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ABBREVIATION LIST

ABH	-	<i>Acanthi balcanici herba</i>
ADR	-	adrenaline
APFC	-	polyphenol carboxylic acid
ATP	-	adenosine triphosphate
CAF	-	<i>Cardui acanthoiditis folium</i>
TLC	-	thin layer chromatography
DM	-	diabetes mellitus
DPH	-	<i>Dorycnii pentaphylli herba</i>
FR. X	-	ten pharmacopoeia
FT-IR	-	Fourier transform infrared spectroscopy
GC-MS	-	Gas chromatography–mass spectrometry
GPx	-	glutathione peroxidase
Gr	-	glutathione reductase
HBA1C	-	glycosylated haemoglobin
HDL	-	high-density lipoprotein
HPLC	-	High-performance liquid chromatography
IR	-	Infrared
LDL	-	low-density lipoprotein
MDA	-	malondialdehyde
<i>M-fol.</i>	-	<i>Myrtilli folium</i>
<i>M-fr.</i>	-	<i>Myrtilli fructus</i>
POL	-	lipid peroxides
ROS	-	reactive oxygen species
SOD	-	superoxide dismutase
STZ	-	streptozotocin
TC	-	total cholesterol
TG	-	triglycerides
TPF	-	<i>Tragoponis pratensis folium</i>
TRFF	-	<i>Tamaricis ramosissimae folium et flos</i>
TTGO	-	oral glucose tolerance test
VIS	-	visible
VLDL	-	very-low-density lipoprotein
WHO	-	World Health Organization

INTRODUCTION

Diabetes mellitus (DM) is the most frequent metabolic disease, with a constantly expanding prevalence and incidence, leading to early disability and high mortality, ranking third in the world in terms of frequency and severity, after cardiovascular and oncological diseases [1]. W.H.O. classifies diabetes into the following main categories: type 1, type 2, other types of diabetes, produced by multiple causes and gestational diabetes. The most common forms are type 1 diabetes and type 2 diabetes. The current treatment for diabetes is made gradually, depending on the severity of the disease: hygienic-dietary diet (balanced diet that involves eating foods with low glycemic index, exercising regularly), herbal therapy, pharmacological treatment with oral antidiabetic agents or insulin injections [2]. Since ancient times people have used the whole plants or the vegetal plant parts that are rich in active ingredients, fresh or processed (in the form of infusion, decoction, tincture, watery macerate) for their healing properties [3]. Many plants show important hypoglycemic therapeutic properties and are widely recognized and accepted in the current treatment of diabetes. In Romanian herbal therapy, the bilberry (*Vaccinium myrtillus*) is traditionally used for maintaining glucose homeostasis.

The objective of the thesis is to determine whether plant extracts that come from plant species least studied: *Tragopogon pratensis* (meadow salsify), *Dorycnium pentaphyllum subsp herbaceum* (prostrate canary clover), *Acanthus balcanicus* (balcanicus bear's breeches), *Tamarix ramosissima* (salt cedar), *Carduus acanthoides* (spiny plumeless thistle) have antidiabetic properties compared with *Vaccinium myrtillus* (bilberry).

THE STAGE OF KNOWLEDGE ON DIABETES MELLITUS AND ITS HERBAL THERAPY

1 GENERAL CONSIDERATIONS ON DIABETES MELLITUS

Through a topical bibliography, in the general part of the thesis we detailed subchapters related to diabetes: definition, classification, prevalence, pancreas and insulin, pathogenesis, causes and risk factors, acute and late complications, the involvement of oxidative stress in microvascular complications of diabetes, medications that have the side effect of producing hyperglycemia and insulin resistance induction, the current management of diabetes, plant products traditionally used to treat diabetes and its complications.

2 KNOWN PHARMACOGNOSTIC AND PHARMACOLOGIC DATA ABOUT THE PLANT PRODUCTS STUDIED IN THE THESIS

In scientific literature there is little information about the medicinal uses of the plant extracts selected in this thesis: *Tragoponis pratensis folium* (TPF); *Dorycnii pentaphylli herba* (DPH); *Acanthi balcanici herba* (ABH); *Tamaricis ramosissimae folium et flos* (TRFF); *Cardui acanthoiditis folium* (CAF). This data is shown with that about *Vaccinium myrtillus*, a plant with a chemical composition and pharmacological effects studied more intensely.

PERSONAL CONTRIBUTIONS

3 RESEARCH CONCERNING THE OBTAINING AND THE PHYSICO-CHEMICAL CHARACTERISATION OF SOME TINCTURES FROM POSSIBLY HYPOGLICEMIC PLANT PRODUCTS, TAKEN UNDER STUDY

3.1 Objectives

-Obtaining dyes by simple leaching, according to F.R. X and then characterizing from a physico-chemical point of view (color, taste, odour, relative density, refractive index, high level of quality - iron content, heavy metals, evaporation residue, concentration of alcohol).

