Therapeutics, Pharmacology and Clinical Toxicology
Vol XIII, Number 2, June 2009
Pages 183-187

ORIGINAL PAPER

EVALUATION OF HYPOGLYCEMIC EFFECT OF SOME VEGETAL SPECIES ON STREPTOZOTOCIN-INDUCED DIABETES IN RATS

Anca Berbecaru-Iovan[1], G. D. Mogoșanu[2], S. Berbecaru-Iovan[3], F. Gorunescu[4], Cristiana Iulia Dumitrescu[1], Florica Popescu[1]

[1.] *Department of Pharmacology, University of Medicine and Pharmacy of Craiova*
[2.] *Department of Pharmacognosy–Phytotherapy, University of Medicine and Pharmacy of Craiova*
[3.] *Department of Internal Medicine, University of Medicine and Pharmacy of Craiova*
[4.] *Department of Mathematics, Biostatics and Informatics, University of Medicine and Pharmacy of Craiova*

Abstract. Diabetes mellitus, the most common endocrine disorder, despite current therapies, has numerous and serious long term complications, leading to significant morbidity and mortality. The traditional methods of treatment of diabetes should be further investigated. A multitude of vegetal remedies have been utilized for the treatment of diabetes throughout the world for many centuries. Phytotherapy offers a valuable opportunity to discover new natural compounds with beneficial effects on glucose homeostasis, compounds that stimulate insulin biosynthesis and secretion or promote peripheral glucose uptake and utilization. The purpose of this study is to investigate the oral hypoglycemic effect of six vegetal products (Cynosbati fructus, Myrtilli folium, Myrtilli fructus, Phaseoli sine seminibus fructus, Trigonellae foenum-graeci semen, Violae tricoloris herba) in streptozotocin-induced diabetic rats. Some of these vegetal remedies are used in the treatment of diabetes mellitus, but for Cynosbati fructus and Violae tricoloris herba, so far, there is no certitude about the hypoglycemic action. This study indicates that the vegetal products used possess hypoglycemic effect, but they are smooth remedies and could offer solutions for the newly discovered patients who only need diet and exercise or as complementary treatment in diabetic patients after initiating pharmacological therapy.
Keywords: diabetes mellitus, phytotherapy, hypoglycemic effect

Introduction

While management of diabetes mellitus includes diet, exercises, oral hypoglycemic agents and insulin, these treatments do not effectively prevent the numerous and serious long term complications. All over the world, for many centuries, several herbal medicines in different oral formulas have been recommended for diabetes mellitus treatment. Trigonella foenum-graecum, Allium sativum, Momordica charantia, Vaccinium myrtillus, Panax Ginseng, Arctium lappa are some of the vegetal species that have been used as herbal medicine for the treatment of diabetes in folk medicine throughout the world. The hypoglycemic effect may be due to the stimulation of insulin biosynthesis and secretion or to the increase in the levels of glucose transporters and stimulation of peripheral glucose uptake and utilization. Phytotherapy offers a valuable opportunity to discover new natural compounds with beneficial effects on glucose homeostasis and with the possibility of developing a few useful drugs from medicinal plants with a long history of human use.

Material and methods

Vegetal species: The vegetal products utilized (Cynosbati fructus, Myrtilli folium, Myrtilli fruc-

Anca Berbecaru-Iovan
Department of Pharmacology,
University of Medicine and Pharmacy of Craiova
Email: ancaberbecaru@yahoo.com

tus, Phaseoli sine seminibus fructus, Trigonellae foenum-graeci semen, Violae tricoloris herba) were purchased from herbalists or collected and left for drying in the shade and then reduced to a powder.

Animals and experimental design: All experiments were performed using eight groups (five animals each) of healthy adult male Wistar rats with body weights ranging from 180 to 220 g. The animal house was well ventilated with 12 hours day and night schedule and maintained at 25±1°C. The animals were fed with standard rat feed and water ad libitum. All animal experiments were performed in accordance with the bioethical rules of scientific research of our institute.

For repeated oral administration, the rats were treated once daily using an intragastric tube for 4 weeks.

Blood glucose level, total cholesterol level and body weight change were monitored weekly between nine and ten o'clock in the morning. Daily food and water consumption were determined by subtracting the remaining quantities from the total amount provided.

