PharmacologyOnLine

Archives • 2021 • vol.2 • 62-67

STUDY OF HYPOGLYCEMIC ACTIVITY OF ANTIDIABETIC HERBAL MIXTURE ON STREPTOZOTOCIN-NICOTINAMIDE-INDUCED RAT MODEL OF TYPE 2 DIABETES

Savych, Alona*[1], Polonets, Olha[2]

[1]Department of Pharmacognosy with Medical Botany, I. Horbachevsky Ternopil National Medical University, Ukraine

[2]Department of Pharmaceutical Chemistry, National Pirogov Memorial Medical University, Ukraine

*alonasavych@gmail.com

Abstract

Diabetes is a global problem today, as the number of patients in the world increases sharply every year. Severe complications of diabetes are very dangerous, as they can lead to disability and high mortality.

The purpose of the study was to establish the hypoglycemic property of new herbal mixture (*Equiseti arvensis herba, Sambuci flores, Inulae rhizomata et radices, Hyperici herba, Tiliae flores, Polygoni avicularis herba, Myrtilli folium, Urticae folia*) in streptozotocin-nicotinamide-induced diabetic rats.

Experimental diabetes type 2 was reproduced on male albino rats of the Wistar strain by injection of streptozotocin (65 mg/kg) and nicotinamide (230 mg/kg).

The results showed that the use of antidiabetic herbal mixture for four weeks reduced the severity of hyperglycemia, prevented the development of secondary insulin resistance and increased glucose tolerance in rats with streptozotocin-nicotinamide-induced diabetes mellitus, which was manifested by an increase of coefficient of sensitivity to insulin and a decrease of hyperglycemia to baseline during the intraperitoneal glucose tolerance test.

Keywords: *diabetes mellitus, herbal mixtures, insulin resistant, streptozotocin, nicotinamide,* hypoglycemic activity

Introduction

Diabetes mellitus is a global social and medical problem caused by the rapid spread of the disease and the development of serious complications such as micro- and macroangiopathies, which significantly reduce the quality and life expectancy of patients [1]. According to the official data from the International Diabetes Federation (2019), the number of patients is projected to increase to 642 million by 2040 [2].

An important problem of pharmacovigilance is that existing pharmacotherapy can effectively reduce hyperglycemia, but it is not always able to stabilize fluctuations in glycemic values during the day and maintain it at an optimal level. This leads to the formation of a cascade of pathological processes - excessive glycation and inactivation of the body's antioxidant defense system, triggering the processes of free radical oxidation of lipids and, as a consequently, the development of oxidative stress, which leads to the development and progression of diabetic angiopathies [3, 4, 5, 6]. In addition, a frequent trigger for the development of diabetic complications is a violation of the lipid profile, manifested by dyslipidemia, hypercholesterolemia and/or hypertriglyceridemia [7, 8, 9]. Therefore, the optimization of pharmacotherapy, search and study of new drugs with hypoglycemic activity for the prevention and treatment of this disease and its dangerous complications is a topical issue of pharmacy and medicine.

One such area is phytotherapy, as it has a number of advantages over traditional therapy with using oral synthetic agents, namely, it is low-toxic, has a mild pharmacological effect and can be used for long periods without significant side effects, is well combined with synthetic drugs, has a complex activity through a number of biologically active compounds [10, 11, 12, 13]. Particular attention deserve the combinations of different medicinal plants, because such herbal mixtures will have more biologically active substances that will influence on the all links of the pathogenetic mechanism of development of diabetes mellitus and its complications [14, 15, 16].

Thus, for this purpose, it is advisable to study the hypoglycemic properties of the investigated herbal mixtures in streptozotocin-nicotinamide-induced diabetic rats, which are used in folk medicine for the prevention and treatment of diabetes mellitus type 2 in Ukraine [14], but do not have a scientific basis.

Methods

Plant materials: It was used the herbal raw materials harvested in June – August 2017 in Ternopil region and Charpathians (*Vaccinium myrtillus* leaf) (Ukraine) during the study. After harvesting, the raw materials were dried, crushed and brought back to standard according to the general GACP requirements [17]. The plants were identified by Department of Pharmacognosy with Medical Botany, I.Horbachevsky Ternopil National Medical University, Ternopil, Ukraine. Samples of herbal raw materials have been deposited in Departmental Herbarium for future record.

For the study was used the herbal mixture with the studied phytochemical composition [18, 19, 20, 21, 22] that is used in folk medicine for the prevention and treatment of diabetes mellitus type 2 in Ukraine [14].

