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Research Article

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Antidiabetic activity of *Poterium sanguisorba* L. extract on streptozotocin-nicotinamide induced diabetic rats

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ABSTRACT

Diabetes mellitus is one of the most progressive disease in the world especially type 2. It is known that injection of streptozotocinin terms of previous injection of nicotinamidecauses the main metabolic violations of diabetes mellitus type 2 in rats. The aim of our study was to evaluate the effect of Poterium sanguisorba extract on body weight, blood glucose, plasma insulin and lipid profile in streptozotocin-nicotinamide induced diabetic rats. Our results showed that in streptozotocin-nicotinamide induced diabetic rats were observed weight loss, decreased insulin sensitivity, hyperinsulinemia, increased insulin resistance coefficient, increased lipid profile and atherogenic index. Poterium sanguisorba extract was able to improve insulin resistance, glucose toleration, rectifies dyslipidemia in streptozotocin-nicotinamide induced diabetic rats.

Key words: *Poterium sanguisorba* L., Blood burnet, insulin resistance, streptozotocin, nicotinamide, rats.

INTRODUCTION

About 347 million people worldwide suffered from diabetes mellitus (DM). In 2014, 9% of the population of 18 years and older had diabetes. In 2012, diabetes was the direct cause of 1.5 million deaths. According to forecasts, the overall death rate from diabetes will increase by more than 50% in the next 10 years and will become the seventh leading cause of death in 2030 [1,2].

There are two main forms of diabetes: type 1 diabetes (characterized by a lack of insulin production) and type 2 diabetes (characterized by ineffective use of insulin). The proportion of patients with type 2 diabetes is about 90% of the total array of diabetics and it is largely is due to overweight and low physical activity of patients [1,2,3].

Plant materials have always played an important role in the traditional treatment of diabetes, particularly type 2. In many parts of the world, herbal remedies are still more accessible for patients than conventional medicines [4]. Since ancient times plants were the major sources of drugs, and a large number of existing drugs, directly or indirectly were isolated from plants. Studies show that herbal remedies have many advantages, due to the presence of a complex of active ingredients [5,6]. For example, plants rich in phenolic compounds, flavonoids, terpenoids, coumarins, and other components can reduce blood glucose level [6,7].

There are many references that such species as *Poterium ancistroides Guir. ex. Nym.* And *Poterium spinosum L.* traditionally used to treat DM type 2 in various parts of the world. *Poterium spinosum L.* is known for its powerful antidiabetic properties from Bedouin's medicinal folklore [4,6,7]. Hypoglycemic action of Poterium ancistroides Guir.ex. Nym. and its triterpenoid compound tormentic acid had confirmed by many studies [8,9]. Among these plants from genus Poterium L. only *Poterium sanguisorba L.* is widespread in Ukraine and, moreover, hypoglycemic properties of this species investigated incompletely.Hypoglycemic activity of *Poterium sanguisorba*

L. herb was claimed only in normo- and hyperglycemic mice. Also tormentic acid was determined in aerial parts of *Poterium sanguisorba L.*[10].

Streptozotocin-nicotinamide (STZ-NA) induced type 2 diabetes is based on nicotinamideprotecting effect against the β-cytotoxic effects of streptozotocin on pancreas. This model was first introduced by Masiello using the 10-week rat male Wistar [11].

This model is characterized by stable moderate hyperglycemia that does not require exogenous insulin to maintenance life; reduction in β-cells by 40%; reduction of pancreatic insulin reserves by 60%; impaired glucose tolerance, mainly due to violations of insulin secretion; available glucose-stimulated insulin secretion; preserving sensitivity to sulfonylureas, and polydipsia polyphagia [11,12,13,14,15].

The aim of our work was to study the antidiabetic activity of *Poterium sanguisorba L.* underground partsin terms of STZ-NA -induced DM type 2.

EXPERIMENTAL SECTION

Plant material

Poterium sanguisorba rhizome was collected from the steppe zone in Zaporizhzhia region, Ukraine. The rhizomes were air dried under shade and powdered using mechanical grinder and stored in air- tight container.