-Qualitative analysis, by TLC and HPLC, of flavonoids (hyperoside, rutoside, isoquercitrozida, apigenol-7-glucoside, quercetol, luteola, kaempferol, apigenol) and polyphenol-carboxylic acids (caffeic acid, chlorogenic acid, rosmarinic acid);

-Qualitative analysis, by GC-MS of the volatile compounds from the tinctures (Methyl ethyl carbonate, ethyl ester of etanimidic acid, 2-propen-1-ol acetate, 3-methoxy-3-methyl-2-butanone, 1-butanol 3-methyl-benzoic acid etc.)

-Spectrophotometric quantitative analysis, in VIS, of phenilpropanic compounds;

-Analysis of the antioxidant capacity of tinctures by determining the polyphenols total and the flavonoids total;

-Quality characterisation of the tinctures from the point of view of the absorption peaks in VIS and the IR-specific vibrations.

3.2 Conclusions

The tinctures obtained contain appreciable amounts of polyphenols, the TRFF tincture having the highest content, followed by the M-fr tincture and the ABH tincture. From the point of view of flavonoid content, the richest tinctures are M-fr and M-fol, followed by TPF. TLC and HPLC analysis confirmed the presence of acid and polyphenol flavonoids in the studied tinctures.

Following the GC-MS analysis we observed that in the CAF tincture the highest number of volatile compounds was identified compared to other tinctures examined.

Spectra analysis in VIS of the tinctures shows the presence of maximum absorption for chlorophyll a (640-670 nm) and two absorption maxima of λ 530-540 nm and 600-610 nm, probably due to flavonoids.

Vibrations of FT-IR spectra of the tinctures are attributable to polyphenol type compounds (flavonoids, polyphenol acids), carotenoids (β -carotene, carotenoid esters), triglycerides, phytosterols, and amino acids.

By the content of flavonoids and polyphenol carboxylic acids, conferring plant products hypoglycaemic and antioxidant properties, the studied tinctures could be recommended as a source of natural polyphenols with an adjuvant role in the prevention and treatment of diseases caused by the presence inside the body of reactive oxygen species, such as diabetes mellitus.

4 PRELIMINARY RESEARCH OF PLANT EXTRACTS

4.1 The determination of acute and subacute toxicity of plant extracts considered for the study

4.1.1 Material and method

In the acute and subacute toxicity testing we created experimental groups of 4 mice, male and female, to whom we administered by gavage a single dose of the studied tinctures (1, 2, 3, 4, 5 g/kg) and 400 mg/kg for 2 weeks. We had a control group that received saline gavage. After administration of the extracts, the animals are constantly analysed for 24 hours, monitoring the toxicity and lethality.

4.1.2 Results and discussions

We noticed no toxicity for ABH, DPH, TPF, TRFF, M-fr tinctures. CAF tincture shows toxicity, LD₅₀ being 4 g/kg, its administration in a dose of 5 g/kg caused the death of the animals by seizure at intervals of time ranging from 30 minutes to one hour. In subacute toxicity testing, plant extracts had no lethal effect for the 400 mg/day dose and animal behaviour was normal. No weight changes occurred.

4.2 Establishing the effective dose for the studied tinctures using oral glucose tolerance test on mice with normal pancreatic function

4.2.1 Material and method

The experiment was conducted on VII batches of nine mice each, groups II-VII being divided each into three subgroups. The pre-treatment administered to mice by gavage was: group I - control, distilled water; group - II DPH tincture; group III - TRFF tincture; group IV - TPF tincture; group V - CAF tincture; group VI - ABH tincture and group VII – control, M-fr tincture. Each subgroup in each group of mice received by gavage the same plant extract, but with increasing dose (100 mg/kg, 150 mg/kg, and 200 mg/kg) dissolved in 0.3 ml of distilled water.

Tinctures were administered to mice 30 minutes prior to glucose administration by gavage (2 g/kg). In the TTGO, we determined glycemia at 30, 60, 90, 120 minutes. The test was conducted during a single day.

