The Pharma Innovation Journal 2017; 6(3): 175-177

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating 2017: 5.03
TPI 2017; 6(3): 175-177

www.thepharmajournal.com
Received: 15-01-2017
Accepted: 16-02-2017

Alona Savych
Department of Pharmacognosy with Medical Botany, Pharmaceutical Faculty, I. Horbachevsky Ternopil State Medical University, Ruska 36, 46001 Ternopil, Ukraine

Svitlana Marchyshyn
Department of Pharmacognosy with Medical Botany, Pharmaceutical Faculty, I. Horbachevsky Ternopil State Medical University, Ruska 36, 46001 Ternopil, Ukraine

Correspondence
Alona Savych
Department of Pharmacognosy with Medical Botany, Pharmaceutical Faculty, I. Horbachevsky Ternopil State Medical University, Ruska 36, 46001 Ternopil, Ukraine

Investigation of pharmacological activity the new antidiabetic plant gathering in streptozotocin-nicotinamide-induced diabetes in the rats

Alona Savych and Svitlana Marchyshyn

Abstract
Diabetes mellitus is a global problem today, because there is a sharp increase the number of patients in the world each year. Severe complications of diabetes are very dangerous because it can lead to disability of patients and high mortality.
The purpose of the study was to establish the antidiabetic property of new plant gathering (consisting of 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 type 2 in the rats.
Experimental diabetes type 2 was reproduced on rats by a single intravenous dose of Streptozotocin at 65 mg/kg. Solution of Streptozotocin was prepared in 0.1 M citrate buffer pH 4.5. To reduce the action of substance that causes diabetes was injected Nicotinamide at a dose of 230 mg/kg for 15 minutes before entering of Streptozotocin.
The results showed that the using of antidiabetic plant gathering for four weeks reduced the expressiveness of hyperglycemia, prevented the development of secondary insulin resistance and increased the tolerance to glucose in rats with Streptozotocin-Nicotinamide -induced diabetes, which showed the normalization coefficient of insulin sensitivity and approximation of glycemic reaction to carbohydrate load to the level of normal control.
The antidiabetic plant gathering was not inferior to reference drug Metformin and prevailed officinal plant gathering "Arphasetynum" for hypoglycemic action.

Keywords: Antidiabetic plant gathering, diabetes mellitus type 2, streptozotocin, nicotinamide

1. Introduction
Diabetes mellitus raNCs 7th among the main causes of mortality in the most countries [1]. Diabetes mellitus - a state of chronic hyperglycemia resulting from absolute or relative lack of insulin caused by the influence of various exogenous, immune, endocrine and genetic factors or a combination thereof [2]. The structure of endocrine diseases, its share reached 70% [3].
According to modern concepts basic pathogenetic mechanisms of diabetes type 2 are insulin resistance, β-cell dysfunction of the pancreas and the formation of excess glucose by the liver. Insulin resistance causes to depletion of the functional activity of β-cells. This process is irreversible and has played a leading role in the progression of diabetes type 2 [4]. Therefore, pharmacotherapy of diabetes type 2 should be directed at preservation or improvement the features of β-cells in pancreas, increase sensitivity of peripheral tissues to the action of insulin, stabilization of blood glucose levels and restoration the metabolism in general. It is necessary to use the drugs with multidirectional action or combination therapy aimed at different pathogenesis of diabetes type 2.
In this respect, the fundamentally new direction of pharmacology is search of new antidiabetic drugs with a comprehensive action which combine several types of activities. The using of medicinal plants in the treatment of diabetes is appropriate, pathogenetically substantiated and helps endocrinologists to solve certain tasks: to reduce the dose of antidiabetic drugs, to neutralize the side effects some of them, to improve sensitivity the target tissue to insulin, to activate regenerative processes in β-cells of the pancreas, to increase general non-specific resistance of the organism.
The aim of our study was to establish the antidiabetic property of new plant gathering (consisting of 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 type 2 in the rats.

2. Materials and methods

2.1 Animals

A total of 40 male albino rats of Wistar strain, obtained from the vivarium of National University of Pharmacy, Kharkov, Ukraine, weighing 170 – 200 g, were used in the present study.

All rats were housed in a room with controlled environment, at the constant temperature of 23±1 ºC, humidity of 60±10% and a 12 h light/dark cycle. The animals were housed in groups and kept at constant nutritional conditions throughout the experimental period.

The studies were conducted in compliance with the principles of the European Convention for the Protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986) and "General ethical principles of animal experiments" (Ukraine, 2001).

2.2 Chemicals and drugs

Streptozotocin (STZ) purchased from Sigma-Aldrich Co. (St. Louis, MO, USA), Nicotinamide (NC), officinal plant gathering "Arphasetynum" of Pharmaceutical factory PrJSC "Viola" (Zaporizhzhya, Ukraine), Metformin SANDOZ® (Germany), test kits of "Lachema" company (Czech Republic). Other chemical reagents for analytical analysis.

