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TESTING THE HYPOGLYCEMIC AND LIPID-LOWERING EFFECT OF *DORYCNIUM HERBACEUM* PLANT POWDER COMPARED TO BILBERRY, USING MICE WITH STREPTOZOTOCIN DIABETES

Varut Renata-Maria*
University of Medicine and Pharmacy of Craiova, Pharmacy, Department 1, physical chemistry, 2-4 Petru Rares Str., 200349, Craiova, Romania,

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Corresponding author*

ABSTRACT

Diabetes mellitus is one of the most serious and most common multisystemic diseases with increasing prevalence and incidence, leading to early disability and mortality. In the pharmaceutical field, there has been an increase in the demand for natural hypoglycemic products, the management of diabetes being a challenge for the medical system. In this context, the present study aims to investigate the hypoglycemic and lipid-lowering action of plant powder derived from *Dorycnium herbaceum* using experimentally streptozotocin-induced diabetic mice. For the induction of diabetes, streptozotocin was injected intraperitoneal in a single dose of 180 mg/kg b.w. The experiment was performed on 4 groups of 5 adult Swiss Albino Mice mice, the animals receiving daily medication at the same time for four weeks. Three control groups were used in the experiment: group I consisting of mice with normal pancreatic function, diabetic group II and diabetic group IV, treated with *Vaccinium myrtillus fructus* powder (50 mg / kg), a recognized product for the hypoglycemic and hypolipidemic effect . diabetic mice Group III diabetic mice were treated with *Dorycnium herbaceum herba* powder (50 mg / kg). At seven-day intervals, after a 12-hour diet, we determined blood glucose, cholesterol, and triglycerides, blood being harvested from the tail vein. The results obtained support the efficacy of the studied powders, normalizing the values of glycemia and lipids level in diabetes.

ORIGINAL RESEARCH ARTICLE

Introduction

Diabetes mellitus is one of the most serious and most common multisystem diseases with increasing prevalence and incidence, leading to early disability and mortality[1]. It is a complex metabolic disorder that may have multiple etiologies characterized by chronic hyperglycemia associated with changes in glucose, lipid, protein and hydroelectrolytic metabolism resulting from poor insulin secretion, insulin resistance, or both[2]. In diabetes, cellular metabolism is deeply disturbed, and glucose is not available for energy metabolism, resulting in exhaustion of liver glycogen stores. The level of serum lipids increases, especially the level of triglycerides, and there is an imbalance in the HDL / LDL cholesterol ratio, with the decrease in HDL cholesterol and the increase in the incidence of atherosclerosis[3]. Management of diabetes with plant extracts is still a challenge for the medical system. In the pharmaceutical field, there has been an increase in the demand for natural anti-diabetic products, which have fewer side effects. Although medicinal plants

have been used throughout the world for centuries to treat diabetes, few have been validated by scientific criteria. Lately, numerous experimental induction protocols of diabetes have been developed and refined to better understand the pathogenesis of diabetes and to test new therapeutic principles[4]. Specialty literature reports that the type of diabetes and its characteristics differ depending on the dose of streptozotocin administered and the species of animal used. The studies demonstrated the efficacy of inducing type 1 diabetes within 24 hours or within one week of streptozotocin injection, intraperitoneally, at a single dose of 180 mg / kg b.w.[5] The reference hypoglycemic product used in the study is the plant powder derived from Vaccinium myrtillus fructus, traditionally used to maintain glucose homeostasis in diabetic patients, the efficacy of the preparations being comparable to that of biguanides[6]. The active principles contained in bilberry powder confers potential lipid-lowering, anti-oxidant, and anti-inflammatory[7], lowering cholesterol,[8] and lowering the plasma levels of triglycerides[9].

Polyphenols have many therapeutic properties, notably antioxidant and antibacterial potential. Regular consumption has beneficial properties on human health, reducing the risk of developing diabetes[10].

Recent studies confirm the rich content of polyphenolcarboxylic acids and flavonoid of the hydroalcoholic extract from DH-h. DPH tincture contains in its traces apigenol-7-glucoside flavonoids, rutin, chlorogenic acid, chlorophylls, volatile compounds (acid ethyl ester ethanimidic, 2-propane-1-ol acetate, 1.1 diethoxy ethane) [11].