4.2.2 Results and discussions

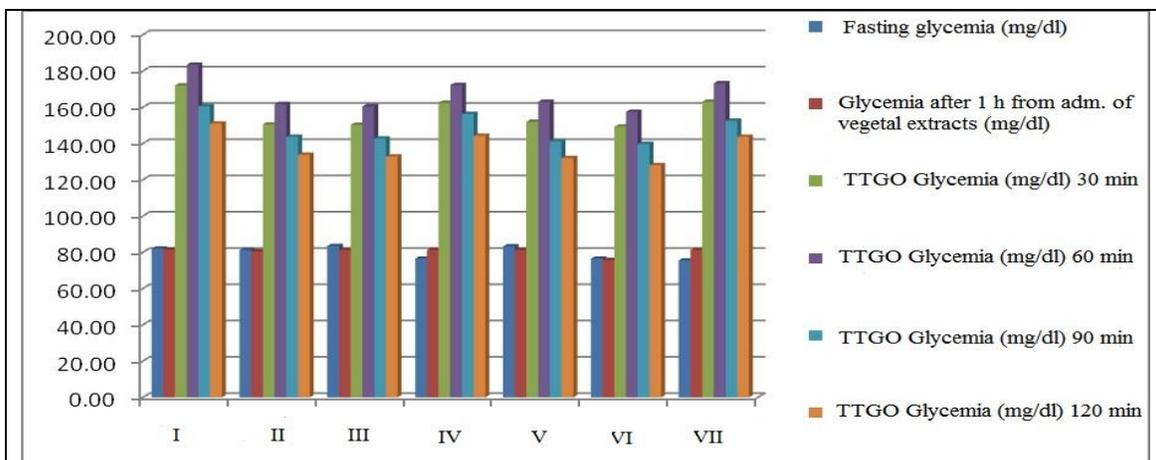


Chart 1. The average value of glucose for groups treated with an effective dose of plant extracts

After the experiment we noticed that the effective therapeutic dose is 200 mg/kg for the DPH tincture and 150 mg/kg for the TRFF, TPF, CAF, ABH and M-fr tinctures.

In the TTGO all plant extracts showed antihyperglycemic capacity, the glucose of groups treated with these being reduced compared to group I. The ABH tincture 150

mg/kg has the strongest anti-hyperglycaemic effect observed at all time intervals during the oral glucose tolerance test.

4.3 Testing the hypoglycemic effect of plant extracts using mice with experimentally adrenaline induced hyperglycemia

4.3.1 Material and method

Hyperglycemia was induced using 0.01 ml of 1 ‰ adrenaline (ADR) solution by subcutaneous administration. Animal testing was conducted over two days, groups receiving the same medication at the same doses.

The pre-treatment administered to mice by gavage one hour before induction of hyperglycemia was as follows: group I – control, distilled water; groups II and III - M-fol tincture and M-fr tincture; respectively, group IV – TPF tincture; group V – DPH tincture; group VI – ABH tincture; group VII – TRFF tincture; group VIII - CAF tincture. Adrenaline administration was also done 24 hours after pre-treatment with the same doses of the tinctures. We determined the blood sugar level one hour and two hours after the administration of adrenaline in the first and the second day.

4.3.2 Results and discussions

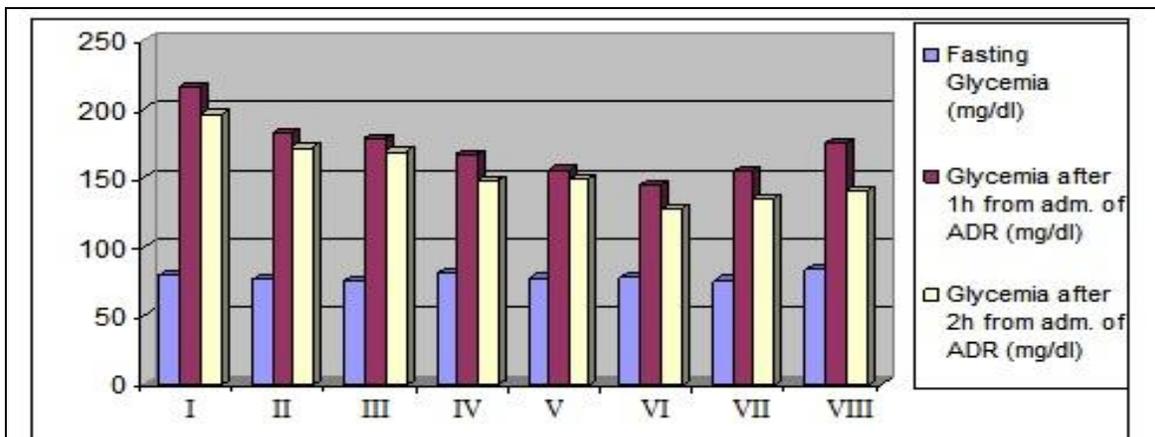


Chart 2. Average value of blood glucose levels of groups I- VIII, in the first day [4]