2.3 Plant materials

All plants were harvested on territory of Western Ukraine. Investigational antidiabetic plant gathering includes:

Equiseti arvensis herba,	2 part;
Sambuci flores,	1 part;
Inulae rhizomata et radices,	1 part;
Hyperici herba,	1 part;
Tiliae flores,	1 part;
Polygoni avicularis herba,	1 part;
Myrtilli folium,	3 parts;
Urticae folia	1 part.

2.4 Preparation of decoction of antidiabetic plant gathering

Dried powdered plant raw materials poured by water in correlation 1:10 and heated in the water bath duringr 30 minutes. Then cooled at room temperature for 10 minutes and filtered through a double layer of cheesecloth.

2.5 Experimental groups and protocol

The experimental model was induced a single intravenous dose of streptozotocin 65 mg/kg, freshly prepared in 0.1 M sodium citrate buffer, pH 4.5 (Wahieb and Godin, 1987). Nicotinamide was dissolved in physiological saline and intraperitoneally administered to at 230 mg/kg, 15 minutes before streptozotocin introduction [5]. Rats of normal control were administered physiological saline.

Antidiabetic plant gathering and reference drugs was administered in the preventive and curative mode: 5 days before STZ -NC-induced diabetes and within 28 days of the experiment. Antidiabetic plant gathering, officinal plant gathering "Arphasetynum" and tablets "Metformin" were introduced intragastric once a day. Animals of pathology control received distilled water on similar scheme.

State of glucose homeostasis in animal with STZ -NC-induced diabetes evaluated the level of basal glucose, which determine in dynamics.

Glucose tolerance was defined by intraperitoneal glucose tolerance test (IPGTT), which was performed in the morning on an empty stomach by load of glucose solution (3 g/kg). The investigated means was administered intragastric, 1 hour before the intraperitoneal administration of 40% glucose solution (3 g/kg). The content of glucose in the blood of animals determined immediately after investigational drugs and 20, 40, 60 90 and 120 minutes after carbohydrate loading.

The short insulin test to determine the degree of sensitivity of peripheral tissues to insulin action, the introduction of which is causing inhibition of glucose production by the liver and increasing glucose utilization by muscles. The degree of sensitivity to insulin action (coefficient of sensitivity to insulin action) was determined as a percentage reduction of basal glucose after 30 minutes after intraperitoneal administration of the hormone in a dose of 1 OD/kg concerning the original level [6].

Afterwards animals were removed from the experiment by decapitation under ether anaesthesia.

2.6 Statistical analysis

All values are expressed as mean ± S.E.M. Statistical analysis was performed by one-way analysis of variance (STATISTICA) and used the method of Newman-Keyls, the data represented as an average (M) and as an average error (m). The results were considered statistically significant if probability factor, $P<0.05$.

3. Results and discussion

According to the data introduction of STZ and NC to rats of control pathology led to the development of a 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 normal control and kept at the same level throughout the experiment.

Table 1: The dynamics of basal glycaemia in rats with STZ -NC-induced diabetes, when applying antidiabetic plant gathering and reference drugs (M ± m), n = 8

Groups of animals	Term of observation			
	2 day	2 week	3 week	4 week
Normal control	4.51±0.25	4.40±0.17	4.40±0.19	3.74±0.25
Control pathology (STZ+NC)	14.72±2.01*	16.47±1.28*	15.49±1.36*	8.65±0.91*
Antidiabetic plant gathering, 9 ml/kg + STZ+NC	3.99±0.17 */**/#^{arph}	6.32±0.38 */**	5.14±0.25 */**	3.59±0.22 **/#^{arph}
Officinal plant gathering "Arphasetynum", 9 ml/kg + STZ+NC	7.28±0.69 */**	5.93±0.91 **	6.54±0.65 */**	5.83±0.63 */**
Metformin, 100 mg/kg +STZ+NC	3.54±0.48**	6.08±0.42 */**	4.80±0.34**	4.54±0.62**

Notes:

1. * – differences statistically significant compared to the normal control, $p<0.05$, according to the method of Newman-Keyls;
2. ** – differences statistically significant compared to the control pathology, $p<0.05$, according to the method of Newman-Keyls;
3. #$^{arph/metf}$ - differences statistically significant compared to the reference drug Arphasetynum/ Metformin, $p<0.05$, according to the method of Newman-Keyls.

