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Metabolic Effects of Goutweed (*Aegopodium podagraria* L.) Tincture and Metformin in Dexamethasone-Treated Rats

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Abstract: The interest in the preclinical studies of the efficacy and safety of herbal drugs combinations with antidiabetic medicines increases. *Aegopodium podagraria* L. (goutweed) is a plant widely used in traditional medicine. The tincture obtained from its aerial part is characterized by antihyperglycemic, lipid-lowering, hepatoprotective and nephroprotective effects and is able to partially increase the efficacy of metformin in the animals receiving dexamethasone (permissive effect in regard to glucose and lipid metabolism normalization). The objective of this study is to determine the influence of goutweed tincture combined with metformin on protein and purine metabolism as well as the state of liver in dexamethasone-treated rats. The animals were divided into 5 groups as follows: intact control, dexamethasone (untreated), dexamethasone + metformin, 50 mg/kg; dexamethasone + *A. podagraria* tincture, 1 ml/kg intragastrically; dexamethasone + metformin, 50 mg/kg intragastrically + *A. podagraria* tincture, 1 ml/kg intragastrically. Dexamethasone was used at a dose of 5 mg/kg subcutaneously for 5 days. Body weight dynamics was registered, total protein and albumin level, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase activity was determined in blood plasma, uric acid and urea content – in blood plasma and urine. Several favourable effects of the combination of goutweed tincture and metformin were seen, namely, the reduction in plasma ALT activity and increase in urea clearance as well as normalization of ALP activity. In contrast to metformin, goutweed tincture limited the dexamethasone-induced increase in plasma albumin concentration and decreased De Ritis ratio. Dexamethasone tended to increase renal uric acid excretion, metformin led to the further increment, in the groups receiving goutweed tincture this value was unchanged, but, proceeding from the normal values of uricemia, extrarenal mechanisms of the influence on purine metabolism were possible in these animals. All of the investigated preparations did not influence on plasma AST activity and caused further decrease in body weight that was reduced under the influence of dexamethasone. In-depth research of the interaction of goutweed tincture with metformin is expedient.

Keywords: *Aegopodium podagraria* L., Goutweed, Dexamethasone, Metformin, Rats, Combined Drugs

1. Introduction

Herbal drugs are used by the human beings since time immemorial, at present more than 80% of the world's population (mainly in the developing countries) rely on plant-derived drugs for their primary health care needs [1], while in the developed countries the patients are becoming more interested in traditional herbal medicines and frequently use them together with the conventional drugs, often without a medical advice. Given that the most of the coadministered herbal products are characterized by complex composition, there is a high risk of herb-drug interactions. Long evolutionary history of interactions between the plants and the herbivorous animals led to the formation of enzymatic systems limiting bioavailability of herbal biologically active substances [2]. From the other point of view, herbal drugs are able to exert favourable pharmacological effects. In this case the complex composition may stipulate synergistic effects that explain the efficacy of such drugs despite the moderate activity and/or concentration of the individual components. Besides, synergism is possible in regard to the conventional drugs allowing the decrease in their dosage. The herbal drugs may reduce toxicity or side effects of the conventional drugs as well as favourably broaden their pharmacodynamics [3].

The aforesaid aspects are of great importance in case of metabolic syndrome and, especially, type 2 diabetes. The latter remains a global problem because of high prevalence, which is constantly increasing, and a need in lifelong treatment with the desirable influence on complications development and quality of life [4]. The preclinical studies of the efficacy and safety of herbal drugs and phytochemical constituents combinations with antidiabetic medicines, including metformin (as the widely prescribed first line agent of the peroral normoglycemic drugs), have intensified recently [5, 6].