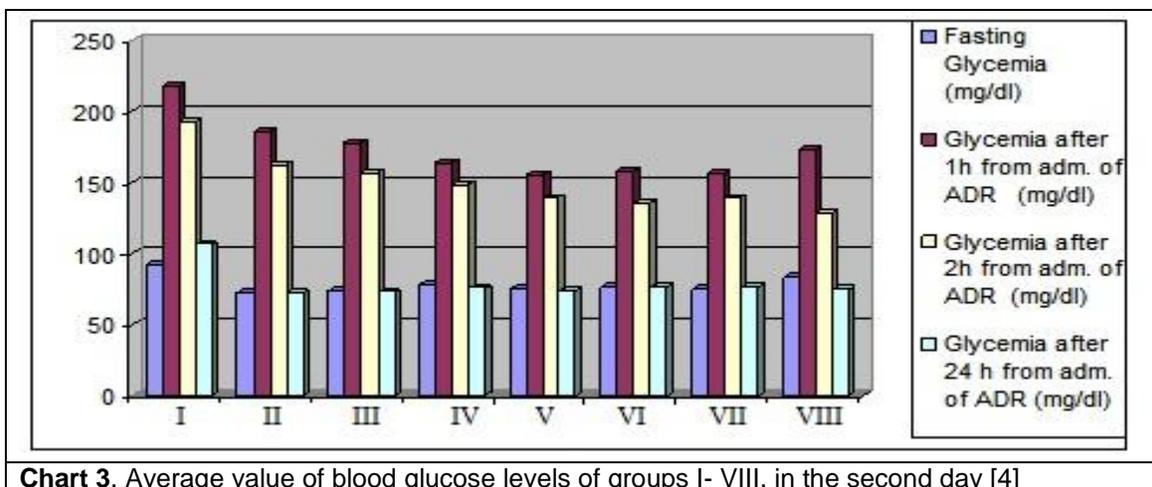


Chart 3. Average value of blood glucose levels of groups I- VIII, in the second day [4]

M-foI and M-fr tincture significantly decreases blood glucose levels in mice with adrenalin induced hyperglycemia, compared to group I, to which we administered only adrenaline.

The studied plant products have significantly lowered induced hyperglycemia, being more effective than extracts M-foI and M-fr, ABH tincture having the highest hypoglycemic efficiency.

5 EVALUATION OF THE HYPOGLYCEMIC, LIPID-LOWERING AND ANTIOXIDANT EFFECT OF STUDIED PLANT EXTRACTS, USING MICE WITH EXPERIMENTAL STREPTOZOTOCIN-INDUCED DIABETES

5.1 Experimental model for determining the hypoglycemic and hypolipidemic potential of plant extracts

To induce experimental diabetes i.p streptozotocin was injected in a single dose of 180 mg /kg, animals with fasting blood glucose levels above 300 mg/dl, confirmed by two measurements, were considered diabetic [5].

We realized VIII batches of 5 animals, being treated daily, differently during five weeks of testing: group I - healthy control group, untreated; group II - diabetic untreated control group; group III – TPF tincture; group IV – DPH tincture; group V – TRFF tincture; group VI – CAF tincture; group VII – ABH tincture; group VIII - M-fr tincture.

Animals were monitored over five weeks; the parameters monitored being the ingestion of water, food, weight, glucose levels, cholesterol and triglyceride levels. At seven days intervals, at the same hour, the animals were monitored after 12 hours of fasting, determining glucose and cholesterol, and triglycerides levels at the start of the study and after 5 weeks.

After five weeks of tests, the animals were sacrificed, blood samples were collected in heparinized tubes, being prepared for plasma separation and erythrocyte hemolysis. Biochemical investigations made addressed determining the level of lipid peroxides and activities of superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione reductase (GR) [6].

5.1.1 Results

Group no.	Water consumed M ± SD (ml/day)	Food consumed M ± SD (g/day)
Group I	10.2 ± 3.65	4.3 ± 0.59
Group II	33.5 ± 10.28	9.3 ± 1.8
Group III	24.3 ± 4.75	7.7 ± 1.3
Group IV	20.1 ± 2.60	5.7 ± 0.9
Group V	17.9 ± 5.15	4.9 ± 1.1
Group VI	18.4 ± 6.6	6.5 ± 1.38
Group VII	15.5 ± 3.5	4.8 ± 0.41
Group VIII	19.2 ± 4.4	5.2 ± 0.88

Table 1. The average value of water and food consumption for groups I-VIII, during the experiment

Group no.	Initial weight (g)	Weight (g) in the first week	Weight (g) week 2	Weight (g) week 3	Weight (g) week 4	Weight (g) week 5
Group I	37 ± 2.34	37.2 ± 2.16	37.2 ± 1.92	38 ± 2.23	39 ± 2.23	39.4 ± 1.51
Group II	35 ± 2.91	34 ± 2.34	33 ± 1.87	32.6 ± 1.14	30.4 ± 1.14	28.6 ± 1.67
Group III	34.6 ± 1.14	33.8 ± 2.28	33.4 ± 2.70	34.2 ± 2.38	35.2 ± 2.38	36 ± 1.73
Group IV	38 ± 2.54	39 ± 2.73	38.6 ± 2.07	39.4 ± 2.40	38.8 ± 2.28	40 ± 2.00
Group V	37.4 ± 4.50	37.2 ± 3.70	37.4 ± 2.70	38.2 ± 1.48	39 ± 1.22	40.2 ± 0.83
Group VI	36.4 ± 3.64	35 ± 3.80	34.6 ± 3.43	36 ± 3.74	36.4 ± 3.64	37 ± 4.30
Group VII	34.8 ± 2.16	36.2 ± 2.16	35.8 ± 1.30	37.8 ± 2.38	38.8 ± 2.16	40.4 ± 1.51
Group VIII	34.8 ± 3.03	34.4 ± 2.07	34.6 ± 2.70	33.8 ± 1.92	34.6 ± 2.07	35.4 ± 1.81