Preparation of ethanolic extract of Blood burnet's rhizome

Dried, powdered rhizomes were extracted with ethanol using soxhlet apparatus for 72 hrs. The *Poterium sanguisorba* extract (PSE) was concentrated under vacuum evaporator and residue extract was stored at 4^0C in refrigerator for further pharmacological studies.

Animals

Healthy male and female Wistar albino rats weighing 120-140 g were purchased from animal house of Zaporizhzhia State Medical University (ZSMU), Ukraine and were kept in cages with standard laboratory conditions (temperature 22 ± 2°C with a 12/12 hr light – dark cycle). The animals were fed with normal laboratory diet and allowed to drink water *ad libitum*. All researches were conducted with ethical standards in accordance to the rules of International Conference on Harmonisation (ICH) –GoodClinical Practice (GCP), Declaration of Helsinki (1964), the European Convention on Human Rights and Biomedicine (ETS164) and the Ukraine laws.

Experimental design for antidiabetic activity

Induction of insulin resistance by streptozotocin-nicotinamide introduction and treatment protocol:

All animals were randomly divided into four groups (n=6). Three groups of overnight fasted rats were firstly administered with a single intra-peritoneal injection of nicotinamide (230 mg/kg bodyweight for each rat) (Sigma Aldrich, USA) dissolved in normal saline. After 15 min the rats received an intra-peritoneal injection of STZ (65 mg/kg BW) (Sigma Aldrich, USA) dissolved in citrate buffer (pH 4.5). Formation of insulin resistance was confirmed by OGTT on the 14s day after STZ-NA administration [11,12,13].

After OGTT current groups were treated daily for 2 weeks as follows: Group I: intact control rats were administered distilled water; Group II: diabetic control rats; Group III: diabetic rats received PSE orally by gastric tube in dose 100 mg/kg body weight and group IV: diabetic rats received metformin (150 mg/kg bodyweight, Aldrich, USA) as standard medication.

Body weight

Body weight was measured weekly throughout the all term of each experiment.

Glucose tolerance test

Animals were loaded with glucose (3 g/kg p.o.) and the blood samples were collected on 0, 30, 60, 120 minutes time interval [13]. The blood glucose levels were determined by using One Touch Select glucometer.

Blood and liver collection

At the end of the treatment period, all of the groups were fasted for 12 h and then anesthetised. The blood was collected into heparinised tubes and centrifuged at 4000 rpm for 10 min at 4^oC, and the serum was collected and stored at -80^oCuntil biochemical estimation was carried out. The liver was dissected out into ice-cold saline, thoroughly rinsedand subjected to the glycogen determination.

Biochemical analysis

Serum triglyceride (TG), Total Cholesterol (TC), LDL-cholesterol (LDL-c) and HDL-cholesterol (HDL-c) were estimated using enzymatic methods (Felicit, Ukraine). Atherogenic Index was calculated using formula ([TC–HDL‑c/HDL‑c]×100 [%]) [13]. Circulating insulin level was evaluated by radioimmunoassay.

Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was calculated using the equation: (insulin (μU/ml)×glucose (mmol/l))/22.5 [16].

Liver glycogen was estimated by the standard method [17]. The glycogen content in liver was expressed in mg %.

Statistical analysis

All results were expressed by the equation mean ± SEM. One-way ANOVA test was used for statistical analysis followed by Mann–Whitney U test. Values of $P<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Body weight

Weight gain was not observed in rats 2 weeks later after STZ-NA injection, in contrast to the intact group of animals in which body weight gain was 4.9%.

