

ЕКСПЕРИМЕНТАЛЬНА ТА КЛІНІЧНА ФАРМАКОЛОГІЯ

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The effects of goutweed (*Aegopodium podagraria* L.) preparations on glycemia in intact rats and against the background of metformin

The preparations obtained from goutweed (*Aegopodium podagraria* L.) are characterized by favorable metabolic effects, including the antidiabetic activity.

Aim. To determine the dose dependence of the effect of the extract and the tincture of goutweed on the glucose metabolism in intact rats, to evaluate the metabolic effects and the possibility of the combined use of the goutweed preparations with metformin.

Materials and methods. The experiments were conducted on intact rats. The influence of the extract and the tincture of goutweed (*per se* and combined with metformin) on the basal glycemia and the results of the glucose tolerance test were determined. The metabolic effects which are possibly interrelated (the changes in uric acid and electrolyte exchange) were also studied.

Results and discussion. The goutweed tincture exhibits the hypoglycemic action in intact rats after administration of single doses of 0.5; 1.0; 5.0 ml/kg, while the goutweed extract (100; 250; 500 mg/kg and 1.0 g/kg) does not demonstrate this action. The correlation analysis results indicate that certain interrelationship may exist between the effect of the extract on the electrolyte homeostasis and glucose metabolism (though these changes are moderate in intact animals), as well as between the effect of goutweed preparations on uric acid and the glucose exchange. The goutweed tincture in the doses of 0.5 and 1.0 ml/kg does not block the effects of metformin, and does not enhance its effect with the excessive decrease of the blood glucose concentration. The absence of a significant decrease of glycemia after combined administration of the tincture and low doses of metformin may reflect synergoantagonism (however, these phenomena were seen in intact animals, and the dose dependence of the metformin action did not change). In the glucose tolerance test the tincture in the dose of 1.0 ml/kg (but not 0.5 ml/kg) combined with metformin allows reducing its effective dose from 400 mg/kg to 200 mg/kg.

Conclusions. Goutweed tincture, in contrast to the extract, shows a dose-dependent hypoglycemic effect and is promising for combined use with metformin.

Key words: *Aegopodium podagraria* L.; glycemia; metformin; rats

O. B. Товчига

Вплив препаратів яглиці звичайної (*Aegopodium podagraria* L.) на глікемію в інтактних тварин і на тлі метформіну

Препарати яглиці звичайної (*Aegopodium podagraria* L.) характеризуються сприятливими метаболічними ефектами, в числі яких антидіабетична активність.

Мета дослідження – визначити дозозалежність впливу настойки та екстракту *Aegopodium podagraria* L. на обмін глюкози в інтактних щурів, оцінити метаболічні ефекти, спряжені з цим впливом, встановити можливість поєднаного застосування препаратів яглиці з метформіном.

Матеріали та методи. Експерименти проведені на інтактних щурах. Досліджено вплив екстракту та настойки яглиці (*per se* та у комбінації з метформіном) на базальну глікемію та результати тесту толерантності до глюкози. Вивчені зміни обміну сечової кислоти та електролітів, які можуть бути взаємопов'язані з впливом препаратів яглиці на обмін вуглеводів.

Результати та їх обговорення. Настойка яглиці чинить гіпоглікемічну дію в інтактних щурів при одноразовому введенні у дозах 0,5; 1,0; 5,0 мл/кг, тимчасом як екстракт яглиці (100; 250; 500 мг/кг і 1,0 г/кг) такої дії не чинить. Результати кореляційного аналізу свідчать про можливий взаємозв'язок впливу екстракту на обмін електролітів та глюкози (хоча ці зміни в інтактних тварин виражені помірно), а також між впливом настойки на вуглеводний та пуриновий обмін. Настойка *Aegopodium podagraria* L. у дозах 0,5 і 1,0 мл/кг не блокує ефекти метформіну та не призводить до різкого посилення його активності із надлишковим зниженням рівня глюкози крові. Відсутність достовірного зниження глікемії після поєднаного введення настойки та низьких доз метформіну може відбивати синергоантагонізм (хоча такі закономірності спостерігали в інтактних тварин, більше того, дозозалежність дії метформіну не змінювалася). В умовах тесту толерантності до глюкози настойка в дозі 1 мл/кг (але не 0,5 мл/кг) при поєднаному застосуванні з метформіном дозволяє знизити його ефективну дозу з 400 мг/кг до 200 мг/кг.

Висновки. Настойка яглиці звичайної, на відміну від екстракту, виявляє дозозалежну гіпоглікемічну дію та є перспективною для комбінованого застосування з метформіном.