Table 2. The average value of weight for groups I-VIII, during the experiment

Group no.	Initial glucose level. M \pm SD (mg/dl)	Glucose level first week. M \pm SD (mg/dl)	Glucose level week 2. M \pm SD (mg/dl)	Glucose level week 3. M \pm SD (mg/dl)	Glucose level week 4. M \pm SD (mg/dl)	Glucose level week 5. M \pm SD (mg/dl)
Group I	87.9 \pm 3.19	91.4 \pm 3.50	90.2 \pm 2.58	91.6 \pm 2.07	91 \pm 2.34	88.8 \pm 1.92
Group II	309.8 \pm 4.65	323.2 \pm 7.56	315.8 \pm 5.26	329.4 \pm 5.12	329.2 \pm 1.64	329.6 \pm 2.88
Group III	312.6 \pm 2.30	304.4 \pm 4.72	260.2 \pm 2.58	207.4 \pm 2.70	159.4 \pm 2.96	120 \pm 3.16
Group IV	308.8 \pm 8.28	282.8 \pm 3.56	239.8 \pm 3.03	191.8 \pm 4.49	133 \pm 2.91	107.2 \pm 8.49
Group V	313 \pm 2.91	281.6 \pm 4.21	237.6 \pm 3.84	187.6 \pm 3.04	130.8 \pm 1.92	109.4 \pm 3.20
Group VI	309.4 \pm 3.07	286 \pm 4.84	252.4 \pm 2.40	191.4 \pm 3.64	135.2 \pm 2.58	116.4 \pm 2.07
Group VII	316.2 \pm 2.77	275.2 \pm 4.14	223.8 \pm 3.70	174.2 \pm 2.77	115.8 \pm 3.27	96.6 \pm 8.29
Group VIII	311.8 \pm 3.03	303.6 \pm 2.07	263.8 \pm 4.14	213.8 \pm 2.86	163.6 \pm 6.26	159.8 \pm 3.49

Table 3. The average values of glucose levels of the groups considered for the study, during the experiment, M=average; SD=standard deviation; [7]

Group no.	Initial cholesterol. M \pm SD (mg/dl)	Cholesterol first week. M \pm SD (mg/dl)	Cholesterol week 2. M \pm SD. (mg/dl)	Cholesterol week 3. M \pm SD. (mg/dl)	Cholesterol week 4. M \pm SD (mg/dl)	Cholesterol week 5. M \pm SD (mg/dl)
Group I	75.6 \pm 2.40	75.4 \pm 2.70	83.4 \pm 2.70	77.2 \pm 2.38	77.4 \pm 1.51	79.2 \pm 2.58
Group II	174 \pm 2.73	174 \pm 5.38	172.2 \pm 4.76	174.4 \pm 3.91	173.6 \pm 2.30	174.2 \pm 1.48
Group III	174 \pm 4.84	186.6 \pm 3.43	177 \pm 2.91	165.8 \pm 2.58	155.8 \pm 3.96	136.2 \pm 3.96
Group IV	169.4 \pm 4.72	172.4 \pm 5.31	168.2 \pm 5.01	166.4 \pm 5.59	160.2 \pm 4.43	153.8 \pm 4.20
Group V	175.2 \pm 2.94	172.8 \pm 3.70	170.8 \pm 3.56	168.4 \pm 2.60	168.4 \pm 3.84	162 \pm 4.52
Group VI	174.4 \pm 1.14	188.2 \pm 3.56	192.2 \pm 2.28	189.4 \pm 2.88	179.8 \pm 1.92	166.4 \pm 3.20
Group VII	163.2 \pm 2.38	157.8 \pm 2.48	152 \pm 4.30	133.2 \pm 3.49	118.4 \pm 3.50	101.6 \pm 3.84
Group VIII	172 \pm 4.18	172 \pm 2.23	170 \pm 3.93	169.8 \pm 1.48	166.4 \pm 1.14	159.8 \pm 3.49

Table 4. The values of total seric cholesterol of the groups considered for the study, during the experiment, M=average; SD=standard deviation; [7]

