pilosa herba have a higher flavonoid content, close to that of Myrtilli folium corroborated with a high hypoglycemic effect.

12. Compared to the blood glucose value on day 7 after the induction of streptozotocin diabetes, the decrease in statistically significant blood glucose occurred from day 14 in Vaccinum Myrtillus, from day 28 in Hesperis matronalis and Agrimonia pilosa, on day 35 in Polygonum persicaria and Polygonum hidropiper, and in Calendula arvensis, the decrease in blood glucose was not statistically significant throughout the study.

13. The antihyperglicemic effect at the end of the study was more intense in Hesperis matronalis, Polygonum hidropiper, Agrimonia pilosa and settled faster in Hesperis matronalis and Agrimonia pilosa.

14. Treatment with extracts from the plants studies fails to determine an absolute control of the hyperglycemia of streptozotocin-induced diabetes.
4.4. The action of pharmaceutical preparations in the plants studied over the body mass, food and fluid intake in mouse streptozotocin diabetes

Body mass, food and fluid intake were analyzed during the 35 days of the study in the 8 groups of mice studied.

Partial conclusions
1. Compared to the control, streptozotocin diabetes caused a statistically significant decrease in the weight of the animals during the experiment.
2. Hydroalcoholic plant extracts partially antagonized the weight loss effect of the animals in the group with untreated diabetes except for Polygonum persicaria extract.
3. The extracts from Calendula arvensis and Polygonum persicaria showed a weight / mouse deficit compared to the group with untreated streptozotocin diabetes by 0.70% and 11.79% higher, respectively.
4. The effect of weight gain on the administration of plant extracts was manifested from 7 days, except for Agrimonia pilosa where the growth started after 3 days.
5. The antagonizing action of the weight loss effect generated by streptozotocin diabetes increased in the order of Hesperis matronalis < Agrimonia pilosa < Polygonum hydropiper < Vaccinium myrtillus.
6. Treatment with Polygonum hydropiper has caused the strongest improvement in the body weight, almost identical to that of Vaccinium myrtillus.
7. Compared to the food consumption in the control group that has increased physiologically once with increasing in age, food consumption by mice in the group with streptozotocin diabetes was significantly lower, while fluid consumption was significantly higher.
8. Food consumption was significantly lower than the control group and decreased in the order of Hesperis matronalis > Polygonum persicaria > Calendula Arvensis > Vaccinium Myrtillus > Polygonum hidropiper > Agrimonia pilosa.
9. Fluid intake was significantly higher than the control group and increased in the order of Vaccinium Myrtillus > Calendula Arvensis Polygonum hydropiper > Hesperis matronalis > Polygonum persicaria > Agrimonia pilosa.
10. Compared to food consumption in the group with streptozotocin diabetes, food consumption was insignificantly higher in the groups treated with hydroalcoholic solutions from plants, consumption which increased in the order of Hesperis matronalis < Calendula Arvensis < Polygonum persicaria < Vaccinium Myrtillus < Polygonum hidg < Agrimonia pilosa.
11. Compared to the fluid intake in the group with streptozocinic diabetes, fluid intake was insignificantly lower in the groups treated with hydroalcoholic solutions from plants and increased in the order of Vaccinium Myrtillus < Calendula Arvensis < Polygonum hydropiper < Hesperis matronalis < Polygonum persicaria.
12. Is there a positive correlation between food intake and weight gain in batches of mice?
1. There is a positive correlation between the decreased fluid intake and weight gain for Vaccinium myrtillus, but not for Calendula arvensis. The correlation is lower in case of Polygonum hydropiper and especially Hesperis matronalis.

4.5. The action of pharmaceutical preparations in the plants studied over the serum lipid profile in the streptozotocin diabetes on mice

1. Partial conclusions

1. Total cholesterol has increased by 13.01% in the group with untreated streptozotocin diabetes compared to the control group during the 35 days of the experiment, a statistically significant difference.

2. Compared to the hypocholesterolemic action of Vaccinium myrtillus, the hydroalcoholic extracts from the 5 plants studied have lower effects, and Agrimonia pilosa and Polygonum persicaria have no hypocholesterolemic effect.

3. Calendula arvensis decreases total cholesterol by 40.88% of its value in the group with untreated diabetes, Polygonum hydropiper by 35.58%, and Hesperis matronalis by 27.09%.

4. Serum triglycerides increased 35 days after the induction of streptozotocin diabetes by 41.30% compared to the value of triglycerides in the control group (141.60% versus 100.30%).

