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INFLUENCE OF SOME HERBAL MIXTURES ON INSULIN RESISTANCE AND GLUCOSE TOLERANCE IN RATS

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Abstract

Insulin resistance and tissue glucose intolerance play a major role in the development and progression of diabetes mellitus type 2. The herbal mixtures might increase the sensitivity of target tissues to insulin and regulate carbohydrate metabolism due to the wide range of biologically active substances. The aim of our research was to study the influence on insulin resistance and glucose tolerance *in vivo* of the herbal mixtures No. 3 (*Urtica dioica* leaf, *Cichorium intybus* roots, *Rosa majalis* fruits, *Elymus repens* rhizome, *Taraxacum officinale* roots), No. 4 (*Arctium lappa* roots, *Elymus repens* rhizome, *Zea mays* columns with stigmas, *Helichrysum arenarium* flowers, *Rosa majalis* fruits), No. 7 (*Inula helenium* roots, *Helichrysi arenarium* flowers, *Zea mays* columns with stigmas, *Origanum vulgare* herb, *Rosa majalis* fruits, *Taraxacum officinale* roots), No. 13 (*Cichorium intybus* roots, *Elymus repens* rhizome, *Helichrysum arenarium* flowers, *Rosa majalis* fruits, *Zea mays* columns with stigmas) and No. 19 (*Urtica dioica* leaf, *Taraxacum officinale* roots, *Vaccinium myrtillus* leaf, *Rosa majalis* fruits, *Mentha piperita* herb). There were used the male albino rats of the Wistar strain for research. The pathology was modeled by intramuscular administration of dexamethasone at a dose of 1 mg/1kg of body weight once a day for 15 days. The aqueous extracts of the herbal mixtures (12 mL/kg/day) and standard drug – metformin (60 mg/kg/day) were administered orally once a day for 15 days. The results found out that the studied phytomixtures significantly (p <0.05) reduced the manifestations of insulin resistance by reducing the value of HOMA-IP index by 45%-58% relative to DEXA group. Metformin as a comparative agent reduced the HOMA-IR index by 60%. The ability to regulate carbohydrate metabolism by phytomixtures and metformin was confirmed by IPGTT results, as the value of AUCglu was 1.6-1.8 times lower in comparison with the DEXA group. According to the results the aqueous extracts of herbal mixtures No. 3, No. 4, No. 7, No. 13 and No. 19 have the ability to reduce insulin resistance and glucose intolerance that precede the development of diabetes mellitus type 2.

Keywords: *diabetes mellitus, herbal mixtures, insulin resistance, glucose tolerance, dexamethasone*

Introduction

Diabetes mellitus is a serious problem in the field of health care, because the number of patients is increasing every year along with the number of deaths and disabilities due to the development of diabetic complications [1]. According to the official data from the International Diabetes Federation (2019), an increase in the incidence of diabetes is projected to 642 million by 2040 [2]. In general, the development of insulin resistance contributes to the spreading of diabetes mellitus type 2. It is a trigger for the development of hyperglycemia and glucose intolerance, which start the cascade of pathological processes, such as lipid peroxidation and accumulation of their products, inactivation the antioxidant defense system, and the development of oxidative stress [3, 4].

Therefore, the optimization of pharmacotherapy of diabetes mellitus type 2 and the state of the insulin resistance, searching and investigation of new drugs with the ability to regulate carbohydrate metabolism is a top issue of pharmacy and medicine.

Phytotherapy is one of such areas, as it has certain number of advantages, namely, it is low-toxic, has a mild pharmacological effect and can be used for longer treatment without significant side effects, is well combined with synthetic drugs, has a complex activity through a variety of biologically active compounds [5, 6, 7, 8]. Particular attention deserve the combinations of different medicinal plants, because such herbal mixtures will have more biologically active substances that will affect all links of the pathogenetic mechanism of diabetes mellitus development, and its complications [9, 10, 11, 12].