Group no.	Initial triglycerides. M \pm SD (mg/dl)	Triglycerides week 5. M \pm SD (mg/dl)
Group I	108.2 \pm 3.42	107.8 \pm 2.38
Group II	207.2 \pm 3.70	208.4 \pm 2.19
Group III	210.6 \pm 3.84	165.4 \pm 3.64
Group IV	209.6 \pm 3.91	133.4 \pm 3.84
Group V	204.8 \pm 3.49	127.4 \pm 2.30
Group VI	201.2 \pm 3.11	134.4 \pm 3.84
Group VII	207.2 \pm 1.78	105.8 \pm 2.94
Group VIII	203.8 \pm 3.83	153.6 \pm 3.20

Table 5. The values of seric triglycerides of the groups considered for the study, during the experiment, M=average; SD=standard deviation; [7]

5.2 *In vivo* testing antioxidant potential of plant extracts, determining the level of superoxide dismutase activity, glutathione peroxidase, glutathione reductase, and the level of substances that react with thiobarbituric acid

5.2.1 Results

Group no.	GR (U/l total blood). M ± SD	GPx (U/l hemolyzed) M ± SD	SOD (U/ml hemolyzed) M ± SD	POL(mmol/l). M ± SD
Group I	60.25 ± 1.91	4251.5 ± 28.99	203.8 ± 1.84	0.5350 ± 0.06
Group II	46.3 ± 2.12	3415.5 ± 98.29	191.7 ± 10.75	2.6937 ± 0.007
Group III	60.3 ± 8.63	5652 ± 1370.37	243.6 ± 1.13	1.1808 ± 0.16
Group IV	61.4 ± 2.26	6269 ± 207.89	220.3 ± 0	0.9594 ± 0.014
Group V	89.6 ± 0	7124 ± 246.07	227.65 ± 10.39	0.3690 ± 0.014
Group VI	57.7 ± 0.14	6580.5 ± 672.46	212.45 ± 5.30	0.7749 ± 0.007
Group VII	59.3 ± 0.70	5755.5 ± 236.88	195.1 ± 2.27	0.3321 ± 0.007
Group VIII	52.5 ± 0.70	4 301 ± 11.31	194.15 ± 3.18	0.5904 ± 0.028

Table 6. The values of antioxidant enzymes and of lipid peroxides of the groups considered for the study at the end of the experiment, M=average; SD=standard deviation; [8]

5.2.2 Conclusion

1. The administration of plant extracts on diabetic animals had positive effects on food and water consumption, also favourably influencing weight.
2. Plant products tested demonstrated a significant hypoglycemic potential in streptozotocin diabetes, *Acanthus longifolius* species having the strongest therapeutic effect.
3. In diabetic mice ABH and TPF tinctures exerted a powerful cholesterol-lowering effect and plant extracts from species DPH, TRFF, CAF, ABH caused a significant decrease in plasma triglyceride levels, compared with the control groups. ABH tincture, as opposed to M-fr, has favourable effects also in the regulation of lipid metabolism in animals with streptozotocin-induced diabetes.
4. TRFF tincture demonstrated a strong antioxidant effect, group V, treated with it, showing the highest values of enzyme activity for glutathione reductase and glutathione peroxidase. Superoxide dismutase highest level was obtained for the group treated with TPF tincture. The plasma level of lipid peroxides was lowest in the diabetic mice treated with ABH tincture.

6 THE HISTOPATHOLOGICAL EVALUATION OF BIOLOGICAL TISSUES SAMPLED FROM MICE WITH INDUCED DIABETES AFTER FIVE WEEKS OF TREATMENT

At the end of the experiment on diabetic mice, after sacrificing the animals, we conducted histological analysis of tissues of interest from each group by taking samples from the pancreas, brain, kidney, and liver.

Microscopic observations revealed the structural abnormalities of the pancreas in diabetic mice compared with normal mice. The pancreas of animals from group VIII treated M-fr has a partially preserved structure, the damage being reduced compared to group II. The pancreas of animals treated with ABH tincture shows unchanged structure. All tested plant extracts proved therapeutic efficacy, limiting the destructive effects of streptozotocin.[9], [10].