5. The hydroalcoholic preparations from Polygonum persicaria and especially from Agrimonia pilosa had no triglyceride lowering effect in the experiment.

6. The hydroalcoholic preparation of Polygonum hydropiper lowered the triglycerides in the group with streptozotocin diabetes in an insignificant manner to close values, but lower than the standard preparation of Vaccinum myrtillus.

7. The hydroalcoholic preparations of Hesperis matronalis and especially Calendula arvensis decreased the streptozotocin hypertriglyceridemia more intensely than the preparation of Vaccinum myrtillus.

8. The hydro-alcoholic preparations of Polygonum hydropiper, Hesperis matronalis and especially Calendula arvensis regulated the lipid metabolism in streptozotocin diabetes in mice.

4.6. Analysis of the antioxidant capacity of the plants studied in experimental chronic diabetes

At the end of the treatment the mice were placed under anesthesia with sodium pentota 60 mg / kg i.p.; The heart blood was rapidly collected in sertas with EDTA, which was processed to obtain blood products for analysis of markers specific to the determination of oxidative stress. The serum prepared and the plasma samples were stored in a freezer at -20 °C until analysis.

The determination of Malondialdehyde (MDA), Superoxidismutase (SOD), Glutathione peroxidase (GPx), Total antioxidant capacity (TAC) was performed in the biochemistry laboratory of the Faculty of Pharmacy Craiova according to the methodology set out in detail in the thesis.
Partial conclusions
1. All the plants studied have proved oxidative anti-stress effects, but for the most part the values are statistically insignificant.
2. The prooxidant activity of Malondialdehyde decreases in the order Streptozotocin > Vaccinium myrtillus > Agrimonia pilosa > Calendula arvensis > Control Saline > Polygonum persicaria > Hesperis matronalis.
3. Polygonum hydropiper increases the activity of malondialdehyde insignificantly. All the 4 other plants have inhibited the activity of malondialdehyde more strongly than the known antidiabetic plant Vaccinium myrtillus, but in an insignificant manner compared to the group with untreated streptozotocin diabetes and the control group.
4. The antioxidant value of superoxide dismutase increased in the order of Streptozotocin < Agrimonia pilosa < Hesperis matronalis < Calendula arvensis < Polygonium persicaria < Physiological control < Vaccinium mirtillus < Polygonum Hydropiper, statistically insignificant, except for significant batch of Polygonum hydropiper untreated and control group.
5. The value of the antioxidant activity of glutathione peroxidase increases statistically significantly in the order Streptozotocin < Vaccinium mirtillus < Polygonum hydropiper < Hesperis matronalis < Calendula arvensis < Polygonium persicaria < Agrimonia pilosa. The increase is manifested compared to the group with untreated diabetes, and for Agrimonia pilosa the increase is also significant for the control group.
6. We highlight the higher glutathione peroxidase activity increases for the 5 plants compared to Vaccinium myrtillus.
7. In case of measuring the total antioxidant capacity, the antioxidant effect increased in the order streptozotocin < Calendula arvensis < Polygonium persicaria < Polygonum Hydropiper < Vaccinium mirtillus < Agrimonia pilosa < Control saline < Hesperis matronalis.
8. For Polygonum hydropiper there is a positive correlation between antioxidant activity, high concentration in polyphenols, flavonoids and less in polyphenolcarboxylic acids (chlorogenic acid and caffeic acid) and a significant antihyperglycemic action.
9. For Calendula arvensis, there is a correlation between low hypoglycemic capacity, the lowest concentration of polyphenols and flavonoids and a decreased antioxidant activity, statistically insignificant.
10. The hypoglycemic effect of Hesperis matronalis is positively correlated with the antioxidant action and phytochemical composition of Hesperis matronalis tincture. There was the largest decrease in MDA activity and the highest increase in CAT compared to other plants and compared to the positive control. It has the highest amount of caffeic acid.

4.7. Analysis of biochemical parameters related to renal function in streptozotocin diabetes treated with hydroalcoholic extracts from the plants studied.
Hypoalbuminemia is considered a strong predictor of death in renal failure. In case of streptozotocin diabetes in mice or rats, plasma albumin levels decrease
significantly with an increase in albumin levels in the urine, proving that albuminuria is associated with impaired renal function [49].

Increased creatinine and serum urea with decreased creatinine excretion in the urine are the indicators of the development of diabetic nephropathy. [49]

At the end of the study, serum creatinine was measured by the kinetic colorimetric method, as well as the serum albumin by the colorimetric method.