Therefore, the aim of our research was to study the influence of some herbal mixtures on insulin resistance and glucose tolerance *in vivo*. According to the literature, it is used in folk medicine for prevention and treatment of diabetes mellitus type 2 in Ukraine [13, 14].

Methods

Plant materials: The herbal raw materials, harvested from June to August 2019 in the Ternopil region and Carpathians (*Vaccinium myrtillus* leaf) (Ukraine), were used. After harvesting, the raw materials were dried, graind and stored according to the general GACP requirements [15]. The plants were identified by Department of Pharmacognosy with Medical Botany, I.Horbachevsky Ternopil National Medical University, Ternopil, Ukraine. The voucher specimens of herbal raw materials have been deposited in the departmental herbarium for future records.

Five different herbal mixtures with reliable hypoglycemic activity established during the screening tests [16, 17, 18, 19] were used for the study. The composition of the mixtures is given in Table 1.

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

Drugs and chemicals: Dexamethasone KRKA, Slovenia was administered, the standard drug – metformin SANDOZ® Lek S.A., Poland, Sodium thiopental for anesthesia - Abbott Park, IL, USA. All chemicals that were used in the research were analytically 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 in vivarium 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 [20]. All protocols for animals experiment were approved by the animal ethical committee of I.Horbachevsky Ternopil National Medical University.

Induction of Insulin Resistance and Experimental Protocol: Induction of insulin resistance by

dexamethasone was performed according to the previously described protocol [21] with some modifications. Rats were randomly divided into eight groups comprising 8 animals in each group (n=8) and received different treatment once a day for 15 days. Group I (Control): received distilled water (12 mL/kg/day) per os (*p.o*) and intramuscular (*i.m.)* injection of NaCl 0.9 % (1 mL/kg/day). Group 2 (DEXA) received intramuscular injection of dexamethasone (1 mg/kg/day) daily and distilled water (12 mL/kg/day, *p.o.*). Group III (DEXA+MET) received dexamethasone (1 mg/kg/day, *i.m.*) and the standard drug – metformin (60 mg/kg/day, *p.o.*). Group IV-VIII (DEXA+EHM) received dexamethasone (1 mg/kg/day, *i.m.*) and the aqueous extracts of the studied herbal mixtures (12 mL/kg/day, *p.o.*). The dose of dexamethasone (1 mg/kg/day) and metformin (60 mg/kg/day) was selected according to previous studies [21, 22]. The effective dose of herbal mixture extracts was established during the previous screening testing [16, 17, 18, 19]. At the end of the experiment, rats were sacrificed by decapitation under anesthesia with Sodium thiopental and the blood was taken for analysis.

Homeostasis Model Assessment of Insulin Resistance index (HOMA-IR): HOMA-IR index was calculated based on fasting glucose and fasting insulin in the blood of experimental animals using the HOMA algorithm [23]. The level of glucose was determined by glucose analyzer (glucometer Accuk-Check, Germany) and the insulin concentration – by enzyme-linked immunosorbent assay using a standard set of reagents "DRG" (Germany).

Measurement of Intraperitoneal Glucose Tolerance Test (IPGTT): After overnight fasting (16-18 hours) on 16th 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, that was obtained from animals tail vein, was determined before the introduction of glucose and after 15, 30, 60 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 $p<0.05$. The value of the integrated glycemic index of the area under glycemic curve (AUC_{glu}, mmol/L min) was calculated using the statistical software package "MedCalk, v.9.3.7.0".

Results

The results of the experimental study established that the introduction of dexamethasone at a dose of 1 mg/kg/day to linear rats for 15 days caused the development of persistent basal hyperglycemia, which was significantly ($p<0.05$) 2 times higher than in animals from the Control group. The oral administration of aqueous extracts of the herbal mixtures No. 3, No. 4, No. 7, No. 13 and No. 19 significantly ($p<0.05$) reduced hyperglycemia from 26% to 36% relative to DEXA group. Metformin, as a comparison drug, reduced glycose level by 43 % relative to DEXA group. It was found out the best result of hypoglycemic activity for the herbal mixture No. 19 (Table 1).