Table 1 Effect of PSE on insulin resistance related parameters in terms of STZ-NA-induced DM type 2

Parameter	Groups			
	Intact	*Control*	*PSE*	*Metformin*
Initial weight (g)	136,67 ± 1,41	136,5 ± 1,5	134,67 ± 1,20	140,33 ± 2,2
Weight after 2 weeks (g)	143,33 ± 0,99	137,17 ± 1,62$^{\#}$	135,0 ± 1,37$^{\#}$	142,17 ± 1,78
Weight after 4 weeks (g)	153,17 ± 1,01	125,33 ± 1,41$^{\#}$	143,67 ± 0,95*$^{\#}$	154,0 ± 1,69*
Insulin (μU/ml)	9,22 ± 0,30	15,08 ± 0,25$^{\#}$	11,88 ± 0,19*$^{\#}$	10,98 ± 0,36*$^{\#}$
Glucose (mmol/l)	4,45 ± 0,14	5,3 ± 0,14$^{\#}$	4,366 ± 0,14*	4,033 ± 0,08*$^{\#}$
HOMA-IR	1,83 ± 0,10	3,56 ± 0,13$^{\#}$	2,31 ± 0,10*$^{\#}$	1,96 ± 0,04$^{\#}$
Glycogen content (mg%)	1977,3 ± 49,7	692,7 ± 29,2$^{\#}$	1176,6 ± 41,2*$^{\#}$	891,6 ± 31,6*$^{\#}$
AUC (mmol/l/min)	95,22 ± 2,21	132,26 ± 2,12$^{\#}$	112,18 ± 4,65*$^{\#}$	107,92 ± 2,62*$^{\#}$
Cholesterol (mmol/l)	0,83 ± 0,03	0,88 ± 0,02	0,70 ± 0,05*$^{\#}$	0,72 ± 0,03*
Triglyceride (mmol/l)	0,40 ± 0,04	0,55 ± 0,02$^{\#}$	0,37 ± 0,04*	0,37 ± 0,05*
HDL-c (mmol/l)	0,47 ± 0,02	0,38 ± 0,02$^{\#}$	0,40 ± 0,04	0,43 ± 0,03
LDL-c (mmol/l)	0,22 ± 0,02	0,27 ± 0,01$^{\#}$	0,163 ± 0,02*$^{\#}$	0,17 ± 0,01*$^{\#}$
Atherogenic index	0,79 ± 0,05	1,32 ± 0,07$^{\#}$	0,77 ± 0,08*	0,68 ± 0,08*

AUC, area under the curve.
Here and in figure 1, 2, 3,4 and 5:
All data are Mean±SEM. (n=6);
** Significant difference with control group (P<0.05);*
 # Significant difference with intact group (P<0.05).

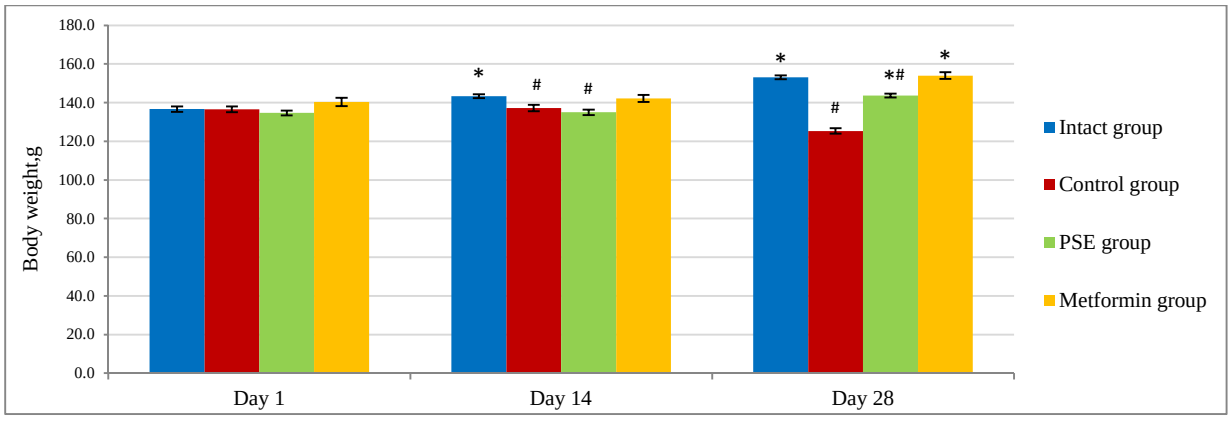

Figure 1 Effect of PSE on body weight in terms of STZ-NA-induced DM type 2

There was a powerful reduce of total body weight by 8.6% in animals of the control group after next 2 weeks. Introduction of PSE not only reliably saved animals from weight loss, but rather contributed to weight gain. The average percentage increase in body weight in PSE treated group amounted to 6.4%. Intact animals grown fat by 6.9%. Comparison drug, metformin, also favorably affected to animal weight, increasing it by 8.3% (Table 1, Figure

1). The results showed the ability of PSE to reverse the metabolic defection STZ cytotoxic effect observed in the control group.