The experimental groups were as follows: group 1, non-diabetic control rats (NDC); group 2, diabetic control rats – untreated (UDC); group 1 and group 2 were orally administered using an intragastric tube with distilled water daily 4 ml; groups 3-8, diabetic rats – treated (TD_{3-8}; categorized by alphabetical order of the vegetal species administered) - fed orally by an intragastric tube with 1.5 g/kg body weight powder (of each vegetal species used, categorized alphabetically) suspended in 4 ml of distilled water.

Experimental induction of diabetes: Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ); the rats were fasted for 12 hours before the induction of diabetes and were injected intraperitoneally with fresh prepared solution of STZ (70 mg STZ/kg body weight; STZ was dissolved in 0.05 M sodium citrate buffer, pH 4.5). Diabetic rats were confirmed by measuring the fasting blood glucose levels using a blood glucose meter Glucotrend on blood samples collected from the tail vein at seventy two hours after injection with STZ. Animals with a blood glucose level above 150 mg/dl were considered to be diabetic and included in the experimental diabetic groups. Blood cholesterol levels were measured using a blood cholesterol meter.

Statistical analysis: the values are presented as means ± SD of 5 rats in each group. All the data were statistically evaluated by *t*-value two-sided test; the limit of statistical significance was set at P-level < 0.05.

Results

At the end of the experimental period (4 weeks), blood glucose level in the untreated diabetic control group G2 was significantly higher ($p < 0.05$) than in the non-diabetic control group G1 (286.5 ± 41.4 vs. 88.3 ± 21.2 mg/dl) and it was significantly lower ($p < 0.05$) in the treated diabetic groups G3-G8 than in the UDC group G2. At the end of the experimental period of 4 weeks, the blood glucose level was significantly lower in the following treated diabetic groups: G4 (106.5 ± 22.9 mg/dl), G5 (116.4 ± 35.6 mg/dl), G6 (108.2 ± 29.3 mg/dl), G7 (105.8 ± 26.4 mg/dl) than in the UDC group G2 (286.5 ± 41.4 mg/dl); in TD groups G3 (210.9 ± 33.5 mg/dl) and G8 (189.9 ± 21.5 mg/dl) the blood glucose level was slightly lower than that of the UDC group G2 (Table I, Table I.1).

The treatment of diabetic animals with the vegetal species powder produced a significant decrease in blood glucose concentrations compared to those of untreated diabetic control animals; the results indicated potent anti-diabetic activity of Myrtilli

Group		Blood glucose level mg/dl	
		1st week	4 weeks
Non-diabetic control NDC – G1		90.24 ± 17.3	88.3 ± 21.2
Untreated diabetic control UDC – G2		170.3 ± 18.2	286.5 ± 41.4
Treated diabetic TD	G3	172.7 ± 20.5	210.9 ± 33.5
	G4	164.6 ± 11.3	106.5 ± 22.9
	G5	168.1 ± 7.8	116.4 ± 35.6
	G6	167.2 ± 9.3	108.2 ± 29.3
	G7	169.7 ± 17.1	105.8 ± 26.4
	G8	170.6 ± 12.5	189.9 ± 21.5

Table I. Blood glucose level at the end of the first week and after 4 weeks experimental period

P-value for the t-value two-sided test			
G2 vs. G1 -1st week	0.0001	G2 vs. G1 -4th week	0.0000
G2 vs. G3 -1st week	0.85	G2 vs. G3 -4th week	0.0131
G2 vs. G4 -1st week	0.57	G2 vs. G4 -4th week	0.0000
G2 vs. G5 -1st week	0.81	G2 vs. G5 -4th week	0.0001
G2 vs. G6 -1st week	0.74	G2 vs. G6 -4th week	0.0000
G2 vs. G7 -1st week	0.96	G2 vs. G7 -4th week	0.0000
G2 vs. G8 -1st week	0.97	G2 vs. G8 -4th week	0.0017

Table I.1 Blood glucose level – comparison

folium (G4), Myrtilli fructus (G5), Phaseoli sine seminibus fructus (G6), Trigonellae foenum-graeci semen (G7) and slightly anti-diabetic activity of Violae tricoloris herba (G8) and Cynosbati fructus (G3) (Figure 1).