Partial conclusions
1. The kidneys function is affected by chronic streptozotocin diabetes, an effect proved by the increased serum creatinine and decreased serum albumin.
2. Serum albumin was statistically significantly lower in the group with streptozotocin diabetes compared to the control group.
3. The value of serum albumin increased in the diabetic groups treated with plant extracts in the order Calendula arvensis < Polygonum persicaria < Polygonum hydropiper < Vaccinium myrtillus < Agrimonia pilosa < Hesperis matronalis.
4. The effect of decreasing creatinine by treating plant extracts of DMD was emphasized in the order: Hesperis matronalis > Polygonum hydropiper = Agrimonia pilosa > Vaccinium myrtillus > Calendula arvensis.
5. The hydroalcoholic extracts from the plants studied have showed an improvement in the kidneys function both by increasing serum albumin, especially for Hesperis matronalis, and by decreasing serum creatinine especially for Calendula arvensis.

4.8. Histopathological assessment of biological tissues taken from mice with induced diabetes after five weeks of treatment with the tinctures studied.
1. The sampling method, the preparation procedure and the highlighting of histopathological changes under the light microscope are set out in the thesis on the toxicity of the five herbal tinctures compared to the
preparations obtained from the control group (subchapter subacute toxicity of the tinctures). analyzed under an optical microscope - histological preparations from the pancreas, liver, heart, kidneys, spleen. Partial conclusions
1. The histopathological aspect at the pancreas level in the group with streptozotocin diabetes not treated with tinctures confirms the induction of the disease by this compound.
2. The group of mice without diabetes treated with saline (control group) did not show histological lesions in the biological samples collected.
3. The positive control group, treated with Myrtilli folium tincture, showed a granular hepatic degeneration, which was actually found in the other tinctures.
4. The tincture of Agrimonia pilosa showed only a granular-vacuolar degeneration, slightly more emphasized than the tincture of Myrtilli folium.
5. In the tinctures of Hesperis matronalis and Polygonum hydropiper, in addition to hepatic granular / vacuolar degeneration, myocardial interfascicular edema was also encountered.
6. Calendula arvensis tincture caused histological lesions in the liver, with granular-vacuolar degeneration of both hepatocytes and red blood cells.
7. The most emphasized histopathological toxicity was found in the treatment with Polygonum persicaria where histopathological changes were found in the liver, myocardium, kidney, spleen.

5. FINAL CONCLUSIONS

1. Obtaining and the physical-chemical characterization of tinctures from products with hypoglycemic properties takes into account the native plant species studied Polygonum persicaria, Polygonum hydropiper, Hesperis matronalis, Calendula arvensis, Agrimonia pilosa and Vaccinium myrtillus (their positive control), their uses and, in some cases, the cumulative long-term toxicity.
2. The in vitro antioxidant capacity of the tinctures was highlighted by the analysis of the polyphenolic total and the flavonoid total. The highest polyphenol content is found in the VMF tincture, followed, in order, by the tinctures PHH, APH, PPH, HMH and CAH.
3. After the analysis of acute toxicity, we consider that the 5 tinctures fall into category 5 of acute toxicity according to the United Nations Globally harmonized classification of chemicals and labeling of chemicals 2011, practically do not show acute toxicity at therapeutic doses.
4. Under the subacute toxicity according to the parameters followed, Hesperis matronalis and Calendula arvensis at a dose of 400 mg / kgc / day the tincture did not show any subacute toxicity. Precautions should
be taken when clinically using tinctures of Polygonum persicaria, Agrimonia pilosa and less tincture of Polygonum hydropiper.

5. The DE50 established for the chronic experiment are:
   Polygonum persicaria 200 mg / kgc
   Polygonum hydropiper 100 mg / kgc
   Hesperis matronalis 200 mg / kgc
   Calendula arvensis 150 mg / kgc
   Agrimonia pilosa 200 mg / kgc
   Vaccinium myrtillus 100 mg / kgc

6. All the plants studied showed an antihyperglycemic effect.

7. The antihyperglycemic effect was close to that obtained with Vaccinium Myrtillus (decrease of about 50%) and increased in the order of Hesperis matronalis <Polygonum hydropiper <Agrimonia pilosa.

8. The highest polyphenol content was recorded in the plants with the strongest hypoglycemic effect Agrimoina pilosa, Polygonum hydropiper. A lower hypoglycemic effect was found in Polygonum persicaria and Calendula arvensis which had the lowest amount of polyphenols.

8. Polygonum hydropiper and Agrimonia pilosa herba had a higher flavonoid content, close to that of Myrtilli folium, corroborated with the high hypoglycemic effect.

