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ESTABLISHING AN EFFECTIVE ANTIHYPERGLYCEMIC DOSE FOR SIX PLANT EXTRACTS USING THE ORAL GLUCOSE TOLERANCE TEST IN MICE WITH NORMAL PANCREATIC FUNCTION

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ABSTRACT

The oral glucose tolerance test is useful for the determination of insulin receptors sensitivity to an excess of glucose in the healthy animal. The test was carried out using mice with normal pancreatic function and the plant extracts was made from very rarely studied plant species: *Tragopogon pratensis, Dorycnium pentaphyllum subsp herbaceum, Acanthus balcanicus, Tamarix ramosissima, Carduus acanthoides*, taking as a reference the proven antihyperglycemic effect of tincture of *Vaccinium myrtillus*. After oral administration of glucose (2g/kg) in previously untreated mice, we observed an increase in plasma glucose within 30 minutes of TTGO administration, reaching a maximum of 60 minutes, with glycemic values progressively decreasing to 90 minutes and 120 minutes. The obtained average blood sugar level to 120 minutes is considerably increased compared to the initial mean before oral administration of glucose. Following testing, we established for the tinctures studied the optimum concentration at which the most powerful antihyperglycemic effect and 200 mg / kg respectively for tincture *Dorycnii pentaphylli herba* and 150 mg/kg for tinctures *Tamaricis ramosissimae folium et flos, Tragoponis pratensis folium, Cardui acanthoiditis folium, Acanthi balcanici herba and Myrtilli fructus*. After the test we concluded that all plant extracts have shown anti-hyperglycaemic capacity, the groups II-VII blood glucose level treated with tincture being reduced in comparison with group I.

INTRODUCTION

The oral glucose tolerance test is a dynamic test, which is recommended to be performed when the patient has blood glucose levels higher than 125 mg / dl[1]. It is also recommended to perform the test in patients with a history of hereditary diabetes, obesity, hypertension, dyslipidemia, chronic pancreatic disease, persistent glycosuria or pregnant women with a history of heredocolateral diabetes mellitus[2].

Interpreting the oral glucose tolerance test involves measuring plasma glucose at time intervals from oral glucose administration. A blood glucose level below 140 mg / dl reveals a normal pancreatic function, while values above 200 mg / dl confirm the diagnosis of diabetes [3].

The test was carried out using mice with normal pancreatic function and the plant extracts originated from very rarely studied plant species: *Tragopogon pratensis*, *Dorycnium pentaphyllum subsp herbaceum*, *Acanthus balcanicus*, *Tamarix ramosissima*, *Carduus acanthoides*, taking as a reference the proven antihyperglycemic effect of tincture of *Vaccinium myrtillus*[4]. In this experiment, we proposed to evaluate the potential antihyperglycemic effect and to determine the effective doses for the plant extracts studied using the oral glucose tolerance test.

MATERIAL AND METHOD

The plant products to be tested were harvested from species from the Botanical Garden of the University of Craiova during April-June. Vegetable products were used in the form of tinctures, obtained by simple percolation, in a plant / solvent ratio (ethanol 70o) of 1: 5 (F.R.X.). The test sample of each tested tincture is found in the Pharmacognosy Laboratory Collection of the Faculty of Pharmacy in Craiova[5].

The animals were kept during the experiment in polypropylene cages containing sawdust as a litter, in a well-ventilated room. In each box, we assigned an experimental group consisting of nine mice, which were kept under standard laboratory conditions: 24-28 ° C, 60-70% relative humidity, 12 hours light / dark alternation. The animals were 12 hour fasting before the experiment, having free access to the water.

The experiment was performed on seven groups, in the first group (control) three mice were studied, in groups II-VII, nine mice for each, Swiss albino, adults with normal pancreatic function. Groups II-VII were each divided into three subgroups of three mice[6].

In the experiment, we used two control groups: group I, consisting of normal pancreatic non-medicated mice, receiving distilled water through gavage, and group VII, which received gavage from a plant extract from *Vaccinium myrtillus*, product recognized for antihyperglycemic effects[7].