Materials and Methods

Preparation of the plant product.

The plant products to be tested were harvested from the Botanical Garden of the University of Craiova. The plants were dried in well-ventilated spaces, the vegetable products were then brought to a suitable degree of crushing with an electric grinder.

Protocol introducing diabetes mellitus.

To induce diabetes mellitus, we used healthy, male Swiss albino mice weighing between 35-45 grams and 6-8 weeks of age, which were obtained from the biobased of the Faculty of Medicine and Pharmacy, Craiova. Animals were kept at dietary (free access to water to maintain optimal hydration) 12 hours prior to streptozotocin injection and three hours after injection. For the induction of experimental diabetes, streptozotocin was injected at a single dose of 180 mg/kg, intraperitoneally, the injected amounts being determined according to the body weight of the animal[12]. Following streptozotocin administration, animals had access to a glucose solution (5%) for 24 hours to avoid hypoglycemia, knowing that pancreatic cell destruction leads to a massive release of insulin.

Mouse weight and glycemia were monitored prior to streptozotocin injection, then 72 hours and one week, the animals were maintained at 12-hour fast before blood samples were collected for biochemical determinations.

Animals with blood glucose values above 300 mg/dl, confirmed by two determinations at 3 days, were considered diabetic and were introduced into the research groups[13].

The experiment was approved by the University and Scientific Ethics and Deontology Commission of the Faculty of Medicine and Pharmacy, Craiova, in accordance with the standards presented in the animal care and use guidelines in experimental models[14].

An experimental model for the determination of the hypoglycemic and hypolipidemic potential of plant extracts

Using experimentally induced diabetic mice we investigated the potential hypoglycemic and lipid-lowering effect of a very rarely studied plant species: *Dorycnium pentaphyllum subsp herbaceum* (spearmint, cat's cheese) taking as a reference the proven hypoglycaemic effect of *Vaccinium myrtillus fructus*. During the experiment, the animals stayed in cages polypropylene with sawdust bedding in a well-ventilated room. Each box contained a test group consisting of five mice, which were maintained at a temperature of

24-28 ° C, relative humidity 60-70%, alternating 12 hours light/dark, Animals were fed standard and water *ad libitum*. The diet of animals contains carbohydrates, proteins, fats, mineral salts, and vitamins. In the test, we performed four groups of 5 mice, each group receiving the same medication daily at 9.00 in the same doses for four weeks. The groups were numbered I-IV for a precise description of the test because each group of animals was given different treatment.

Group I - the normal control group, consisting of mice with normal pancreatic function, did not receive medication and had free access to normal diet and distilled water.

Group II - the diabetic control group, consisting of diabetic mice, did not receive medication and had access to a normal diet and distilled water.

Group III - made up of diabetic mice, to which we administered 50 mg/kg of *Dorycnium herbaceum herbal* by gavage, suspended in 0.3 ml of distilled water daily, once daily for 4 weeks.

Group IV - consisting of diabetic mice, to which we have administered 50 mg/kg of *Vaccinium myrtillus fructus* powder gavage suspended in 0.3 ml of distilled water daily, once daily for 4 weeks.

Before the plant extracts were administered, the animals were weighed and their blood sugar, cholesterol, and triglycerides determined. The animals were monitored over four weeks, the parameters being glucose, cholesterol and plasma triglycerides. At seven-day intervals at the same time, the animals were monitored after a 12-hour fast. Glucose was determined using an eBensensor meter, cholesterol and plasma triglycerides were determined using an AccuTrend device, blood being harvested from the tail vein. [15,16]

Results and Discussions

Table 1 Monitoring the water and food consumption, during the experiment

Groups	**Water consumed**	**Food consumption**
	M ± SD (ml/zi)	**M ± SD (g/zi)**
Group I	10 ± 1,29	4,57 ± 0,97
Group II	32 ± 2,16	9,71 ± 1,38
Group III	21 ± 2,16	5,42 ± 0,78
Group IV	21,42 ± 1,90	5,85 ± 0,89

Table 2- Serum glucose levels in the groups studied during the experiment.