Ключові слова: *Aegopodium podagraria* L.; глікемія; метформін; щури

О. В. Товчига

Влияние препаратов сныти обыкновенной (*Aegopodium podagraria* L.) на гликемию у интактных животных и на фоне метформина

Препараты сныти обыкновенной (*Aegopodium podagraria* L.) характеризуются благоприятными метаболическими эффектами, в т. ч. антидиабетической активностью.

Цель исследования – определить дозозависимость влияния настойки и экстракта *Aegopodium podagraria* L. на обмен глюкозы у интактных крыс, оценить метаболические эффекты, сопряженные с этим влиянием, установить возможность сочетанного применения препаратов сныти с метформином.

Материалы и методы. Опыты проведены на интактных крысах. Исследовано влияние экстракта и настойки сныти (*per se* и в комбинации с метформином) на базальную гликемию и результаты теста толерантности к глюкозе. Изучены изменения обмена мочевой кислоты и электролитов, которые могут быть взаимосвязаны с влиянием препаратов сныти на углеводный обмен.

Результаты и их обсуждение. Настойка сныти проявляет гипогликемическое действие у интактных крыс при однократном введении в дозах 0,5; 1,0; 5,0 мл/кг, тогда как экстракт сныти (100; 250; 500 мг/кг и 1,0 г/кг) такого действия не оказывает. Результаты корреляционного анализа свидетельствуют о возможной взаимосвязи влияния экстракта на обмен электролитов и глюкозы (хотя эти изменения у интактных животных выражены умеренно), а также между влиянием настойки на углеводный и пуриновый обмен. Настойка *Aegopodium podagraria* L. в дозах 0,5 и 1,0 мл/кг не блокирует эффекты метформина и не приводит к резкому усилению его активности с чрезмерным снижением уровня глюкозы крови. Отсутствие достоверного снижения гликемии после сочетанного введения настойки и низких доз метформина может отражать синергоантагонизм (тем не менее такие закономерности наблюдали у интактных животных, и кроме того дозозависимость действия метформина не изменялась). В условиях теста толерантности к глюкозе настойка в дозе 1 мл/кг (но не 0,5 мл/кг) при сочетанном применении с метформином позволяет снизить его эффективную дозу с 400 мг/кг до 200 мг/кг.

Выводы. Настойка сныти обыкновенной, в отличие от экстракта, проявляет дозозависимое гипогликемическое действие и является перспективной для сочетанного применения с метформином.

Ключевые слова: *Aegopodium podagraria* L.; гликемия; метформин; крысы

The metabolic syndrome and type 2 diabetes are generally considered as pandemic of the modern society with the need in lifelong treatment. Herbal drugs attract much attention since they may increase the efficacy of the conventional antidiabetic drugs and favorably broaden their pharmacodynamics [1]. From another point of view, the unwanted herb-drug interactions are highly possible due to the complex composition of the herbal medicines, and the verification of the expediency of such combinations is strongly needed.

Our efforts are focused on the pharmacological studies of the preparations obtained from the aerial part of goutweed (GW, *Aegopodium podagraria* L., Apiaceae). It is a ubiquitous perennial plant widely used in traditional medicine and consumed as vegetable. A dry extract and the tincture of GW are standardized by the content of hydroxycinnamic acids and exhibit a favorable effect on the purine metabolism along with the expressed nephroprotective and hepatoprotective properties and low toxicity [2, 3]. The activity of GW preparations is mediated by hydroxycinnamic acids, flavonoids, protein-polysaccharide complex, micro- and macroelements [2]. The additional benefit of GW preparations, especially the tincture, is the ability to normalize the carbohydrate metabolism proven in rats receiving the excess fructose combined with hydrochlorothiazide [4] and in dexamethasone-treated rats; on this model the tincture also increased the efficacy of metformin [5]. Both GW preparations have a favorable effect on the course of alloxan-induced diabetes in mice [6]. The study of the promising antidiabetic medicines pre-

supposes determination of their influence on glycemia in intact animals [7]. Therefore, it is expedient to broaden such data concerning GW preparations [8] and analyze if there is a relationship between their effect on the metabolism of electrolytes, uric acid (UA), and carbohydrates in intact animals (since these relationships are established in rats receiving fructose and hydrochlorothiazide [4], and the changes in electrolyte intake are not indifferent to the carbohydrate exchange [9]).

Besides, there is a need to confirm safety (in the context of the possible hypoglycemia) of GW preparations combined with the agents normalizing the blood glucose level. Among the latter metformin deserves special attention as the widely prescribed first line agent of the oral normoglycemic drugs possessing numerous benefits and advantageously combined with the GW tincture in diabetic animals [5].

Therefore, the aim of this work was to determine the dose dependence of the effect of the extract and the tincture of goutweed on the glucose metabolism in intact rats, to evaluate the metabolic effects and the interrelated metabolic effects use of the goutweed preparations with metformin.