Extraction procedure: The sample of 10 g of powdered herbal mixture was put into a 100 mL conical flask and 120 mL of distilled water was added to each. The aqueous extract was obtained by heating in the boiling water bath for 30 minutes. The extract was filtered using Whatmann filter paper No. 1. Then the filtrate was evaporated by rotary evaporator and was lyophilized to dryness. The lyophilized powder of herbal mixture was stored at 4 °C for further use.

Drugs and chemicals: Streptozotocin (STZ) and nicotinamide (NA) was purchased from Sigma-Aldrich Chemical Company (Germany), the standard drug – metformin SANDOZ® from Lek S.A., Poland, Sodium thiopental for anesthesia from Abbott Park, IL, USA. All chemicals that were used in the research were analytical graded.

Experimental Animals: The study was performed on male albino rats of the Wistar strain weighing between 180 g and 200 g, which were bred at the animal house of the Central Research Laboratory of I.Horbachevsky Ternopil National Medical University, where they were kept under appropriate

conditions (at a constant room temperature of 22 ± 1°C, 40-70% humidity conditions and a 12-hour light/dark cycle). Throughout the experimental period, the animals received standard rat diet and water *ad libitum.* The animals were treated in accordance with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community Guidelines [23]. All protocols for animals experiment were approved by the animal ethical committee of I.Horbachevsky Ternopil National Medical University.

Experimental Protocol: Rats were randomly divided into four groups of eight animals (n=8) each and received different treatments once daily for 28 days. Group I (Control): received per os (*p.o*) distilled water (12 mL/kg/day). Group 2 (STZ-NK) received single intravenous injection of STZ (65 mg/kg), freshly prepared in 0.1 M sodium citrate buffer (pH 4.5) and intraperitoneally injection of NK (230 mg/kg), 15 minutes before STZ introduction and distilled water (9 mL/kg/day, *p.o.*). Group III (STZ-NK+MET) received STZ (65 mg/kg), NK (230 mg/kg) and the standard drug – metformin (60 mg/kg/day, *p.o.*). Group IV (STZ-NK+AHM) received STZ (65 mg/kg), NK (230 mg/kg) and the aqueous extracts of the studied herbal mixture (9 mL/kg/day, *p.o.*). The effective dose of herbal mixture extract was established during the previous screening testing [10]. At the end of the experiment, rats were sacrifice by decapitation after anesthesia with Sodium thiopental and the blood and the liver were collected.

Measurement of Intraperitoneal Glucose Tolerance Test (IPGTT): After overnight fasting (16-18 hours) on 28th day of the experiment, rats were injected intraperitoneally with glucose solution (2 g/kg, *i. p.*) in the morning [24]. The level of glucose in blood, which was obtained from tail vein, was determined before the introduction of glucose and after 20, 40, 60, 90 and 120 minutes using a glucose analyzer (glucometer Accuk-Check, Germany).

Statistical Analysis: The values were expressed as mean ± SEM. The data were analysed by using GraphPad Prism software version 5.03. The results were compared by using the ANOVA-One-Way test followed by *Mann-Whitney U test*. The difference was considered statistically significant at $p<0.05$.

Results and Discussion

According to the results of the study, the introduction of STZ and NK to rats led to the development of stable and moderate hyperglycemia (Table. 1). On the second day after the induction of diabetes basal glucose level was 3.6 times higher than in the animals of Control group and kept at the same level throughout the experiment.

The results of the study showed that the administration of antidiabetic herbal mixture for 28 days significantly ($p<0.05$) reduced hyperglycemia in animals with STZ-NK-induced diabetes mellitus. The level of basal glycemia was 2.4 times lower in the group STZ-NK+AHM than in the group STZ-NK on 28th day of the experiment. Moderate hyperglycemia developed only up to 2 weeks. However, by the end of the experiment, the blood glucose level dropped to the level of normal control (Table. 1). There was an improvement in glucose tolerance and a decrease in insulin resistance, as evidenced by the results of IPGTT (Table 2) and a short test for insulin (Table 3). The glycemic response of rats injected with antidiabetic herbal mixture did not differ from the usual Control group during IPGTT (Table 2). Similar effects were observed with the use of Metformin tablets.

Therefore, the oral administration of aqueous extract of the herbal mixture (*Equiseti arvensis herba, Sambuci flores, Inulae rhizomata et radices, Hyperici herba, Tiliae flores, Polygoni avicularis herba, Myrtilli folium, Urticae folia*) have shown its ability to reduce the manifestations of hyperglycemia and to normalize the impaired glucose tolerance in in STZ-NK-induced diabetic rats.