Total cholesterol level, at the end of the 4 weeks experimental period, was significantly higher ($p < 0.05$) in the UDC group G2 than in the NDC group G1 (229.8 ± 28.7 vs. 164.3 ± 14.6 mg/dl). All the values of total cholesterol for TD groups G3-G8

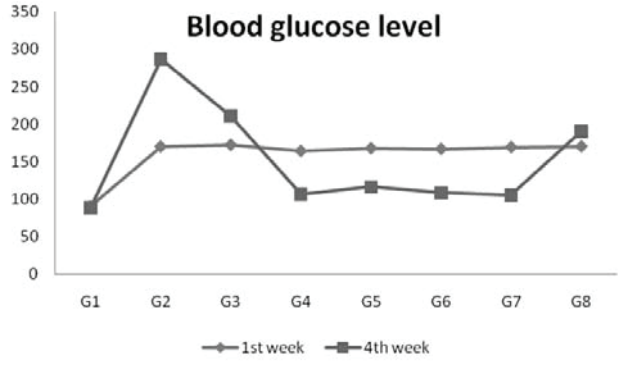

Figure 1. Changes in blood glucose level at the end of first week and after 4 weeks experimental period

Group		Total cholesterol level mg/dl	
		1st week	4 weeks
Non-diabetic control NDC – G1		158.6 ± 16.5	164.3 ± 14.6
Untreated diabetic control UDC – G2		221.8 ± 29.6	229.8 ± 28.7
	G3	209.6 ± 27.8	196.4 ± 16.5
	G4	216.3 ± 24.6	204.8 ±12.3
Treated diabetic TD	G5	220.7 ± 26.1	210.4 ± 22.1
	G6	222.4 ± 18.6	215.4 ± 20.2
	G7	209.7 ± 30.5	186.4 ± 10.3
	G8	224.3 ± 19.6	220.6 ± 13.3

Table II. Total cholesterol level at the end of the first week and after 4 weeks experimental period

P-value for the t-value two-sided test			
G2 vs. G1 -1st week	0.0031	G2 vs. G1 -4th week	0.0019
G2 vs. G3 -1st week	0.52	G2 vs. G3 -4th week	0.0541
G2 vs. G4 -1st week	0.76	G2 vs. G4 -4th week	0.1112
G2 vs. G5 -1st week	0.95	G2 vs. G5 -4th week	0.27
G2 vs. G6 -1st week	0.97	G2 vs. G6 -4th week	0.39
G2 vs. G7 -1st week	0.54	G2 vs. G7 -4th week	0.0129
G2 vs. G8 -1st week	0.88	G2 vs. G8 -4th week	0.53

Table II.1 Total cholesterol level - comparison

were also significantly higher than those of the NDC group. As for the treated diabetic groups, we observed a significant decrease ($p < 0.05$) in the G3 (196.4 ± 16.5 mg/dl) and G7 (186.4 ± 10.3 mg/dl) than in the UDC group G2 (Table II, Table II.1).

A significant decrease in the total cholesterol was determined through oral administration of the Trigonellae foenum-graeci semen powder (G7), followed by Cynosbati fructus powder (G3) and a slight decrease was observed in animals treated orally with Myrtilli folium powder (G4) (Figure 2).

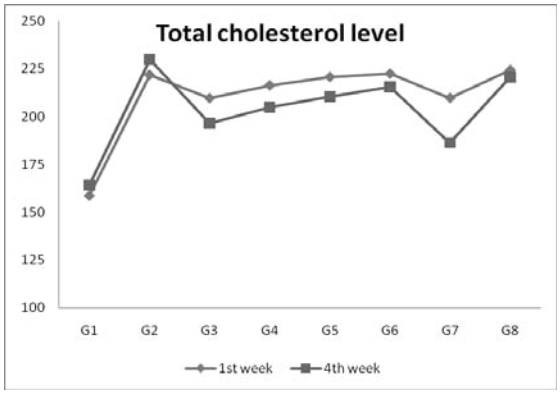

Figure 2. Changes in total cholesterol level at the end of first week and after 4 weeks experimental period

During the experimental period, water intake and food intake in diabetics groups G2-G8 were significantly higher ($p < 0.05$) than those in the NDC group G1; as for water intake, in the TD groups there were significantly lower values ($p < 0.05$) for G4, G5, G6, G7 than in the UDC group G2. Body weight gain in diabetic groups G2-G8 was significantly lower ($p < 0.05$) than in the NDC group G1; also body weight gain is higher in the TD groups G3-G8 than in the UDC group G2 (Table III).