Materials and methods

All the experimental protocols were approved by the Bioethics Commission of the National University of Pharmacy and were in accordance with “Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.” Noninbred albino rats from the

Central Research Laboratory of the National University of Pharmacy were kept under controlled standard conditions [7] on the natural light-dark cycle.

The first series of experiments was conducted using male rats with the body weight of 160 to 210 g randomly divided into 6 groups ($n = 6-9$ in each group) as follows: intact control (IC), four groups of rats receiving GW preparations (in the doses substantiated previously [2, 3, 6], namely the extract in the doses of 100 mg/kg and 1 g/kg, the tincture (after the removal of ethanol) in the doses of 1 and 5 ml/kg intragastrically), as well as the animals receiving the reference drug – officinal hypoglycemic collection “Arfasetinum” in the dose of 18 ml/kg [10]. The last dose of these preparations (or the same amount of water in the IC group) was given 30-40 min before the biochemical tests. On days 8-9, after an overnight fast, the basal glycemia was determined in the samples obtained from a cut at the tip tail, the oral glucose tolerance test [7] was carried out (the glucose load in the dose of 2.0 g/kg, glycemia determination at 0, 30, 60 and 120 min), the total area under the blood glucose curve was calculated. The glucose concentration in all samples was measured using the glucose oxidase method.

To determine the possible relationship between the changes in the glucose and electrolyte metabolism (which might be expected considering the data [4]) the excretory renal function was analyzed under the conditions of the 24-hour spontaneous diuresis (days 10-11) by the generally accepted method. On the following day the blood samples were taken from anaesthetized animals, the plasma (the anticoagulant heparin *in vitro*) was separated immediately by centrifugation. The sodium and potassium levels in the urine and plasma were determined using the flame photometry method, the uric acid level – by the reaction with a phosphotungstic reagent. Commercially-available kits from Filisit-Diagnostika (Ukraine) were used.

In the second series of experiments the influence of the dose dependence of GW preparations on glycemia was determined, and their interaction with metformin was studied. Female rats with the body weight of 160 to 220 g were randomly divided into the groups ($n = 5-8$ in each group) according to the preparations they received; the extract and the tincture were introduced within the following doses: 0.1; 0.25; 0.5; 1.0 g/kg, and 0.25; 0.5; 1.0; 2.5; 5.0 ml/kg, respectively (the range included the doses used in the previous studies and the intermediate ones). Metformin was used in the doses of 50, 100, 200, 400, 600 mg/kg (since the dose dependence is typical for this drug, especially in intact animals). Because of the wide range of doses supposed to be studied, a shortened scheme of the experiment was used with the measurement of glycemia at baseline, 60 min after the administration of a single dose of the preparation studied (or the combination with the interval between the preparations to minimize the interaction at the level of absorption), and 60 min after the subsequent intragastric glucose load (3.0 g/kg).

Medians, 25 % and 75 % percentiles (the upper and lower quartiles), as well as the arithmetic means and their

standard errors ($M \pm m$), were calculated. The comparison of the dependent values (dynamics of glycemia) was performed by the Wilcoxon criterion \tilde{T} , the central tendencies of independent samples – by the Mann-Whitney U-criterion. To determine the relationship between the individual parameters the Spearman's correlation coefficient ρ was used.

Results and discussion

The GW tincture, in contrast to the GW extract, showed the hypoglycemic effect in intact rat after the course administration (the novelty of this results was confirmed [8]) with a statistically significant decrease in the basal glycemia and the area under the glycemic curve in the dose of 5 ml/kg. The close results were obtained in the group receiving the reference drug “Arfasetinum,” while the dose of the tincture of 1 ml/kg did not change the values mentioned (Tab. 1). It is supposed that the activity of the GW tincture is to a great extent mediated by hydroxycinnamic acids [2], and our results are consistent with the data in the literature concerning the dose dependence of the hypoglycemic effect of chlorogenic acid observed in the dose of 27 mg/kg, but not 9 mg/kg [11], while the antidiabetic activity of chlorogenic acid was demonstrated within the doses of 5-20 mg/kg [12].

It was shown previously that the extract, when administered to rats receiving the excess fructose combined with hydrochlorothiazide and a relatively low sodium diet might cause an unfavorable hyperaldosteronism (as the result of the high potassium load exacerbated by the relative sodium deficiency and thiazide co-administration) [4]. The increased aldosterone level is known to worsen the glucose metabolism [13], and the potassium exchange is believed to be an important link between the carbohydrate metabolism and the renin-angiotensin-aldosterone system [14]. Evidently, in intact rat these effects were not manifested, there were no changes in the parameters undergoing a strict homeostatic regulation (such as plasma sodium and potassium), and the plasma and urine Na^+/K^+ ratios did not indicate hyperaldosteronism (Tab. 1). The plasma Na^+/K^+ ratios only tended to the increase in animals receiving both doses of the extract, the urine Na^+/K^+ ratio was unchanged against the background of its higher dose (natriuresis and kaliuresis were equally elevated in this group, differing from the data obtained previously [3], after a shorter course of administration). The significant increment of this ratio was seen in rats receiving the dose of 100 mg/kg due to intensified natriuresis and unaltered kaliuresis, in this group a statistically significant positive correlation between glycemia in anesthesia and the plasma Na^+/K^+ ratio also appeared.