9. The antihypergicemic effect at the end of the study was more intense in Hesperis matronalis, Polygonum hidropiper, Agrimonia pilosa and settled faster in Hesperis matronalis and Agrimonia pilosa.

10. The treatment with extracts from the plants studied fails to determine an absolute control over the hyperglycemia of streptozotocin-induced diabetes.

11. The antagonizing action of the weight loss effect generated by streptozotocin diabetes increased in the order of Hesperis matronalis <Agrimonia pilosa <Polygonum hydropiper <Vaccinium myrtillus.

12. Compared to the hypocholesterolemic action of Vaccinium myrtillus, the hydroalcoholic extracts from the 5 plants studied have lower effects, and Agrimonia pilosa and Polygonum persicaria have no hypocholesterolemic effect.

13. The hydroalcoholic preparations of Hesperis matronalis and especially Calendula arvensis (at a statistically significant value) decreased the streptozotocin hypertriglyceridemia more intensely than the preparation of Vaccinium myrtillus.

14. At the end of the experiment all the plants studied have proved oxidative anti-stress effects, but for the most part the values were insignificant.

15. The prooxidant activity of Malondialdehyde decreased in the order Streptozotocin> Vaccinium myrtillus> Agrimonia pilosa> Calendula arvensis> Control Saline> Polygonum persicaria> Hesperis matronalis.
16. The antioxidant value of Superoxidismutase increased in the order of Streptozotocin <Agrimonia pilosa <Hesperis matronalis <Calendula arvensis <Poligonium persicaria <Physiological control <Vaccinum mirtilus <Poligonum Hydropiper, statistically insignificant, except for significant polyp and the control group).

17. The value of the antioxidant activity of glutathione peroxidase increased statistically significantly in the order of Streptozotocin <Vaccinum mirtilus <Physiological control <Poligonum hydropiper <Hesperis matronalis <Calendula arvensis <Poligonium persicaria <Agrimonia pilosa. The increase is manifested compared to the group with untreated diabetes, and for Agrimonia pilosa the increase is also significant for the control group.

18. We highlighted the higher glutathione peroxidase activity increases for the 5 plants compared to Vaccinium myrtillus.

19. In case of measuring the total antioxidant capacity, the antioxidant effect increased in the order streptozotocin <Calendula arvensis <Poligonium persicaria <Poligonum Hydropiper <Vaccinum mirtilus <Agrimonia pilosa <Control saline <Hesperis matronalis.

20. For Polygonum hydropiper there is a positive correlation between the antioxidant activity, high concentration in polyphenols, flavonoids and less in polyphenolcarboxylic acids (chlorogenic acid and caffeic acid) and the significant antihyperglycemic action.

21. For Calendula arvensis there is a correlation between low hypoglycemic capacity, the lowest concentration of polyphenols and flavonoids and the decreased antioxidant activity, statistically insignificant.

22. The hypoglycemic effect of Hesperis matronalis is positively correlated with the antioxidant action and phytochemical composition of Hesperis matronalis tincture. There was the largest decrease in MDA activity and the highest increase in CAT compared to other plants and compared to the positive control. It has the highest amount of caffeic acid.

23. The hydroalcoholic extracts from the studied plants showed an improvement of the kidneys function both by increasing serum albumin, especially for Hesperis matronalis, and by decreasing serum creatinine especially for Calendula arvensis.

24. The histopathological aspect at the level of the pancreas in the group with streptozotocin diabetes not treated with tinctures confirms the induction of the disease by this compound.

25. The most emphasized histopathological toxicity was found in the treatment with Polygonum persicaria where histopathological changes were found in the liver, myocardium, kidney, spleen.

26. Noting from 1 to 6 the actions of the 6 studied plants, we highlighted that the best effects are presented by Vaccinium myrtillus (60 points) followed by Polygonum hydropiper and
Agrimonia pilosa with 55 points each, Hesperis matronalis (50 points), Calendula arvensis 41 points and Polygonum persicaria 35 points.

27. Of the 5 tinctures studied, compared to the tincture of Myrtilli folium, known for its hypoglycemic properties, the most recommended as adjuvants in diabetes are the tincture of Polygonum hydropiper and secondly the tincture of Hesperis matronalis.

28. The subacute toxicity and death of 2 mice during the experiment with streptozotocin diabetes makes us cautiously recommend Agrimonia pilosa tincture as an antihyperglycemic. Polygonum persicaria also has high toxicity, especially histopathologically in mice with streptozotocin diabetes.