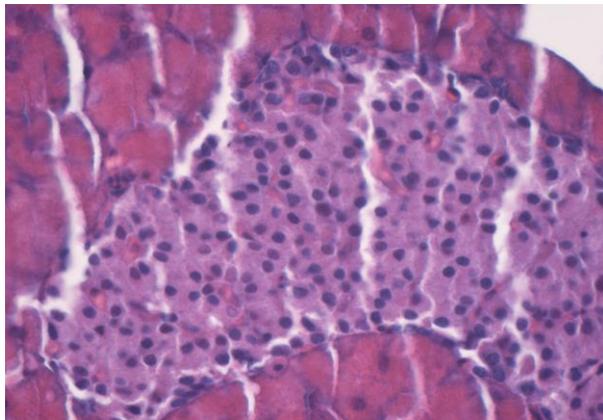


Image 1. Group I (control group, healthy) pancreas with normal structure, x400

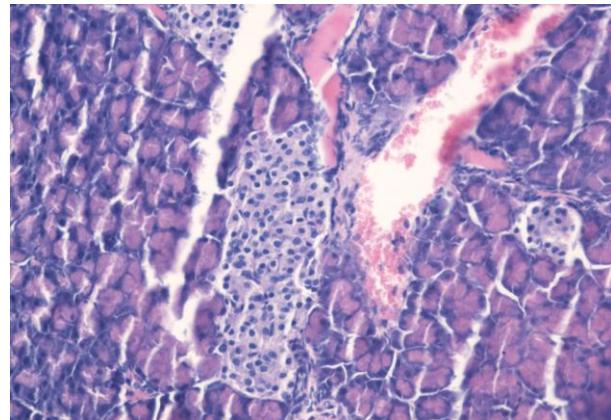


Image 2. Group II (control, diabetic, untreated) – Langerhans islets; necrotic lesions of haemorrhagic nature, dissociative acinar, x200

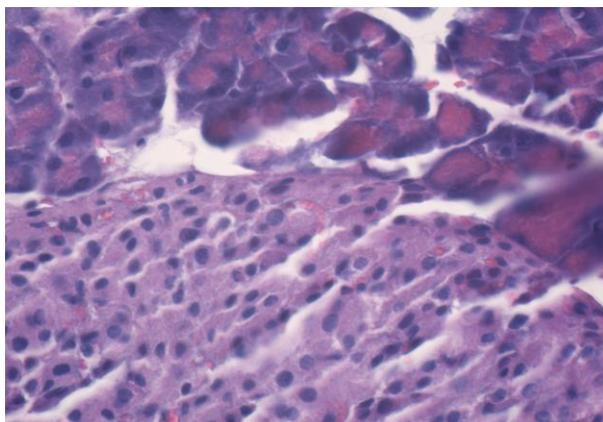


Image 3. Group VII, treated with *Acanthi balcanici herba* tincture – unchanged acino-islet structure, x400

7 DETERMINATION OF THE ANTIBACTERIAL EFFECT OF PLANT EXTRACTS

7.1 Material and method

To test antibacterial effects for the six tinctures analysed the disc diffusion method with nutrient agar discs was used (*Kirby-Bauer*), in accordance with the provisions of F.R. X.

Testing the antibacterial effect of tinctures was performed taking as reference the acknowledged antibacterial effect of elective antibiotics (control +) on reference strains. Positive control was chosen based on the sensitivity of the bacterial species: amoxicillin + clavulanic acid 20 mg - (*Staphylococcus aureus*); levofloxacin 5 mg - (*Escherichia coli*); amikacin 30 mg - (*Proteus vulgaris*); ceftazidime 30 mg - (*Pseudomonas aeruginosa*); cefotaxime 30 mg - (*Klebsiella pneumoniae*).

We also observed the synergistic/antagonistic effects arising between tested plant extracts and the antibiotics used for election (control +) with known antibacterial effect on the bacterial species tested.

7.2 Results and discussions

The antibacterial properties of plant products have varied greatly, depending predominantly on the plant species as well as the bacterial species. ABH and DPH tinctures showed no antibacterial activity on any tested microorganism. Also, DPH and ABH tinctures cancel the effect of the standard antibacterial antibiotic (amoxicillin + clavulanic acid, levofloxacin, amikacin), when associated with them. Our testing has shown that TPF and M-fr tinctures show an antimicrobial effect against a broad spectrum of microorganisms.

GENERAL CONCLUSIONS AND PERSPECTIVES

1. Diabetes mellitus is a disease with heterogeneous etiology, characterized by chronic hyperglycemia, and other metabolic disorders that are due to insulin deficiency, insulin resistance, or both.
2. Diabetes can be improved with the help of plant extracts containing hypoglycemic active ingredients. Vegetable products can be used separately or in mixtures.
3. The plant products used to maintain glucose homeostasis contain hypoglycemic active ingredients represented by: polyphenol carboxylic acids, anthocyanosides, carotenoids, essential oils, triterpenes, tiorheterozides, tannins, sterols, triterpenoid saponins, proantocianidols, bitter substances, flavonoids, coumarins.
4. By "*in vitro*" and pharmacognosy research we obtained and characterized physico-chemical tinctures from products with possible hypoglycemic properties of some plant species: *Tragopogon pratensis*, *Dorycnium pentaphyllum subsp herbaceum*, *Acanthus balcanicus*, *Tamarix ramosissima*, *Carduus acanthoides*, compared with *Vaccinium myrtillus* (plant known as hypoglycaemic) to further the "*in vivo*" study for use as medicinal products.
5. By the content of flavonoids and polyphenol carboxylic acids conferring plant products hypoglycaemic and antioxidant properties, the tinctures obtained could be recommended as a source of natural polyphenols with adjuvant role in the prevention and treatment of diseases like diabetes, in the pathogenesis of which are also involved reactive oxygen species.
6. The acute toxicity study of the extracts tested showed no toxicity for the tinctures of: *Acanthi balcanici herba*, *Dorycnii pentaphylli herba*, *Tragoponis pratensis folium*, *Tamaricis ramosissimae folium et flos*, *Myrtilli fructus*, even at the administration by gavage of a dose of 5 g/kg and the toxicity of *Cardui acanthoiditis folium* tincture at a dosage of 4 g/kg.
7. Following the oral glucose tolerance test, we determined the optimal concentration for the studied tinctures where the strongest hypoglycemic effect appears: 200 mg/kg for *Dorycnii pentaphylli herba* and 150 mg/kg for *Tamaricis ramosissimae folium et flos*,