In addition, against the background of hyperglycemia caused by dexamethasone, there was the development of hyperinsulinemia, which was 29% higher than in the Control group. Treatment with the aqueous extracts of the herbal mixtures reduced the basal insulin concentration in the blood of animals by 22% -29% relative to DEXA group. Metformin showed similar result. The herbal mixtures No. 19 proved the greatest reduction of hyperinsulinemia (Table 1).

Stable hyperglycemia on the background of hyperinsulinemia indicates the development of insulin resistance in experimental animals in DEXA group. Therefore, HOMA-IR index was determined that characterizes the degree of insulin resistance. According to the results of calculation, it was found that the introduction of dexamethasone at a dose of 1 mg/kg/day to linear rats for 15 days simulates the state of insulin resistance, as the HOMA-IR index increased by 3.7 times relative to the Control group. Oral administration of aqueous extracts of the herbal mixtures No. 3, No. 4, No. 7, No. 13 and No. 19 significantly ($p<0.05$) reduced the manifestations of insulin resistance, as the HOMA-IR index decreased by 55%, 49%, 47%, 45% and 58% relative to DEXA group, respectively. Metformin, as comparison drug reduced the HOMA-IR index by 60% (Table 1).

The IPGTT can detect disorders of carbohydrate metabolism and the degree of tissue glucose

intolerance in experimental rats. The ability of the investigated herbal mixtures and comparison drug – metformin to reduce the manifestations of glucose intolerance is evidenced by the decrease in blood glucose levels at the 15th minute of the test (during the maximum rise in blood glucose levels of experimental rats in response to intraperitoneal carbohydrate load). The results of the IPGTT showed the difference in blood glucose of rats from DEXA group was in 2 times higher than that of healthy control animals 15 minutes after glucose load. At the same time, the difference in blood glucose levels of rats receiving concomitantly dexamethasone (1 mg/kg/day, *i.m.*) and aqueous extracts of the herbal mixtures No. 3, No. 4, No. 7, No. 13 and No. 19 (12 mL/kg/day, *p.o.*) were 38%, 35%, 34%, 35% and 38% lower, respectively, as compared to that of the DEXA group. The difference in blood glucose in the metformin-treated animals was 39% lower than in the DEXA group. After 2 hours of glucose loading, a return to baseline blood glucose values was observed in all experimental groups (Figure 1).

It was found out that the area under glycemic curve (AUCglu) of the Control group was 228.8 (mmol/L min) and AUCglu of DEXA group was 473.4 (mmol/L min) during the determination of integrated glycemic index based on the results of IPGTT. The value of AUCglu was 1.7 times lower in the herbal mixture No. 3; 1.6 times lower in the herbal mixtures No. 4, No. 7 and No. 13; 1.8 times lower in the herbal mixture No. 19 and in the comparison drug – metformin (Figure 2).

Discussion

The results of the study showed that the introduction of dexamethasone (1 mg/kg/day, *i.m.*) for 15 days caused a state of insulin resistance and glucose intolerance in rats, which precedes the development of diabetes, metabolic syndrome and obesity. However, oral administration of aqueous extracts of the herbal mixtures No. 3, No. 4, No. 7, No. 13 and No. 19 (12 mL/kg/day) caused the increase of tissue sensitivity to insulin and reduction of manifestations of carbohydrate metabolism disorders.