Conclusions

The results of the present study showed that aqueous extract of the herbal mixture (9 mL/kg/day) (*Equiseti arvensis herba, Sambuci flores, Inulae rhizomata et radices, Hyperici herba, Tiliae flores, Polygoni avicularis herba, Myrtilli folium, Urticae folia*) have potential hypoglycemic effects and reduce the development of impaired glucose tolerance, which was caused by STZ and NK administration. In addition, the studied plant mixture showed the ability to increase the coefficient of insulin sensitivity in rats with STZ-NK-induced diabetes. Established pharmacological properties make these

herbal mixtures perspective remedies for the prevention and treatment of of diabetes mellitus type 2.

References

1. American Diabetes Association (2020). Standards of Medical Care in Diabetes. *Diabetes care*, *43*, 1212.
2. International Diabetes Federation (2019). *IDF Diabetes Atlas, 9th ed.* Brussels, Available at: https://www.diabetesatlas.org
3. Skyler, J. S., Bakris, G. L., Bonifacio, E., Darsow, T., Eckel, R. H., Groop, L., Groop, P. H., Handelsman, Y., Insel, R. A., Mathieu, C., McElvaine, A. T., Palmer, J. P., Pugliese, A., Schatz, D. A., Sosenko, J. M., Wilding, J. P., & Ratner, R. E. (2017). Differentiation of Diabetes by Pathophysiology, Natural History, and Prognosis. *Diabetes*, 66(2), 241–255.
4. Ndjaboue, R., Farhat, I., Ferlatte, C. A., Ngueta, G., Guay, D., Delorme, S., Ivers, N., Shah, B. R., Straus, S., Yu, C., & Witteman, H. O. (2020). Predictive models of diabetes complications: protocol for a scoping review. *Systematic reviews*, 9(1), 137.
5. Savych, A.O., Marchyshyn, S.M., Kozyr, H.R., & Skrinchuk, O.Y. (2019). Basic principles of the using of medicinal plants and their collections for treatment and prevention of diabetes mellitus type 2. *Phytotherapy* chasopys, *4*, 43–46.
6. Marchyshyn, S., Polonets, O., Savych, A., & Nakonechna, S. (2020). Determination of carbohydrates of *Chrysanthemum morifolium L.* leaves and flowers by GC-MS. *Pharmakeftiki*, 32(4), 202-212.
7. Budniak, L., Slobodianiuk, L., Marchyshyn, S., Klepach, P., Honcharuk, Ya. (2021). Determination of carbohydrates content in *Gentiana cruciata* L. by GC/MS method. *International Journal of Applied Pharmaceutics*, 13(1), 124-128.
8. Budniak, L., Slobodianiuk, L., Marchyshyn, S., Demydiak, O. (2020). Determination of *Arnica foliosa* Nutt. fatty acids content by gc/ms method. *ScienceRise: Pharmaceutical Science*, 6(28), 14-18.
9. Marchyshyn, S., Slobodianiuk, L., Budniak, L., Skrynchuk, O. (2021). Analysis of carboxylic acids of *Crambe cordifolia* Steven. *Pharmacia,* 68(1), 15–21.
10. Savych, A., Marchyshyn, S., Harnyk, M., Kudria, V., & Ocheretniuk, A. (2021). Determination of amino acids content in two samples of the plant mixtures by GC-MS. *Pharmacia*, *68*(1), 283-289.
11. Savych, A., Marchyshyn, S., Kyryliv, M., Bekus, I. (2021). Cinnamic acid and its derivatives in the herbal mixtures and their antidiabetic activity. *Farmacia*, 69(3), 595-601.
12. Marchyshyn, S., Budniak, L., Slobodianiuk, L., Ivasiuk, I. (2021). Determination of carbohydrates and fructans content in *Cyperus esculentus* L. *Pharmacia*, 68(1), 211-216.
13. Slobodianiuk, L., Budniak, L., Marchyshyn, S., Basaraba, R. (2020). Investigation of the hepatoprotective effect of the common cat's foot herb dry extract. *PharmacologyOnLine*, 3, 310-318.
14. Savych, A., Marchyshyn, M., & Basaraba, R. (2020). Screening study of hypoglycemic activity of the herbal mixtures (Message 1). *ScienceRise: Pharmaceutical Science,* 4(26), 40-46.
15. Savych, A., Marchyshyn, M., Basaraba, R., & Lukanyuk, M. (2020). Antihyperglycemic, hypolipidemic and antioxidant properties of the herbal mixtures in dexamethasone-induced insulin resistant rats. *PharmacologyOnLine*, 2, 73-82.
16. Savych, A., Marchyshyn, M., & Naconechna, S. (2021). Influence of some herbal mixtures on insulin resistance and glucose tolerance in rats. *PharmacologyOnLine*, 1, 356-364.
17. WHO Guidelines on good agricultural and mixture practices (GACP) for medicinal plants (2003). *World Health Organization*, Geneva, Switzerland, 72.
18. Savych, A., Marchyshyn, S., Kozyr, H., & Yarema, N. (2021). Determination of inulin in the herbal mixtures by GC-MS method. *Pharmacia*, *68*(1), 181-187.
19. Savych, A., Marchyshyn, S., & Basaraba, R. (2020). Determination of fatty acid composition content in the herbal antidiabetic collections. *Pharmacia*, 67(3), 153–159.