Groups	**Initial blood glucose level, (mg/dl)**	**First week blood glucose, (mg/dl)**	**Glycemia week 2, (mg/dl)**	**Glycemia week 3, (mg/dl)**	**Glycemia week 4, (mg/dl)**
Group I	85.2 ± 1.92	86.8 ± 2.86	87.2 ± 3.70	84 ± 3.16	88.8 ± 1.92
Group II	314.6 ± 3.57	312.8 ± 4.76	323 ± 4.52	317,6 ± 4,87	318 ± 5,14
Group III	317.4 ± 5.27	265.4 ± 9.37	224.2 ± 8.28	146.6 ± 5.31	133 ± 2.91
Group IV	316.2 ± 6.53	294.6 ± 5.02	263.86 ± 8.01	212.8 ± 3.86	163. 6 ± 6.26

Data is represented as Mean ± Standard Deviation

Table 3: Values of serum cholesterol in the groups studied during the experiment.

Groups	Initial cholesterol, day 0, (mg / dl)	Cholesterol first week, (mg / dl)	Cholesterol Week. 2, (mg / dl)	Cholesterol Week. 3, (mg / dl)	Cholesterol Week. 4, (mg / dl)
Group I	76 ± 1.58	74.6 ± 1.14	78.8 ± 1.78	77.4 ± 1.51	75.4 ± 1.14
Group II	174 ± 2	175 ± 1.58	177.6 ± 2.88	176.6 ± 1.51	175 ± 1.58
Group III	173.2 ± 1.64	172± 1.58	168 ± 1.58	160.4 ± 4.03	149.8 ± 3.56
Group IV	173.8 ± 2.86	173.2 ± 1.3	169.4 ± 1.14	167.2 ± 2.68	159.46 ± 1.34

Data are represented as Mean ± Standard Deviation

Table 4 Values of plasma triglycerides in the groups studied during the experiment.

Groups	Initial triglycerides. M ± SD (mg / dl)	Triglycerides Week. 5. M + SD (mg / dl)
Group **I**	105.8 ± 2.58	105.6 ± 4.61
Group **II**	209.8 ± 1.92	209.2 ± 2.58
Group **III**	209.2 ± 2.58	131.4 ± 3.04
Group **IV**	206.8 ± 3.19	150.6 ± 4.15

Data is represented as Mean ± Standard Deviation

The administration of vegetable powders limits the polyphagia and polydipsia. characteristic of type 1 diabetes. It can be seen that the non-medicated diabetic animals of the second group show the highest consumption of water (32 ± 2.16 ml/day) and food (9. 71 ± 1.38 g / day).

All animals brought in lots of work as diabetes have blood glucose levels exceeding 300 mg/dl by measuring morning on an empty stomach. During the four weeks. there was a tendency to normalize blood glucose levels in the DP-h and VM-f treated groups. At the end of the experiment. group III treated with DP-h powder showed the lowest mean blood sugar (133 ± 2.91 mg/dl). VM-f treated group IV had higher mean blood glucose levels compared with DP-h (163.6 ± 6.26 mg/dl).

The group III treated with DP-h (149.8 ± 3.56 mg/dl) had lower mean cholesterol compared to untreated diabetic group II (175 ± 1.58 mg/dl) and group IV treated with VM-f (159.46 ± 1.34 mg/dl).

Average triglyceride values at the start of the study for diabetic animals are around 200 mg/dl. At the end of the experiment. the mean value for animals of group III (131.4 ± 3.04 mg/dl) and IV (150.6 ± 4.15 mg/dl) was decreased.

Conclusions

Dorycnium herbaceum is a little-studied plant known to have an antibacterial and antifungal effect.

In recent decades. *in-vivo* researches on the hypoglycemic action of plant extracts have received particular attention. active principles from plants being an effective alternative to treating diabetes. All animals develop diabetes after a single injection of streptozotocin (180 mg/kg. i.p.). with hyperglycemia remaining during the experiment. The plant products to be tested

have demonstrated a significant hypoglycemic potential. the *Dorycnium herbaceum* species having the strongest effect. Both vegetal powders belonging to the species *Dorycnium herbaceum* and *Vaccinium myrtillus* caused a very significant decrease in plasma triglyceride levels.

The administration of plant extracts in diabetic animals has had positive effects on water and food consumption. influencing favorably body weight.

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