Preventive and therapeutic introduction of antidiabetic plant gathering inhibited the carbohydrate metabolism. Basal glucose level in the group of animals, which injected the decoction of antidiabetic plant gathering, was statistically significant lower than the control pathology. The moderate hyperglycemia was developed only by 2 week. However, by the end of the experiment the blood glucose decreased to the level of normal control (Table. 1). There was improvement of glucose tolerance and reduce insulin resistance, as evidenced by the results IPGTT (Table. 2) and a short insulin test (Table. 3). The glycemic reaction of rats which were administered antidiabetic plant gathering, not different from normal control during the load of carbohydrates (Table. 2). Similar effects were observed when using tablets "Metformin", but the officinal plant gathering "Arphasetynum", found a similar, but less expressive effect on carbohydrate metabolism. Basal level of glucose in the application of officinal plant gathering "Arphasetynum" was lower than in the control pathology, but remained higher than in normal control.

Table 2: The impact of antidiabetic plant gathering on dynamics of glucose during the intraperitoneal glucose tolerance test in rats with STZ - NC-induced diabetes, n=8

Groups of animals	Term of observation, minutes (M±m)					
	Output data	20	40	60	90	120
Normal 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
Control pathology (STZ+NC)	8.65± 0.91*	24.05± 1.75*	22.90± 1.52*	19.88± 1.52*	17.70± 1.88*	16.14± 1.06*
Antidiabetic plant gathering, 9 ml/kg + STZ+NC	3.59± 0.215 **/#arph	14.85± 0.60**	11.64± 0.92**	8.63± 0.915*/**	5.54± 0.51**	5.01± 0.56**
Officinal plant gathering "Arphasetynum", 9 ml/kg + STZ+NC	5.83± 0.63*/**	16.42± 0.97**	14.53± 2.04*/**	9.30± 1.68**	7.53± 1.18**	5.50± 0.67**
Metformin, 100 mg/kg +STZ+NC	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:
1. * – differences statistically significant compared to the normal control, $p<0.05$, according to the method of Newman-Keyls;
2. ** – differences statistically significant compared to the control pathology, $p<0.05$, according to the method of Newman-Keyls;
3. # arph/metf - differences statistically significant compared to the reference drug Arphasetynum/ Metformin, $p<0.05$, according to the method of Newman-Keyls.

Table 3: The impact of antidiabetic plant gathering on insulin sensitivity in rats with STZ -NC-induced diabetes (M±m), n = 8

Groups of animals	Coefficient of sensitivity to insulin, %
Normal control	47.20±2.38
Control pathology (STZ+NC)	17.34±3.29*
Antidiabetic plant gathering, 9 ml/kg + STZ+NC	40.44±3.28**
Officinal plant gathering "Arphasetynum", 9 ml/kg + STZ+NC	34.72±6.11*/**
Metformin, 100 mg/kg +STZ+NC	49.37±1.43**/#arph

Notes:
1. * – differences statistically significant compared to the normal control, $p<0.05$, according to the method of Newman-Keyls;
2. ** – differences statistically significant compared to the control pathology, $p<0.05$, according to the method of Newman-Keyls;
3. # arph/metf - differences statistically significant compared to the reference drug Arphasetynum/ Metformin, $p<0.05$, according to the method of Newman-Keyls.

4. Prospects for further research

The proved efficiency of antidiabetic plant gathering on model of STZ -NC-induced diabetes that determines the appropriateness and provides opportunities for further study of the present plant collection to be used in the treatment of diabetes type 2.

5. Conclusions

The use of antidiabetic plant gathering for four weeks reduced the expressiveness of hyperglycemia, prevented the development of secondary insulin resistance and increased the tolerance to glucose in rats with STZ -NC-induced diabetes, which showed the normalization coefficient of insulin sensitivity and approximation of glycemic

1. reaction to carbohydrate load to the level of normal control.
2. For efficiency the antidiabetic plant gathering was not inferior to reference drug Metformin and prevailed officinal plant gathering "Arphasetynum"

6. References

1. Wild S, Roglic G, Green A. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2014; 27(5):1047-1053.
2. Боднар ПМ, Приступюк ОМ, Щербак ОВ. Ендокринологія. Київ, 2012, 512.
3. Robertson RP, Harmon J, Phuong OT. Glucose toxicity in β-cells: type 2 diabetes, good radicals gone bad, and the glutathione connection. Diabetes. 2013; 52(3):583-589.
4. Belcaro G, Cesarone MR, Errichi BM. Improvement of diabetic microangiopathy with Pycnogenol: a prospective, controlled study. Angiology. 2015; 57(1):431-436.
5. Masiello P, Broca C, Gross R. Development of a new model in adult rats administrated streptozotocin and nicotinamide. Diabetes. 1998; 47:224-229.
6. Полторак ВВ, Горбенко НІ. Методичні рекомендації з експериментального вивчення нових гіпоглікемічних засобів. Доклінічні дослідження лікарських засобів, 2001, 396-408.