20. Savych, A., Marchyshyn, S., & Milian, I. (2021). Determination of carbohydrates in the herbal antidiabetic mixtures by GC-MC. *Acta Pharmaceutica*, *71*(3), 429-443.
21. Savych, A., Marchyshyn, S., Basaraba, R., & Kryskiw, L. (2021). Determination of carboxylic acids content in the herbal mixtures by HPLC. *ScienceRise: Pharmaceutical Science*, *2*(30), 33-39.
22. Savych, A., Basaraba, R., Muzyka, N., & Ilashchuk, P. (2021). Analysis of fatty acid composition content in the plant components of antidiabetic herbal mixture by GC-MS. *Pharmacia*, *68*(2), 433-439.
23. EEC. "Council directive 2010/63/EU, of the 22nd September 2010 on the approximation of laws, regulations and administrative provisions of the member states regarding the protection of animals used for experimental and other scientific purposes". Offical Journal of the European Communities, 2010:1–29.
24. Jørgensen MS, Tornqvist KS, Hvid H. Calculation of glucose dose for Intraperitoneal Glucose Tolerance Tests in lean and obese mice. J Am Assoc Lab Anim Sci 2017; 56(1):95-97.

Table 1. Effect of aqueous extracts of the herbal mixtures and the comparison drug metformin on basal glycaemia in rats with STZ-NK-induced diabetes

Groups of animals	Blood glucose level, mmol/L			
	2 day	2 week	3 week	4 week
Control	4.51±0.25	4.40±0.17	4.40±0.19	3.74±0.25
STZ+NK	14.72±2.01*	16.47±1.28*	15.49±1.36*	8.65±0.91*
STZ+NK+AHM	3.99±0.17*/**	6.32±0.38*/**	5.14±0.25*/**	3.59±0.22**
STZ+NK+MET	3.54±0.48**	6.08±0.42*/**	4.80±0.34**	4.54±0.62**

Notes: Values are expressed as mean ± SEM from 8 rats; * p<0.05 with respect to Control group; ** p<0.05 with respect to STZ+NK group.

Table 2. Effect of aqueous extract of the herbal mixture and the comparison drug metformin during IPGTT in rats with STZ-NK-induced diabetes on 28-th day of treatment

Groups of animals	Blood glucose level, mmol/L					
	0 min	20 min	40 min	60 min	90 min	120 min
Control	3.74±0.25	13.77±0.75	9.44±0.45	6.25±0.40	5.67±0.33	5.24±0.51
STZ+NK	8.65±0.91*	24.05±1.75*	22.90±1.52*	19.88±1.52*	17.70±1.88*	16.14±1.06*
STZ+NK+AHM	3.59±0.215**	14.85±0.60**	11.64±0.92**	8.63±0.915*/**	5.54±0.51**	5.01±0.56**
STZ+NK+MET	4.54±0.62**	16.48±1.88**	13.30±0.98*/**	9.62±1.26*/**	6.73±1.23**	5.61±0.62**

Notes: Values are expressed as mean ± SEM from 8 rats; * p<0.05 with respect to Control group; ** p<0.05 with respect to STZ+NK group.

Table 3. Effect of aqueous extract of the herbal mixture and the comparison drug metformin on insulin sensitivity in rats with STZ-NK-induced diabetes

Groups of animals	Coefficient of sensitivity to insulin, %
Control	47.20±2.38
STZ+NK	17.34±3.29*
STZ+NK+AHM	40.44±3.28**
STZ+NK+MET	49.37±1.43**

Notes: Values are expressed as mean ± SEM from 8 rats; * p<0.05 with respect to Control group; ** p<0.05 with respect to STZ+NK group.