With the increased dose of the extract the correlation coefficient between plasma sodium and potassium showed an increment (Tab. 2). In the groups receiving this preparations there was a change in directionality of the relationship between plasma potassium and glycemia (during anaesthesia and, especially, at the basal state). Interestingly, the extract at a higher dose also caused appearance of the significant positive correlation between plasma potassium and the basal glycemia in rats receiving

Table 1

The effect of goutweed preparations and "Arfasetinum" on the glucose and electrolyte metabolism values in rats (n = 6-9)

Indices	Intact control	GW extract, 100 mg/kg	GW extract, 1.0 g/kg	GW tincture, 1.0 ml/kg	GW tincture, 5.0 ml/kg	"Arfasetinum," 18 ml/kg
Basal glycemia, mmol/l	4.22 ± 0.18 4.33 (3.81-4.61)	4.81 ± 0.33 4.90 (4.07-5.36)	4.76 ± 0.23 4.52 (4.31-5.03)	4.37 ± 0.26 4.29 (3.91-4.99)	3.81 ± 0.13*/**/* 3.69 (3.48-4.24)	3.90 ± 0.31 3.81 3.13-4.58
Area under the glycemic curve, mmol × min/l	644 ± 15.2 637 (620-673)	658 ± 64.7 576 (570-785)	603 ± 45.4 589 (545-629)	649 ± 58.9 631 (556-742)	563 ± 29.9* 557 (539-598)	585 ± 13.3* 572 (571-579)
Glycemia in anaesthetized rats, mmol/l	7.52 ± 0.52 7.43 (7.29-8.55)	7.58 ± 0.55 7.06 (6.43-8.47)	8.21 ± 0.44 8.37 (7.17-9.34)	9.36 ± 0.60*/**/* 8.86 (7.98-10.9)	8.38 ± 0.10 8.33 (8.20-8.50)	8.06 ± 0.40 7.75 (7.40-8.28)
Plasma sodium, mmol/l	145 ± 3.95 147 (140-148)	146 ± 4.78 144 (135-156)	155 ± 4.47 154 (147-161)	156 ± 5.94 153 (143-173)	152 ± 4.43 148 (144-159)	151 ± 2.53 150 (146-157)
Plasma potassium, mmol/l	4.37 ± 0.20 4.28 (4.02-4.64)	4.27 ± 0.21 4.22 (3.95-4.62)	4.49 ± 0.19 4.58 (4.28-4.82)	4.46 ± 0.30 4.49 (4.00-4.82)	4.73 ± 0.52 4.48 (3.84-5.69)	3.98 ± 0.29 4.03 (3.55-4.23)
Plasma Na ⁺ /K ⁺ ratio	33.8 ± 1.36 33.1 (30.9-35.4)	34.5 ± 1.43 35.9 (33.7-37.2)	34.8 ± 1.12 35.3 (33.5-36.4)	35.5 ± 2.38 32.4 (31.6-39.4)	34.4 ± 3.62 34.9 (28.4-40.9)	38.9 ± 3.05 38.8 (34.6-43.8)
Urine Na ⁺ /K ⁺ ratio	0.56 ± 0.13 0.55 (0.34-0.64)	1.22 ± 0.09** 1.12 (1.10-1.41)	0.54 ± 0.08**** 0.54 (0.42-0.61)	0.80 ± 0.18 0.82 (0.54-1.15)	0.52 ± 0.09**** 0.55 (0.36-0.72)	Not determined

Notes: * – p < 0.05 compared to the intact control; ** – p < 0.02 compared to the intact control; *** – p < 0.05 compared to the group receiving the extract in the dose of 100 mg/kg; **** – p < 0.02 compared to the group receiving the extract at a dose of 100 mg/kg; ***** – p < 0.05 compared to the group receiving "Arfasetinum"; ***** – p < 0.005 compared to the group receiving the extract in the dose of 1 g/kg. GW – goutweed. Medians are highlighted in bold.