Oral glucose tolerance test

It was found that insulin binding to membranes of liver cells under the conditions of this model is progressively reduced from 15 days after induction of diabetes [13].

The analysis of the OGTT reliably confirmed the development of IR and glucose intolerance as well as on 60 min of the test blood glucose level of control animal increased almost on 100% when in intact animals blood glucose level increased on 45.8% (Figure 2).

Two week's introduction of PSE to animals significantly improved the sensitivity of cells to insulin. Results of OGTT conducted at the end of the study showed significant regression of blood glucose levels at 30, 60 and 120 minute test by 52.0%, 33.7% and 28.4%, respectively, compared with the previous test in that group. The maximum rise of glucose was on 60 min by 60.8% and it is lower by 25.8% when compared to control group and higher by 21.1% when compared to intact group(Figure 2).

The area under the curve (AUC) of glucose during OGTT of control group was significantly elevated when compared to intact group ($P<0.05$). The AUC in PSE group was significantly lower than that of control group by 17.8% (Table 1, Figure 2).

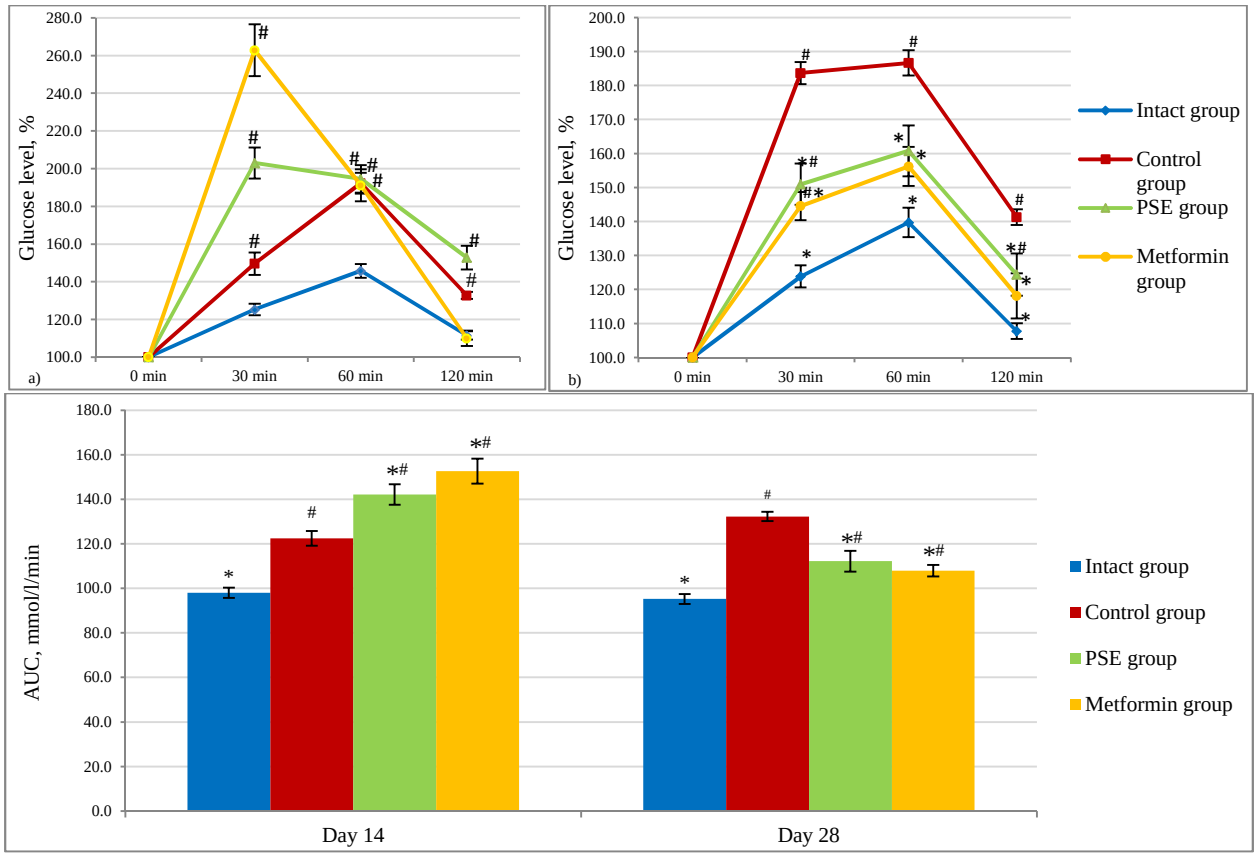

Figure 2 Effect of PSE on glucose tolerance in terms of STZ-NA-induced DM type 2: a) after two weeks of STZ-NA injection; b) after two weeks of treatment