Groups II-VII, received by gavage a plant extract to be analyzed. Each subgroup of mice in each group received the same plant extract by gavage, but in increasing amounts (100mg / kg, 150mg / kg, 200mg / kg) dissolved in 0.3ml distilled water.

Tinctures were given to laboratory animals half an hour before glucose gavage (2 g / kg). The animals received a single dose of vegetal tincture taken in the study. The test was conducted according to the protocol developed by Du Vigneaud and Kaar in 1925, over a single day[8].

In each group the subgroups were marked with batch number and subgroup number (eg I.1) followed by the name of the plant species and the administered dose.

The groups had the following structure:

Group I (control) - received 0.3 ml of distilled water through gavage

Group II - consisting of 3 subgroups of 3 mice, received *Dorycnii pentaphylli herba* tincture in different doses (100 mg / kg, 150 mg / kg, 200 mg / kg) through gavage.

Group III - consisting of 3 subgroups of 3 mice, received *Tamaricis ramosissimae folium et flos* tincture at different doses (100mg / kg, 150mg / kg, 200mg / kg).

Group IV - consisting of 3 subgroups of 3 mice, received *Tragoponis pratensis folium* in different doses (100mg / kg, 150mg / kg, 200mg / kg).

Group V - consisting of 3 subgroups of 3 mice, received *Cardui acanthoiditis folium* in different doses (100mg / kg, 150mg / kg, 200mg / kg).

Group VI - consisting of 3 subgroups of 3 mice, received *Acanthi balcanici herba* in different doses (100mg / kg, 150mg / kg, 200mg / kg).

Group VII - consisting of 3 subgroups of 3 mice, received *Myrtilli fructus* tincture at different doses (100mg / kg, 150mg / kg, 200mg / kg) used as reference group, *Myrtilli fructus* being known as an antihyperglycemic plant extract.

The blood glucose level of the animals was determined on fasting and one hour after the distilled water was administrated for group I and respectively

tinctures for groups II-VII. After glucose administration, we determined blood glucose level at 30, 60, 90, 120 minutes. Blood was collested from the tail vein, blood glucose level being determined using an eBensensor glucometer. For oral administration of the studied tinctures, we used an intragastric tube.

The data were statistically evaluated using the Two Sample t-test. Differences between the measured parameter values were interpreted as statistically significant if the significance level $p < 0.05$. P values much lower than 0.0001 denote very significant differences between batches[9].