Tragoponis pratensis folium, *Cardui acanthoiditis folium*, *Acanthi balcanici herba* and *Myrtilli fructus* tinctures.

8. *Acanthi balcanici herba* 150 mg/kg tincture, shows the strongest hypoglycemic effect compared with the other tested extracts observed at all time intervals within the oral glucose tolerance test (30, 60, 90, 120 minutes).
9. After inducing hyperglycemia by injecting adrenaline, the studied plant products have significantly lowered induced hyperglycemia, being more effective than the *Myrtilli folium* and *Myrtilli fructus* extracts, the *Acanthi balcanici herba* tincture having the highest hypoglycemic efficacy.
10. The plant extracts of species *Acanthus balcanicus* and *Tragopogon pratensis* exerted a powerful cholesterol-lowering effect, and plant extracts from the species *Dorycnium herbaceum*, *Tamarix ramosissima*, *Carduus acanthoides*, *Acanthus balcanicus* caused a significant decrease in plasmatic triglyceride levels compared to control groups.
11. The pharmaceutical solution from *Acanthus balcanicus* unlike that of *Vaccinium myrtillus*, (known hypoglycemic) has favourable effects in regulating lipid metabolism in animals with diabetes induced by streptozotocin.
12. The administration of plant extracts on diabetic animals had positive effects on food and water consumption, also favourably influencing weight.
13. After the study it can be seen at the group of untreated diabetic mice that increased levels of glucose, maintained for long, induces oxidative stress, that is reflected by increased concentration of malondialdehyde and decreased levels of antioxidant enzymes.
14. *Tamarix ramosissima* showed after the experiment a strong antioxidant effect, in mice with streptozotocin diabetes treated with it, showing the highest values of enzyme activity for glutathione reductase and glutathione peroxidase.
15. The highest level of superoxide dismutase was obtained at the group of diabetic mice treated with the plant extract from the species *Tragopogon pratensis*.
16. The plasma level of lipid peroxides was lowest in the diabetic mice treated with the extract derived from the species *Acanthus balcanicus*.
17. Plant extracts showed significant antioxidant effects, limiting the effects of oxidative stress in diabetes.

18. Microscopic observations revealed structural abnormalities of the pancreas in diabetic mice compared with normal mice. Langerhans islets were irregular in size and shape and in diabetic mice islet amyloidosis was observed.
19. In the groups with streptozotocin diabetes treated with the studied tinctures an improvement of the histological aspect of the Langerhans islets from the pancreas was observed, especially in the case of the plant extract derived from the species *Acanthus balcanicus*, hence the potential for regenerating pancreatic tissue of the tincture.
20. A normal histological structure in the brain is found in the group of diabetic mice treated with alcoholic extract derived from *Acanthus balcanicus*.
21. Improvements in histological aspects were noted in the liver rather than the kidney, with a less degraded structure from the effects of streptozotocin.
22. Slightly modified liver structure appears in the group of diabetic mice treated with plant extract originating from *Acanthus balcanicus*, at this level having been observed binucleated hepatocytes, markers of regeneration processes.
23. *Acanthi balcanici herba* and *Dorycnii pentaphylli herba* tinctures showed no antibacterial activity on any tested microorganism.
24. Also *Acanthi balcanici herba* and *Dorycnii pentaphylli herba* tinctures cancel antibacterial effect of standard antibiotics (amoxicillin + clavulanic acid, levofloxacin, amikacin), when coupled with them.
25. Bigger inhibition zones were recorded for the studied extracts in the case against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.
26. Making the antibiogram showed that *Tragoponis pratensis folium* and *Myrtilli fructus* tinctures shows an antimicrobial effect against a broad spectrum of microorganisms (gram positive and negative).
27. *Acanthi balcanici herba* tincture proved to have the strongest therapeutic effects on streptozotocin diabetes in mice, favourably influencing carbohydrate metabolism, lipid metabolism, and oxidative stress. Biochemical data were consistent with the relevant histological improvement of the endocrine pancreas, liver, brain and less at kidney level.
28. This research requires deeper study of the action of *Acanthus balcanicus* for patenting the medicinal product.

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