Serum level of insulin, blood glucose and homeostasis model assessment of insulin resistance

Serum level of insulin in PSE and metformin groups was significantly lower in comparison with the control group by 21.2% and 27.2% respectively ($P<0.05$). IR rate calculated by HOMA model showed the ability of PSE to reliably inhibit the formation of IR in these experimental conditions almost at the level of the comparison drug, Thus, HOMA-IR index in animals treated by PSE exceeds only by 26.6% when compared to intact animals and by 94.5% when compared to control animals. Metformin almost completely leveled formation of IR. HOMA-IR ratio in the group that received it was only on 7.4% higher than in the intact group (Table 1, Figure 3).

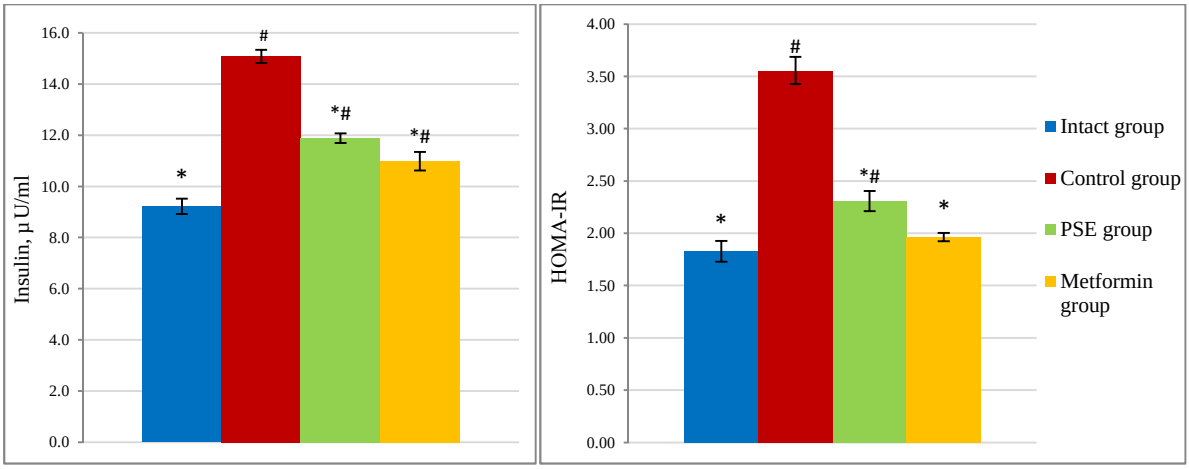

Figure 3 Effect of PSEon serum level of insulin, and HOMA-IRin terms of STZ-NA-induced DM type 2

Liver glycogen

Control rats showed significantly decreased glycogen content in liver by 65.0% when compared to intact group (P<0.05). Treatment with PSE significantly increased the liver glycogen content compared to that of control group by 41.1%. Metformin treated animals also showed significantly increased levels of liver glycogen by 22.3% when compared to control group (Table 1, Figure 4).