Obviously, the effectiveness of the studied herbal mixtures is due to the presence of various biologically active substances. The main groups of biologically active substances, which can regulate carbohydrate metabolism are polysaccharides, especially inulin that has the ability to increase glucagon-like peptide-1 (GLP-1) and insulin secretion, to inhibit the glucagon secretion, to stimulate the β-cells proliferation and neogenesis [25, 26]. These herbal mixtures contain herbal raw materials that are rich in carbohydrates, such as *Cichorii radices* (the herbal mixtures No. 3 and No. 13), *Taraxaci radices* (the herbal mixtures No. 3, No. 7 and No. 19), *Elymi repens rhizomata* (the herbal mixtures No. 3, No. 4 and No. 13), *Inulae rhizomara cum radicibus* (the herbal mixture No. 7), *Arctii lappae radices* (the herbal mixture No. 4).

In addition, medicinal plants that are part of the studied herbal mixtures, contain polyphenolic compounds, which exhibit antidiabetic activity by different mechanism of actions, including stimulation of insulin secretion, improvement of pancreatic β-cell functionality, inhibition of gluconeogenesis, intensification of glucose uptake, delay of carbohydrate digestion and glucose absorption, inhibition of protein glycation and insulin fibrillation [27, 28, 29]. Their antioxidant activity plays important role also in the treatment and prevention of diabetes and its complications, because it might include suppression of reactive oxygen spesies (ROS) formation either by inhibition of enzymes or by chelating trace elements involved in free radical generation; scavenging ROS; inhibition the enzymes involved in ROS generation – microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase, nicotinamide adenine dinucleotide phosphate (NADH) oxidase, and so forth [29, 30]. Medicinal plant raw materials containing phenolic compounds are *Urticae folia* (the herbal mixtures No. 3 and No. 19), *Rosae fructus* (the herbal mixtures No. 3, No. 4, No. 7, No. 13 and No. 19), *Maydis style cum stigmatis* (the herbal mixtures No. 4, No. 7 and No. 13), *Helichrysi arenarii flores* (the herbal mixtures No. 4, No. 7 and No. 13), *Origani herba* (the herbal mixture No. 7), *Myrtilli folia* (the herbal mixture No. 19), *Menthae folia* (the herbal mixture No. 19).

Conclusions

The results of this study showed that aqueous extracts of the herbal mixtures No. 3, No. 4, No. 7, No. 13 and No. 19 (12 mL/kg/day) have the ability to reduce insulin resistance and glucose intolerance that precede the development of diabetes mellitus type 2. Established pharmacological properties make these herbal mixtures perspective remedies for the prevention and treatment of diabetes and insulin-resistant states.

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Table 1. Composition of the herbal mixtures

Herbal mixtures	Herbal drug component	Portion in the mixture, %	Relative ratio
No. 3	*Urtica dioica* leaf	26.32	5
	Cichorium intybus roots	26.32	5
	Rosa majalis fruits	21.05	4
	Elymus repens rhizome	15.79	3
	Taraxacum officinale roots	10.52	2
No. 4	*Arctium lappa* roots	26.32	5
	Elymus repens rhizome	26.32	5
	Zea mays columns with stigmas	21.05	4
	Helichrysum arenarium flowers	15.79	3
	Rosa majalis fruits	10.52	2
No. 7	*Inula helenium* rhizome with roots	10.0	1
	Helichrysi arenarium flowers	20.0	2
	Zea mays columns with stigmas	20.0	2
	Origanum vulgari herb	20.0	2
	Rosa majalis fruits	20.0	2
	Taraxacum officinale roots	10.0	1
No. 13	*Cichorium intybus* roots	26.32	5
	Elymus repens rhizome	26.32	5
	Helichrysum arenarium flowers	21.05	4
	Rosa majalis fruits	15.79	3
	Zea mays columns with stigmas	10.52	2
No. 19	*Urtica dioica* leaf	20.0	1
	Taraxacum officinale roots	20.0	1
	Vaccinium myrtillus leaf	20.0	1
	Rosa majalis fruits	20.0	1
	Mentha piperita herb	20.0	1

Table 2. Effect of aqueous extracts of the herbal mixtures and the comparison drug metformin on insulin resistance caused by dexamethasone in rats.