Table 2

Spearman's coefficients of correlation between the individual biochemical parameters in rats receiving goutweed preparations and "Arfasetinum" (n = 6-9)

Indices	Intact control	GW extract, 100 mg/kg	GW extract, 1.0 g/kg	GW tincture, 1.0 ml/kg	GW tincture, 5.0 ml/kg	"Arfasetinum," 18 ml/kg
Plasma sodium – plasma potassium	+0.24 NS	+0.67 NS	+0.83 p < 0.05	+0.49 NS	+0.14 NS	-0.50 NS
Plasma potassium – basal glycemia	-0.60 NS	-0.07 NS	+0.70 NS	-0.60 NS	-0.49 NS	-0.71 NS
Plasma potassium – glycemia in anaesthetized rats	-0.62 NS	-0.57 NS	0	-0.14 NS	-0.30 NS	+0.04 NS
Basal glycemia – plasma Na ⁺ /K ⁺ ratio	+0.55 NS	-0.21 NS	-0.40 NS	+0.54 NS	+0.09 NS	+0.71 p=0.11
Glycemia in anaesthetized rats – plasma Na ⁺ /K ⁺ ratio	+0.33 NS	+0.67 p = 0.07	+0.14 NS	-0.11 NS	+0.30 NS	+0.14 NS
Plasma uric acid – basal glycemia	-0.76 p < 0.05	+0.32 NS	-0.26 NS	-0.21 NS	+0.29 NS	Not determined

Notes: NS – not significant GW – goutweed.

the excess fructose combined with hydrochlorothiazide [4]. This correlation may indicate that in animals with higher kalemia there is more intense activation of the aldosterone system (with the subsequent changes in glucose homeostasis). The biochemical values studied showed more distinct interrelations with the basal glycemia than with glycemia in anaesthetized rats where there were additional factors of homeostasis disturbance.

Correlation analysis also revealed some intensification of the aldosterone control in "Arfasetinum" group, namely the negative interrelationship between plasma sodium and potassium and appearance of the significant positive correlation between the basal glycemia and the plasma Na⁺/K⁺ ratio, presumably indicating higher glycemia in animals with the most activated aldosterone system. It can be noted that high doses of the herbal drugs are commonly used in preclinical research (because of interspecies differences in pharmacokinetics and sensitivity), but they are generally extrapolated in much lower doses in clinics. The latter are hardly able to cause homeostasis disturbances indirectly supported by the absence of the data about side effects of "Arfasetinum" associated with the aldosterone system despite its wide use.

At the same time, the GW tincture was characterized by low risk of aldosterone-associated side effects [3, 4] and no principal differences with the data of the IC were registered after its administration (only the relationship between the basal glycemia and the plasma Na⁺/K⁺ ratio was weakened against the background of the tincture at a higher dose (Tab. 2). As described above, the basal blood glucose level was moderately and dose-dependently reduced. Unexpectedly, in anaesthetized rats the increase in glycemia was seen against the background of the tincture in the dose of 1 ml/kg. This can be explained taking into account the ability of the GW tincture just in this dose to counteract the effect of thiopental [15]. The current study did not address this issues directly still it was observed that in rats receiving the tincture with the higher doses of this agent were needed to obtain anesthesia; therefore, the dose-dependent effect of thiopental on glycemia was more expressed. The latter have been known for a long time [16], and it was shown that it was not associated with the changes in insulin signaling [17]. Thus, this change in glycemia does not possibly indicate the negative effects of the tincture.

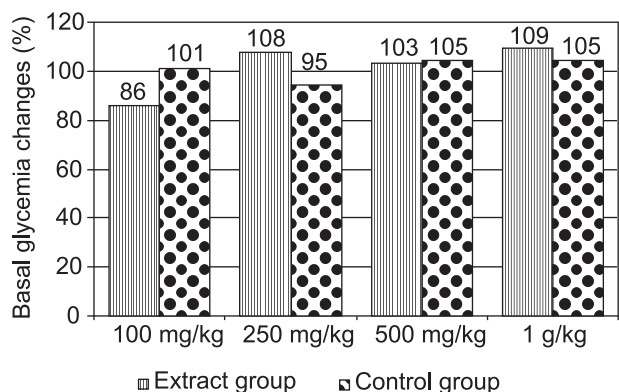


Fig. 1. The effect of the goutweed extract (single dose) on the basal glycemia dynamics in rats

Much attention was paid recently to the interrelationships between glycemia and uricemia. Links between hyperinsulinemia, glucose levels, and tubular glucose uptake and uric acid excretion were described (in the context of the uricemia decrease by sodium-glucose cotransporter 2 (SGLT2) inhibitors) [18]. The relationships of different directions between uricemia and glycemia were seen in clinics [19, 20], and their mechanisms were discussed. It is supposed that in association of hyperglycemia, hypertriglyceridemia and hyperuricemia the latter is caused by the decreased renal uric acid excretion together with the enhancement of its synthesis (including an increased activity of the pentose phosphate pathway and thereby purine biosynthesis) [21].