The results for water intake, food intake and body weight gain indicated that the administration of potent anti-diabetic vegetal species Myrtilli

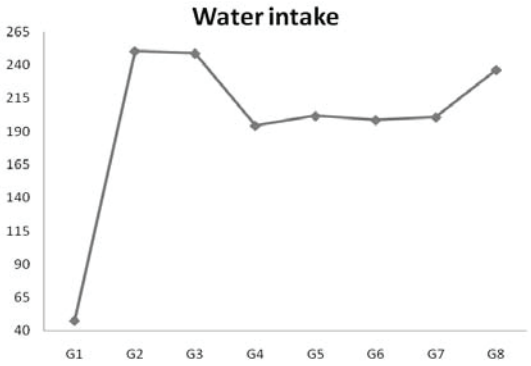

Figure 3a. Water intake during the experimental period

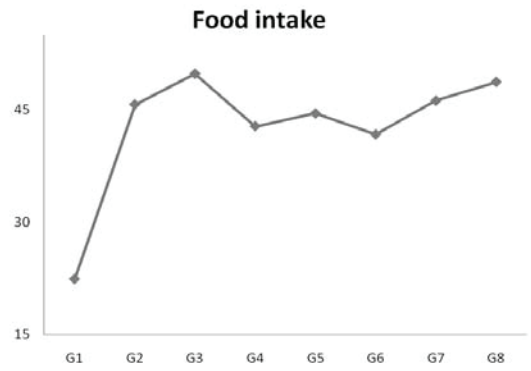

Figure 3b. Food intake during the experimental period

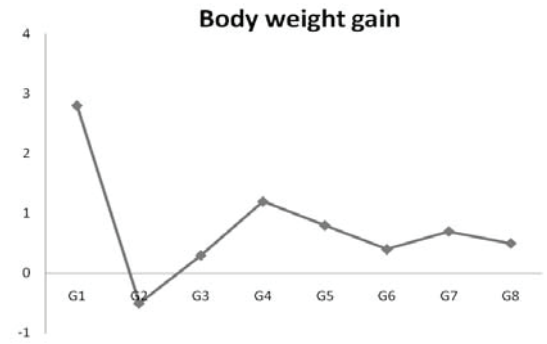

Figure 3c. Body wight gain during the experimental period

Group		Water intake (ml/day)	Food intake (g/day)	Body weight gain (g/day)
Non-diabetic control NDC – G1		47.5 ± 3.5	22.4 ± 4.2	2.8 ± 1.2
Untreated diabetic control UDC – G2		250.2 ± 24.6	45.7 ± 3.5	–0.5 ± 0.3
Treated diabetic TD	G3	248.6 ± 36.5	49.8 ± 2.9	0.3 ± 0.2
	G4	194.2 ± 26.4	42.8 ± 1.7	1.2 ± 0.6
	G5	201.5 ± 19.2	44.5 ± 2.6	0.8 ± 0.5
	G6	198.4 ± 24.9	41.7 ± 2.4	0.4 ± 0.3
	G7	200.6 ± 28.4	46.2 ± 3.7	0.7 ± 0.5
	G8	236.2 ± 39.8	48.7 ± 3.7	0.5 ± 0.3

Table III. Water intake, food intake and body weight gain during the experimental period

folium (G4), Myrtilli fructus (G5), Phaseoli sine seminibus fructus (G6), Trigonellae foenum-graeci semen (G7) might also be effective in metabolic disorders; furthermore, the experiment showed that the administration of slightly anti-diabetic vegetal species Violae tricoloris herba (G8) and Cynosbati fructus (G3) did not determine effective actions regarding daily needs (Figure 3a, 3b, 3c).

Discussion

Since the introduction in diabetes oral treatment of metformin, a drug based on a compound extracted from Galega officinalis known as goat's rue, a traditional European medicinal plant, drew the attention of researchers throughout the world towards medicinal plants for searching and isolating the hypoglycemic agents. Herbal medicines in different oral formulas have been recommended for diabetic patients but the mechanisms of hypoglycemic activities remain without certain elucidation. There is a wide range of possible mechanisms of action: significant increase of beta-cells number or deterrence of the beta-cells' death and permission of the recovery of partially destroyed beta-cells, increase in insulin secretor activity; improvement of insulin sensitivity and increase of glucose uptake by tissues; some vegetal remedies prevent the long-term diabetes' complications possibly by decreasing the oxidative stress and modulating the xenobiotics' metabolism, the depression of gluconeogenic enzymes or by simply reducing the intestinal absorption of glucose.