Group of animals	Fasting glucose, mmol/L	Fasting insulin, μU/mL	HOMA-IR, %
Control	3.98±0.17**	9.86±0.12**	1.74±0.14**
DEXA	10.52±0.36*	13.89±0.22*	6.49±0.15*
DEXA+EHM 3, 12 mL/kg/day	6.38±0.26*/**	10.21±0.21**	2.89±0.11*/**
DEXA+EHM 4, 12 mL/kg/day	7.31±0.16*/**	10.12±0.14**	3.29±0.16*/**
DEXA+EHM 7, 12 mL/kg/day	7.19±0.13*/**	10.87±0.32**	3.47±0.21*/**
DEXA+EHM 13, 12 mL/kg/day	7.55±0.21*/**	10.52±0.28**	3.53±0.19*/**
DEXA+EHM 19, 12 mL/kg/day	6.21±0.17*/**	9.92±0.16**	2.74±0.12*/**
DEXA+MET, 60 mg/kg/day	5.92±0.15*/**	9.88±0.15**	2.60±0.19*/**

Values are expressed as mean ± SEM from 8 rats; * p<0.05 with respect to Control group; ** p<0.05 with respect to dexamethasone (DEXA) group.

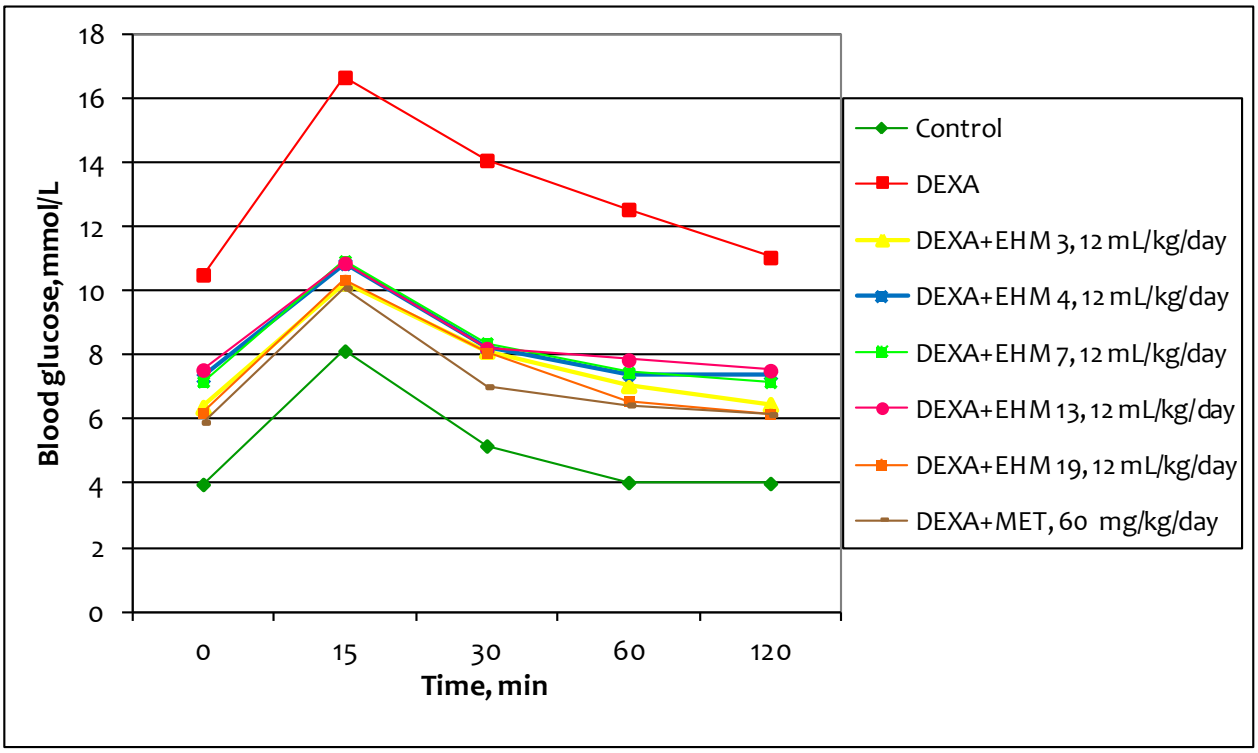

Figure 1. Blood glucose level during IPGTT in dexamethasone-induced insulin resistant rats. Values are expressed as mean ± SEM from 8 rats, $p<0.05$.