In our study, glycemia in anaesthetized rats did not correlate with the plasma uric acid in all of the experimental groups, still the negative interrelationship was observed between uricemia and the basal glycemia ($\rho = -0.76$, $p < 0.05$) in intact animals, both GW preparations eliminated this correlation (in all the doses studied). It may reflect their influence on the synthesis and/or transport systems for uric acid. The latter were seen under the effect of GW preparations [2] despite the absence of uricemia changes. Uric acid excretion tended to the increase against the background of the both doses of the extract and remained unchanged in the animals receiving the tincture at a low dose (that is in complete accordance with our previous data [2, 3]), while at a high dose the tincture decreased this value (the differences with the previous data may be associated with the increase of the term of administration from 3 to 10 days with the possible enhancement of the inhibitory influence on xanthine oxidase, as well as with transitory changes in the renal transport).

The results of the second series of experiments confirmed that the GW extract in all the doses used did not reveal unfavorable shifts of the glucose metabolism (as well as the hypoglycemic action) in intact rat. At the same time, no decrease in the basal glycemia was seen (Fig. 1), and the blood glucose level after the glucose load did not differ significantly from the control value (Fig. 2). For this reason the further study of the extract was not conducted.

The goutweed tincture tended toward the reduction of the basal glycemia in the doses of 0.50 and 2.5 ml/kg

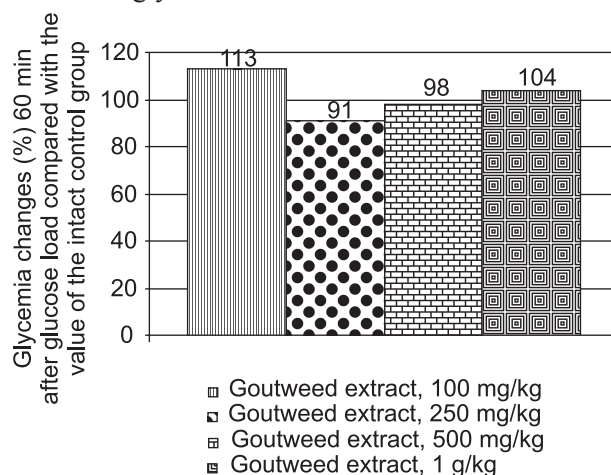


Fig. 2. The effect of the goutweed extract (single dose) on glycemia in rats 60 min after the glucose load

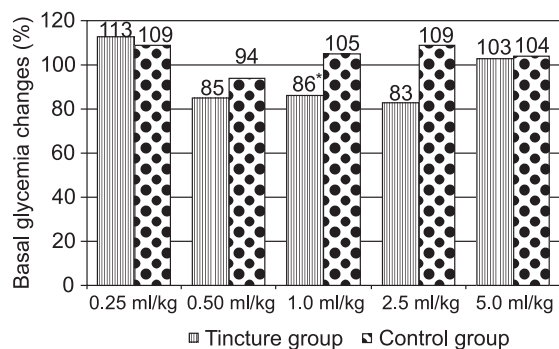


Fig. 3. The effect of the goutweed tincture (single dose) on the basal glycemia in rats

Notes: * – $p < 0.05$ compared to the baseline value.

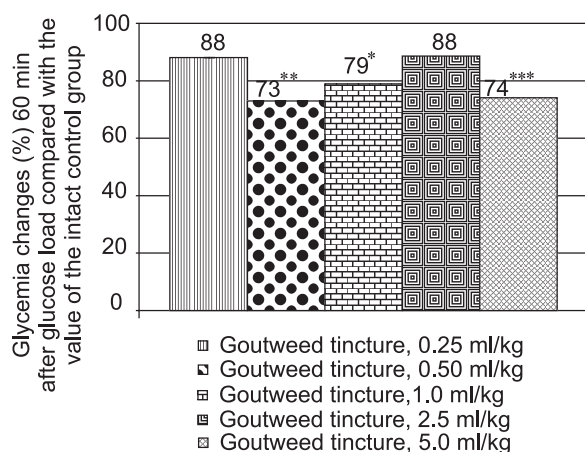


Fig. 4. The effect of the goutweed tincture (single dose) on glycemia in rats 60 min after the glucose load

Notes: * – $p < 0.05$ compared to the simultaneous value of the intact control group; ** – $p < 0.02$ compared to the simultaneous value of the intact control group; *** – $p < 0.01$ compared to the simultaneous value of the intact control group.

and decreased this value in the dose of 1 ml/kg (Fig. 3). The difference was statistically significant when compared to the baseline value of this group, but did not reach the level of statistical significance compared to the data of the synchronous control. The latter corresponded to the results of the previous series of experiments, in which just intergroup differences were analyzed. Besides, the extent of glycemia the decrease was moderate, and the values were within the normal physiological range. As for the higher dose of the tincture, it can be supposed that the course administration is needed for the complete development of the hypoglycemic effect. After the glucose load this effect reached the level of statistical significance in the doses of 0.5; 1.0; 5.0 ml/kg (Fig. 4). Unexpectedly, the dose of 2.5 ml/kg failed to demonstrate any activity allowing us to suppose a biphasic dose-response curve. The doses of 0.5 and 1.0 ml/kg were chosen for further studies.