RESULTS

Table 1 The mean plasma glucose levels in the OGTT

Groups	Blood sugar à jeun (mg/dl)	Blood glucose 1 h after tinctures administration (mg/dl)	OGTT Blood glucose level (mg/dl)			
			30 min	60 min	90 min	120 min
Group I	82,00 ± 1,91	81,50 ± 3,16	172,00 ± 3,65	183,50 ± 4,2	160,50 ± 3,10	151,00 ± 1,82
Group II.1	82,00 ± 2,58	80,25 ± 2,98	160,25 ± 3,30	170,75 ± 4,57	150,75 ± 3,77	143,25 ± 2,21
II.2	79,25 ± 2,21	80,00 ± 4,08	156,50 ± 2,08	167,25 ± 3,30	148,50 ± 2,64	140,25 ± 2,21
II.3	81,25 ± 1,70	80,50 ± 2,88	150,50 ± 3,51	161,75 ± 3,40	143,75 ± 2,21	133,75 ± 3,86
Group III.1	86,25 ± 3,59	86,75 ± 3,30	159,00 ± 4,08	175,50 ± 2,38	151,75 ± 3,77	145,00 ± 2,58
III.2	83,50 ± 1,91	81,25 ± 2,62	150,25 ± 3,86	160,50 ± 3,51	142,75 ± 3,30	132,75 ± 3,86
III.3	76,50 ± 1,29	77,00 ± 0,81	158,50 ± 3,69	173,75 ± 3,86	153,75 ± 3,86	142,25 ± 3,50
Group IV.1	83,25 ± 2,21	81,75 ± 2,75	165,75 ± 2,75	173,25 ± 3,30	155,00 ± 4,08	144,75 ± 1,70
IV.2	76,50 ± 2,08	75,00 ± 1,63	162,50 ± 2,38	172,25 ± 5,43	156,25 ± 3,20	144,25 ± 2,62
IV.3	80,00 ± 2,16	79,25 ± 2,62	159,75 ± 3,20	169,00 ± 1,15	155,75 ± 2,21	144,00 ± 1,82
Group V.1	75,50 ± 2,64	76,25 ± 1,50	165,25 ± 2,21	175,75 ± 2,06	157,75 ± 0,95	146,50 ± 1,29
V.2	83,25 ± 3,30	81,50 ± 3,00	152,00 ± 2,16	163,00 ± 1,82	141,25 ± 2,21	132,00 ± 0,81
V.3	85,75 ± 3,09	85,50 ± 2,38	164,25 ± 4,19	175,50 ± 2,88	159,00 ± 2,94	145,25 ± 2,21
Group VI.1	80,00 ± 2,16	78,50 ± 2,08	159,25 ± 3,86	175,25 ± 2,75	148,50 ± 3,51	138,50 ± 3,51
VI.2	76,50 ± 2,08	75,75 ± 3,30	149,25 ± 2,50	157,50 ± 2,08	139,75 ± 5,31	128,00 ± 2,58
VI.3	83,50 ± 2,38	83,00 ± 3,37	160,50 ± 3,51	170,75 ± 3,5	150,25 ± 2,97	132,50 ± 3,70
Group VII.1	78,25 ± 2,98	77,50 ± 3,87	168,00 ± 2,58	178,25 ± 3,30	156,75 ± 1,70	146,00 ± 1,82
VII.2	75,50 ± 2,38	75,25 ±1,71	163,00 ± 2,58	173,25 ± 3,87	152,50 ± 3,11	143,75 ±3,20
VII.3	77,33 ± 2,08	77,00 ± 2,16	167,00 ± 1,83	177,75 ± 3,50	155,50 ± 2,65	145,00 ± 2,94

OGTT - oral glucose tolerance test

DISCUSSIONS

Testing was performed using mice with normal pancreatic function, the antihyperglycaemic effect of plant extracts being due to the following mechanisms: blocking glucose uptake, increasing glucose tissue sensitivity, increasing the rate of synthesis and insulin secretion or insulinomimetic effect[10].

Increased doses of tinctures (100 mg / kg, 150 mg / kg, 200 mg / kg) in the oral glucose tolerance test, help us to determining the effective dose. (Table 1)

Following the experiment, we noticed that the therapeutically effective dose is 200 mg / kg for tincture *Dorycnii pentaphylli herba* and 150 mg/kg for tincture *Tamaricis ramosissimae folium et flos, Tragoponis pratensis folium, Cardui acanthoiditis folium, Acanthi balcanici herba* și *Myrtilli fructus*. The administration of plant extracts at these concentrations had the most potent antihyperglycemic effect.