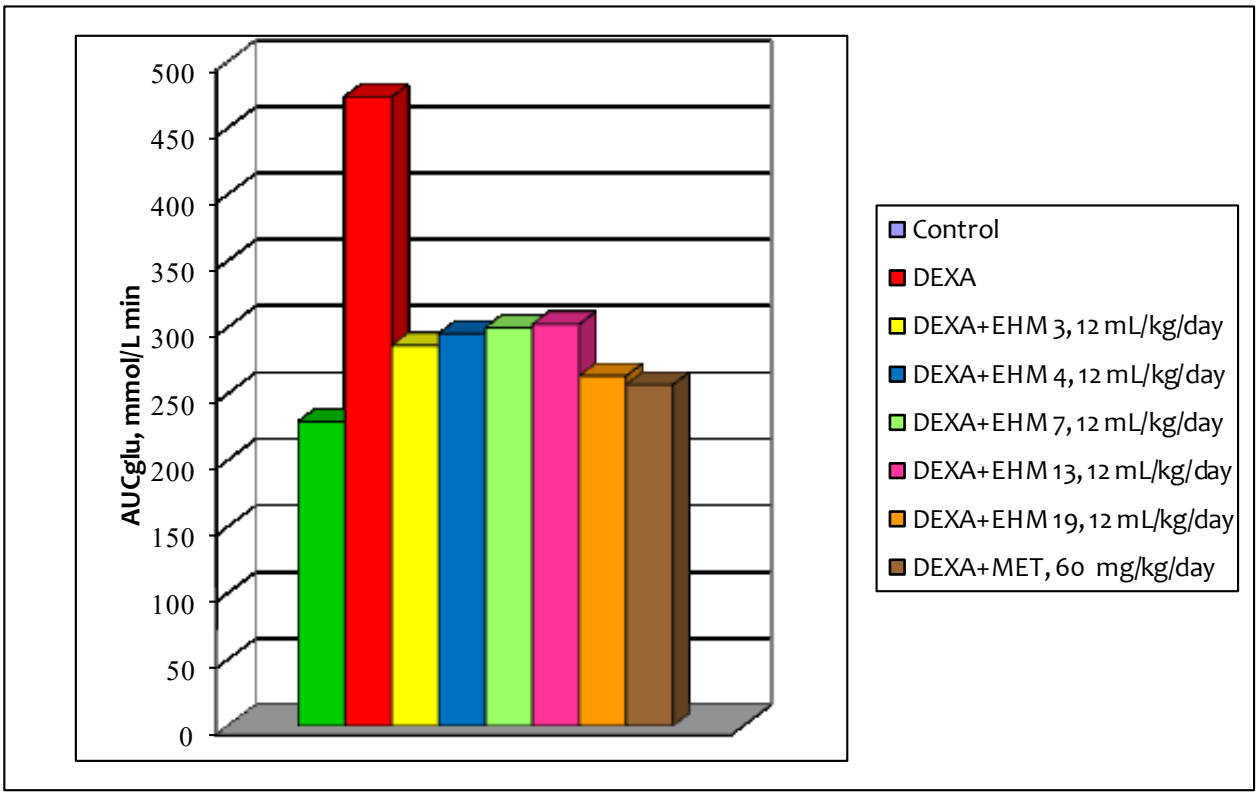

Figure 2. Glucose area under the curve during IPGTT in dexamethasone-induced insulin resistant rats. Values are expressed as mean ± SEM from 8 rats, $p<0.05$.