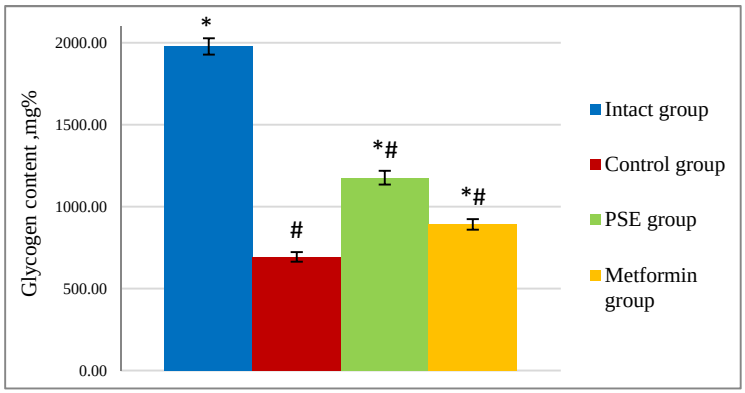

Figure 4 Effect of PSE on glycogen content in terms of STZ-NA-induced DM type 2

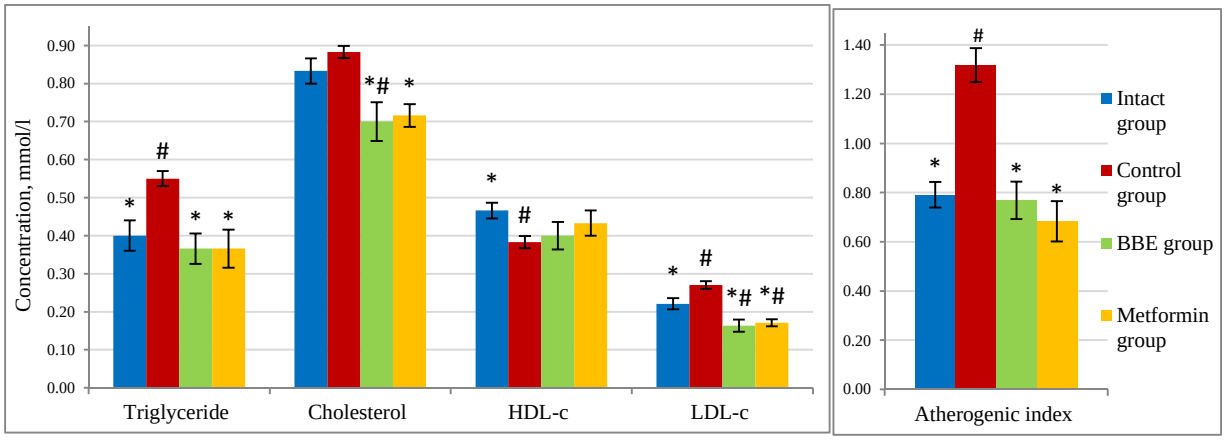

Figure 5 Effect of PSE on lipid profile and atherogenic index in terms of STZ-NA-induced DM type 2

Lipid profile

The serum TG and LDL-c values were significantly increased by 37.5% and 22.7% respectively, and HDL-c was significantly decreased by 17.8% in control group, when compared to intact group. Treatment with PSE significantly normalized TG and LDL-c levels (decreased by 32.7% and 39.6% respectively) when compared to control group (P<0.05). Serum HDL-c concentrations of PSE group, like in metformin group, was higher than those of control

group but this results were not statistically different (P<0.05). There was no significant difference in TC levels of control group than those of intact group but TC levels in PSE group was statistically decreased when compared to both control and intact group (Table 1, Figure 5).

The atherogenic index of PSE and metformin groups was statistically lower when compared to control group (P<0.05).

CONCLUSION

In conclusion, the present study has demonstrated that PSE has significant antidiabetic and antihyperlipidemic activities against STZ-NA- induced diabetes mellitus DM type 2 in rats. PSE is able to improve dyslipidemia, and to abolish the metabolic disorders in glucose homeostasis under STZ influence. Therefore, PSE could be useful as an adjuvant for the prevention or management of IR and disturbed glucose tolerance.

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