The object of our study is goutweed (*Aegopodium podagraria* L., GW). This perennial plant of the Apiaceae family has long been consumed as vegetable and used in folk medicine. It is indigenous to Europe, Siberia, the Caucasus, Kazakhstan and Central Asia mountainous regions and has been naturalized in North America and Australia. Dry extract and tincture obtained from GW aerial part are standardized on hydroxycinnamic acids content and are characterized by low toxicity level confirmed experimentally [7, 8]. Together with hydroxycinnamic acids, flavonoids (Figure 1), protein-polysaccharide complex, micro- and macroelements contribute to pharmacological activity of GW preparations [9–11]. A beneficial effect of these drugs on purine metabolism has been proven as well as nephroprotective and hepatoprotective properties that can be of great value in metabolic syndrome and diabetes type 2 [7–9, 12]. The influence of GW preparations on carbohydrate metabolism has also been partially characterized and it has been shown that the tincture and the extract exert protective action in alloxan-induced diabetic mice [7]. GW tincture appeared to be especially effective in carbohydrate metabolism disorders. It renders hypoglycemic effect in rats receiving excess fructose combined with hydrochlorothiazide [12, 13] and mildly decreases glycemia level in intact rats (the hypoglycemic effect in the intact animals is dose-dependent and in the study [14] it was manifested at a dose of 5 ml/kg). These data substantiate the choice of the tincture as a preparation with a significant influence on glucose metabolism for the next studies. As the dose of 1 ml/kg intragastrically was considered to be safe (not causing hypoglycemia in the intact animals [14]) and highly effective in animals with carbohydrate metabolism disorders (there was no advantage of increasing the dose of the tincture in rats receiving fructose and hydrochlorothiazide [12, 13]), just this dose was used in further studies in dexamethasone-treated rats [11]. It has been shown that the tincture partially increases the efficacy of metformin that was used at a dose of 50 mg/kg intragastrically. This respectively low dose was chosen because the study was aimed at exploring the possibility of synergism (still metformin at this dose was found to be effective on the model of diabetes [5]). Such indices of carbohydrate metabolism as glycemia (basal and its changes in insulin tolerance test as well as in oral glucose tolerance test), liver glycogen level, lipid metabolism values of blood plasma were characterized [11]. As there is a need in the multidirectional influence on the pathogenesis of metabolic syndrome and diabetes type 2, it is expedient to complete these results with the data concerning protein and purine metabolism, as well as the state of the liver as the target organ of diabetes.

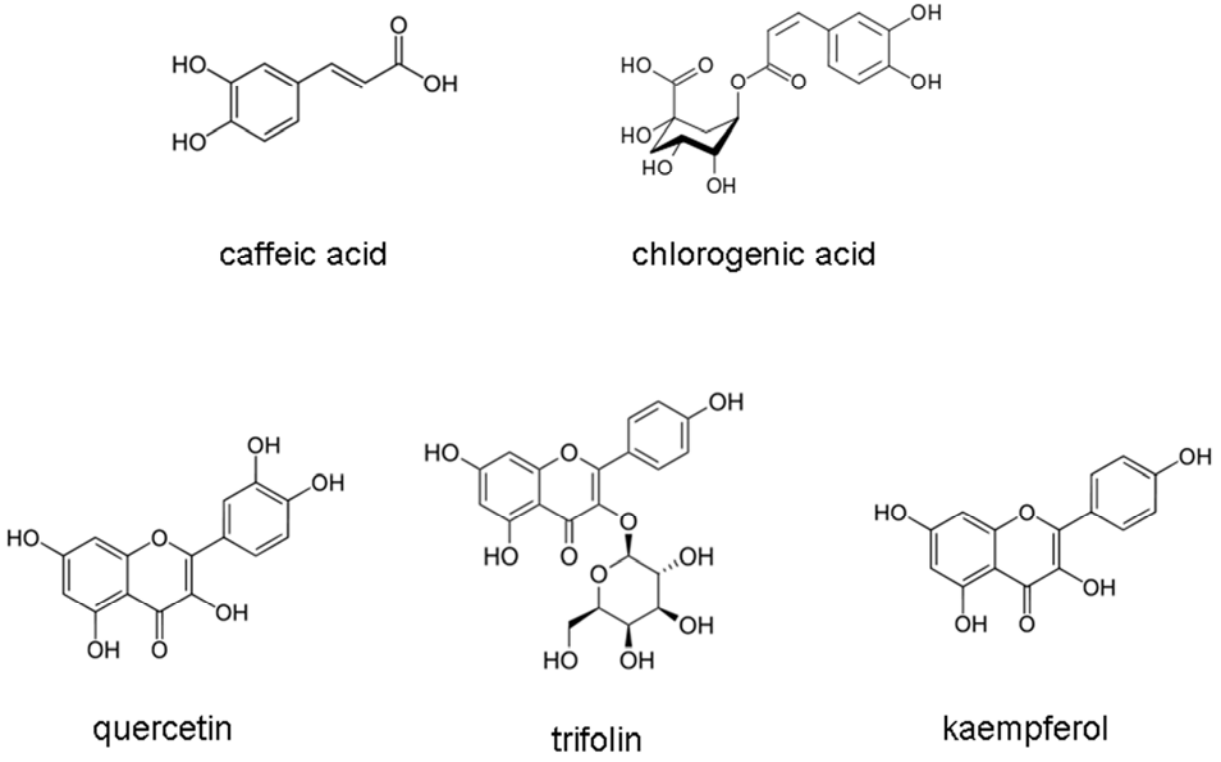


Figure 1. *Phytochemical constituents of goutweed.*

2. Materials and Methods

Noninbred albino rats breeded in the Central Scientific-Research Laboratory of National University of Pharmacy were used. Male rats with 180 to 240 g body weight were kept under controlled standard conditions, on a natural light-dark cycle [15]. All the 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."