Before the estimation of a possible interaction between the GW tincture and metformin it was necessary to determine the effective doses of the latter under the conditions of our study. Its ability to decrease the basal glycemia was observed only in the dose of 600 mg/kg (in the dose of 400 mg/kg a tendency was seen, Fig. 5). It is in accordance with the data given in the literature [22].

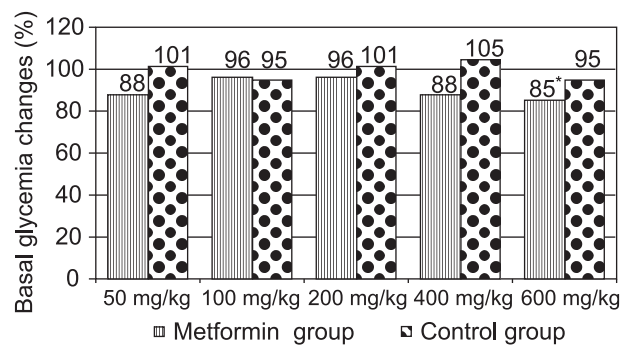


Fig. 5. The effect of metformin (single dose) on the basal glycemia in rats

Notes: * – $p < 0.05$ compared to the baseline value.

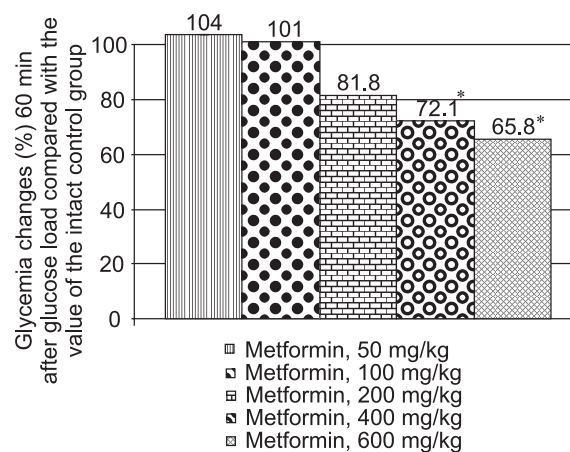


Fig. 6. The effect of metformin (single dose) on glycemia in rats 60 min after the glucose load

Notes: * – $p < 0.02$ compared to the simultaneous value of the intact control group.

As seen from Fig. 7-10, the tincture in the doses of 0.5 and 1.0 ml/kg neither block the effects of metformin, nor enhance its effect with the excessive decrease of the blood glucose concentration. The basal glycemia decreased significantly when metformin was co-administered with the tincture in both doses (Fig. 7, Fig. 9), except for the combinations with the lowest dose of metformin. As for glycemia after the glucose load the tincture in the dose of 0.5 ml/kg did not change the dose dependence of the metformin action (Fig. 8) although it decreased the blood glucose level when administered *per se*. When the dose of the tincture was increased up to 1.0 ml/kg, the statistically significant hypoglycemic effect of metformin was observed in the dose of 200 mg/kg (Fig. 10), i.e. it was possible to decrease the effective dose twice (compared to metformin *per se*, Fig. 6). The absence of a significant glycemia decrease after combined administration of the tincture and low doses of metformin may reflect synergioantagonism. However, these phenomena were seen in intact animals, against the background of the balanced processes of the carbohydrate metabolism.

The possibility of the permissive effect of the goutweed active components (predominantly hydroxycinnamic acids and flavonoids) on the action of metformin was discussed [5].

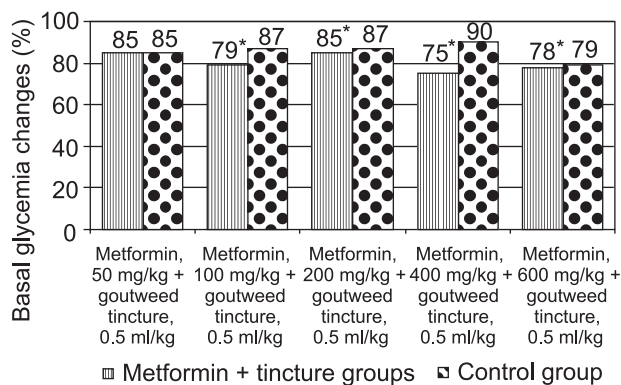


Fig. 7. The effect of the goutweed tincture in the dose of 0.5 ml/kg combined with metformin (single dose) on the basal glycemia in rats

Notes: * – p < 0.05 compared to the baseline value.