Conclusion

This study was carried out to evaluate the hypoglycemic effect of six vegetal products (Cynosbati fructus, Myrtilli folium, Myrtilli fructus, Phaseoli sine seminibus fructus, Trigonellae foenum-graeci semen, Violae tricoloris herba) and the results indicated the potent anti-diabetic activity of Myrtilli folium, Myrtilli fructus, Phaseoli sine seminibus fructus, Trigonellae foenum-graeci semen and the slightly anti-diabetic activity of Violae tricoloris herba and Cynosbati fructus. The results also indicated hypolipidemic effects of Trigonellae foenum-graeci semen, followed by Cynosbati fructus; that indicated the possibility that these vegetal species could be effective for preventing or delaying the development of diabetes' complications due to metabolic disorders.

Currently, the mechanisms involved in the hypoglycemic effects of anti-diabetic vegetal species are not established and we know little about them; regardless of the mechanism of action, the anti-diabetic vegetal species could safely be prescribed to diabetic patients, since there is a long-term use of natural plant substances which even slightly reduce hyperglycemia with no known harmful side effects at recommended doses. Diabetic patients often assume some products without medical advice and the physicians and pharmacists have to consider these suggestions, and especially explain that the complementary treatment is not sufficient and specify the adequate treatment. Furthermore, we have to consider or should not forget that the efficacy of placebo is often high, especially in diabetic patients who are permanently concerning about their medical condition; doctors and pharmacists have the most important role in advising diabetic patients and they need to be familiar with this type of medicine including vegetal remedies and dietary supplements.

References

1. **Abdel-Hassan IA, Abdel-Barry JA, Tariq Mohammeda S T**he hypoglycaemic and antihyperglycaemic effect of citrullus colocynthis fruit aqueous extract in normal and alloxan diabetic rabbits. *J Ethnopharmacol* **71(1–2):325–330.**

2. **Andrade-Cetto A, Heinrich M** Mexican plants with hypoglycaemic effect used in the treatment of diabetes. *J Ethnopharmacol* **99:325–348.**

3. **Badami S, Om Prakash, Dongre SH, Suresh B** In vitro antioxidant properties of Solanum pseudocapsicum leaf extracts. *Indian J Pharmacol* **37:251–252.**

4. **Basch E, Gabardi S, Ulbricht C** Bitter melon (Momordica charantia): a review of efficacy and safety. *Am J Health Syst Pharm* **60(4):356–359.**

5. **Diatewa M, Samba CB, Assah TC, Abena AA** Hypoglycemic and antihyperglycemic effects of diethyl ether fraction isolated from the aqueous extract of the leaves of Cogniauxia podoleana Baillon in normal and alloxan-induced diabetic rats. *J Ethnopharmacol* **92 (2–3):229–232.**

6. **Hsu WT, Tsai LY, Lin SK, Hsiao JK, Chen BH** Effects of diabetes duration and glycemic control on free radicals in children with type 1 diabetes mellitus. *Ann Clin Lab Sci* **36(2):174–178.**

7. **Mann JI** Can dietary intervention produce long-term reduction in insulin resistance? *Br J Nutr* **83 [Suppl 1]: S169–S172.**

8. **Mukherjee PK, Maiti K, Mukherjee K, Houghton PJ** Leads from Indian medicinal plants with hypoglycemic potentials. *J Ethnopharmacol* **106:1–28;**

9. **Nthangeni G, Steyn NP, Alberts M et al.** Dietary intake and barriers to dietary compliance in black type 2 diabetic patients attending primary health-care services. *Public Health Nutr* **5:329–338.**

10. **Ryan GJ, Wanko NS, Redman AR, Cook CB** Chromium as adjunctive treatment for type 2 diabetes. *Ann Pharmacother* **37(6):876–885.**

11. **Szkudelski T** The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiol Res* **50:536–546.**

12. **Vats V, Yadav SP, Grover JK** Effect of T. foenumgraecum on glycogen content of tissues and the key enzymes of carbohydrate metabolism. *J Ethnopharmacol* **85 (2–3):237–242.**