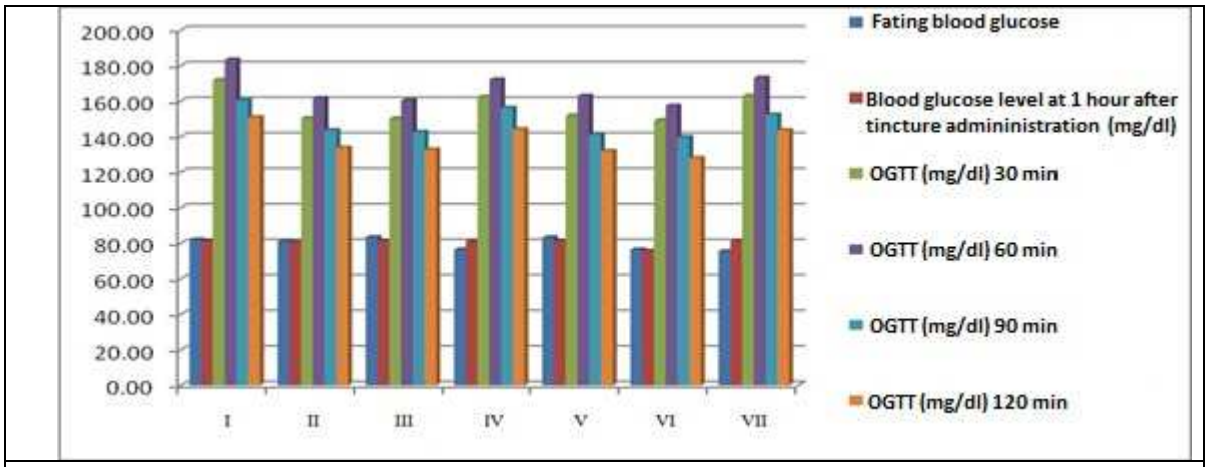

Figure 1 Average blood glucose level for groups treated with the effective dose of plant extracts

The mice of group I showed the highest mean blood glucose values of all the groups tested, at 60 and 120 minutes being 183.50 ± 4.2 mg / dl, respectively 151.00 ± 1.82 mg / dl. In the case of group I, the most pronounced increase in the mean plasma glucose level was observed, with a maximum of 60 minutes (increase by 123.7% compared to baseline).

Within the TTGO, the mean plasma blood glucose levels for the II-VII groups treated with the plant extracts to be tested are lower at all time intervals (30, 60, 90, 120 minutes) compared to the mean blood glucose values obtained for the group I. Exposure was performed in descending order of antihyperglycemic therapeutic efficacy.

Tragoponis pratensis folium tincture (150 mg / kg) has anti-hyperglycaemic potential seen during OGTT (60 minutes), the mean blood glucose of the treated animals being 172.25 ± 5.43 mg / dl, significantly lower than group I (183.50 ± 4.2 mg / dl, $p<0,016964736$) and sensibly equal to control group VII (173.25 ± 3.87 mg / dl; $p<0.774435335$). At 120 minutes after the administration of oral glucose, group treated with *Tragoponis pratensis folium* has mean blood glucose values of 144.25 ± 2.62 mg / dl, lower compared to group I (151.00 ± 1.82 mg / dl; $p<0.005581652$) and comparable to batch VII (143.75 ± 3.20 mg / dL; $p<0.005581652$).

Myrtilli fructus tincture limits induced hyperglycaemia, with mean blood glucose levels of 173.25 ± 3.87 mg / dL at 60 minutes and 143.75 ± 3.20 mg / dL at 120 minutes, lower compared to group I (183.50

± 4.2; p<0,011485417at 60 minutes), (151.00 ± 1.82; p<0,007674929, 120 minutes), but higher than in groups II-VI. **(Figure 1)**

By performing the TTGO and using statistical analysis, we can conclude that the tincture of *Tragoponis pratensis folium* has antihyperglycemic potential similar to *Myrtilli fructus* tincture.

Dorycnii pentaphylli herba tincture has antihyperglycemic efficacy superior to *Myrtilli fructus* tincture, the animals treated with it having mean blood glucose levels of 161.75 ± 3.40 mg / dl at 60 minutes and 133.75 ± 3.86 mg / dl at 120 minutes.

The mice from group II, who received the tincture *Dorycnii pentaphylli herba*, showed glycemic values significantly low at 60 minutes (161.75 ± 3.40 mg / dl) versus group I (183.50 ± 4.2 mg / dl, p<0,000197478) and group VII (173.25 ± 3.87 mg / dl, p<0,011485417) and 120 minutes (133.75 ± 3.86 mg / dl) versus of lot I (151.00 ± 1.82 mg / dl, p<0,000193079) and group VII (143.75 ± 3.20 mg / dl, p<0,007674929). **(Figure 1).**

Tamaricis ramosissimae folium et flos tincture has antihyperglycemic properties superior to those of *Tragoponis pratensis folium, Myrtilli fructus, Dorycnii pentaphylli herba*, the animals treated with it had mean 60-minute blood glucose values of 160.50 ± 3.51 mg / dl and at 120 minutes of 132.75 ± 3.86 mg / dl. For *Tamaricis ramosissimae folium et flos* in the TTGO, we observed at 60 minutes (160.50 ± 3.51 mg / dl) glycemic values significantly lower compared to group I (183.50 ± 4.2 mg / dl , p<0,000155222) and group VII (173.25 ± 3.87 mg / dl, p<0,011485417), and at 120 minutes (132.75 ± 3.86 mg / dl) potentially greater anti-hyperglycaemia than group I (151.00 ± 1.82 mg / dl, p<0,000140993) and group VII (143.75 ± 3.20 mg / dl, p<0,007674929). **(Figure 1)**