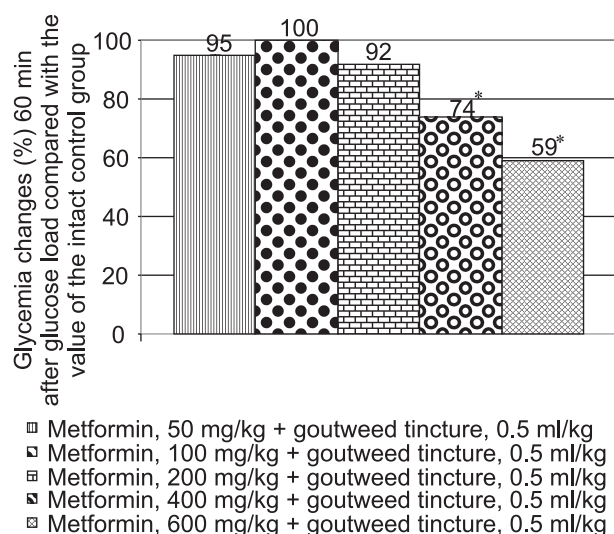


Fig. 8. The effect of the goutweed tincture in the dose of 0.5 ml/kg combined with metformin (single dose) on glycemia in rats 60 min after the glucose load

Notes: * – p < 0.05 compared to the simultaneous value of the intact control group.

Hydroxycinnamic acids are among the most important herbal components counteracting the metabolic syndrome. Chlorogenic acid and ferulic acid show the synergistic effect with metformin on the glucose uptake by myotubes through different mechanisms in relation to PI3K [23]. Caffeic acid can activate AMPK [24]. Such mechanisms might be implemented in our studies, still further research is needed.

CONCLUSIONS

1. The goutweed tincture can exhibit the hypoglycemic action in intact rats after introduction of single doses of 0.5; 1.0; 5.0 ml/kg, while the goutweed extract

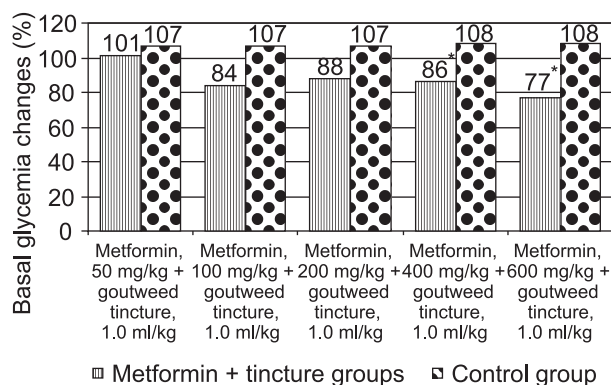


Fig. 9. The effect of the goutweed tincture in the dose of 1.0 ml/kg combined with metformin (single dose) on the basal glycemia in rats

Notes: * – p < 0.05 compared to the baseline value.

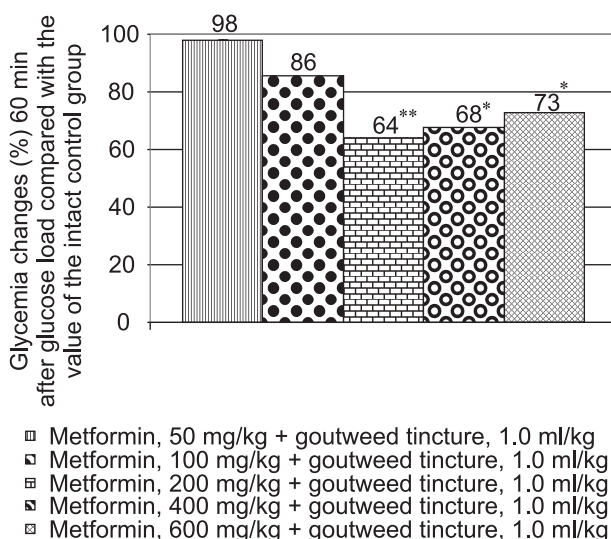


Fig. 10. The effect of the goutweed tincture in the dose of 1.0 ml/kg combined with metformin (single dose) on glycemia in rats 60 min after the glucose load

Notes: * – p < 0.05 compared to the simultaneous value of the intact control group; ** – p < 0.01 compared to the simultaneous value of the intact control group.

does not demonstrate this action in the doses of 100; 250; 500 mg/kg and 1.0 g/kg.

2. The goutweed tincture in the doses of 0.5 and 1.0 ml/kg does not block the effects of metformin, and does not enhance its effect with the excessive decrease of the blood glucose concentration. In rats receiving the glucose load the tincture in the dose of 1.0 ml/kg (but not 0.5 ml/kg) co-administered with metformin allows reducing its effective dose from 400 mg/kg to 200 mg/kg.

Conflict of Interests: authors have no conflict of interests to declare

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