Cardui acanthoitidis folium tincture has notable therapeutic efficacy, limiting the induced hyperglycemia, the mice treated with it having mean blood glucose values at 60 and 120 minutes (163.00 ± 1.82 mg / dl and 132.00 ± 0.81 mg / dl) compared to mice treated with *Tamaricis ramosissimae folium et flos* (160.50 ± 3.51 mg / dl and 132.75 ± 3.86 mg / dl respectively).

Also, *Cardui acanthoiditis folium* tincture showed average blood glucose values at 60 minutes significantly lower than group I (p<0,000108839) and group VII (p<0,003004778), and at 120 minutes than group I (p<0,000400498) and group VII (p<0,000388337). **(Figure 1)**

Acanthi balcanici herba tincture had the lowest mean plasma glucose values, having the most antihyperglycemic effect among the test tinctures.

Acanthi balcanici herba tincture has antihyperglycaemic therapeutic efficacy, mice treated with it have mean blood glucose values at 60 and 120 minutes (157.50 ± 2.08 mg / dl and 128.00 ± 2.58 mg / dl respectively) lower comparative with the mice treated with *Cardui acanthoiditis folium.* In OGTT we obtained lower blood glucose values for mice treated with *Acanthi balcanici herba* tincture at 60 minutes (157.50 ± 2.08 mg / dl) compared to group I (183.50 ± 4.2 mg / dl , p<0,000225185) and group VII (173.25 ± 3.87 mg / dl, p<0,000369002) and 120 minutes (128.00 ± 2.58 mg / dl) versus group I (151.00 ± 1.82 mg / dl, p<0,000848052) and group VII (143.75 ± 3.20 mg / dl, p<0,000258952). **(Figure 1)**

CONCLUSIONS

1. The oral glucose tolerance test is used to determine the sensitivity of insulin receptors to an excess of glucose in the healthy animal.
2. Administering the tinctures one hour before performing the oral glucose tolerance test assesses their potential to improve or alter glucose tolerance.
3. After oral administration of glucose (2g/kg) in previously untreated mice, we observed an increase in plasma glucose within 30 minutes of TTGO administration, reaching a maximum of 60 minutes, with glycemic values progressively decreasing to 90 minutes and 120 minutes. The obtained average blood sugar level to 120 minutes is

considerably increased compared to the initial mean before oral administration of glucose.

4. Following testing, we established for the tinctures studied the optimum concentration at which the most powerful antihyperglycemic effect and 200 mg / kg respectively for tincture *Dorycnii pentaphylli herba* and 150 mg/kg for tinctures *Tamaricis ramosissimae folium et flos, Tragoponis pratensis folium, Cardui acanthoiditis folium, Acanthi balcanici herba* and *Myrtilli fructus*.
5. In the OGTT all plant extracts have shown anti-hyperglycaemic capacity, the groups II-VII blood glucose level treated with tincture being reduced in comparison with group I.
6. Comparative with *Myrtilli fructus*, *Tragoponis pratensis folium tincture* has similar hypoglycaemic efficacy, while *Cardui acanthoiditis folium, Acanthi balcanici herba, Tamaricis ramosissimae folium et flos* and *Dorycnii pentaphylli herba* have superior efficacy.
7. *Acanthi balcanici herba* tincture 150 mg / kg of has the strongest antihyperglycemic effect comparative with the other extracts tested, observed at all time intervals in the oral glucose tolerance test (30, 60, 90, 120 minutes).
8. Tinctures were administered as pretreatment, one hour prior to TTGO initiation, their possible antihyperglycemic mechanisms targeting: blocking glucose uptake, increasing glucose tissue sensitivity, increasing the ^{rate} of synthesis and insulin secretion or insulinomimetic effect.

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