The animals were divided into 5 groups as follows:

intact control,

dexamethasone (untreated);

dexamethasone + metformin, 50 mg/kg;

dexamethasone + GW tincture, 1 ml/kg intragastrically;

dexamethasone + metformin, 50 mg/kg intragastrically + GW tincture, 1 ml/kg intragastrically (n=6–7 in each group).

Dexamethasone (solution for injection, 4 mg/ml, KRKA, d. d., Slovenia) was used at a dose of 5 mg/kg subcutaneously for 5 days as characterized previously [11]. The interval between the administration of GW tincture and metformin equalled 40 min to minimize interaction at the level of absorption. On day 5, 40 min after the drugs administration (the rats fasted for 12 hours before), the status of excretory renal function (ERF) was determined: after administration of water loading at a rate of 3% of body weight, urine was collected for two hours. The animals were previously adapted to the conditions of the experiment. After this, heparinized blood samples were drawn by exsanguination from barbiturate-anesthetized animals and plasma was separated immediately by centrifugation. The generally accepted routine biochemical methods were applied for blood plasma and urine analysis. Plasma total protein concentration was measured by biuret method [16], albumin level – by the bromcresol green procedure [17]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity in plasma were determined according to the method of Reitman and Frankel and De Ritis ratio was calculated [18]. Alkaline phosphatase (ALP) activity was assayed by measurement of the amount of phenol liberated from the hydrolysed substrate [19], gamma-glutamyl transferase (γ-GT) activity – by the kinetic method using γ-L-glutamyl-3-carboxy-4-nitroanilide as a substrate and glycylglycine as an acceptor [20]. Uric acid content in urine was measured by reaction with the phosphotungstic reagent [21], in blood plasma – by the enzymatic method [22], urea concentration – by the reaction with diacetyl monooxime [23]. Using the generally accepted formula, urea and uric acid excretion was determined.

Medians, 25% and 75% percentiles (upper and lower quartiles) were calculated as well as the traditionally used arithmetic means and their standard errors (M±m). Taking into account a problematical character of multiple comparisons in pharmacology and toxicology [24], the comparison of the central tendencies of independent samples was performed by the Mann-Whitney U-criterion. To determine the relationship between the individual parameters, the Spearman's correlation coefficient of ρ was used.

3. Results and Discussion

Dexamethasone-induced model is considered to reproduce the pathogenetic mechanisms of the metabolic syndrome and type 2 diabetes [15]. It has been previously shown that dexamethasone at the dose of 5 mg/kg under the conditions of our experiment causes a significant increment of basal glycemia, as well as the reduction of insulin sensitivity and tolerance to glucose load. A dramatic increase in liver glycogen level was also seen. The lipid metabolism changes were manifested in the elevated plasma triglycerides, total lipids and total cholesterol content tended to the increase [11].

According to the results obtained in the current study (Figure 2), the significant increment was seen in AST and especially ALT activity that is a generally regarded as a marker of hepatocellular injury [25]. Still, apart from the other steroids, glucocorticoids and particularly dexamethasone are generally considered to be non-hepatotoxic and no signs of hepatocytes injury were seen in animals receiving dexamethasone, even at extremely high doses 8 and 30 mg/kg [26]. It can not be excluded that mildly elevated activity of an enzyme in plasma may result from the direct activation or from the increase in its expression in tissues which subsequently leads to the higher degree of its appearance in plasma. Given that dexamethasone interferes protein metabolism, such changes in regard to the investigated transferases are possible. Furthermore, it has been shown in vitro that both ALT isoforms in the hepatocytes are induced by dexamethasone directly increasing glucose output. In gluconeogenic conditions in vivo, namely in obese and diabetic animals, protein levels of ALT are elevated in the liver and presumably are involved into the development of insulin resistance and diabetes [27]. Much attention is paid to the clinically observed mild-to-moderate increase in ALT plasma activity in the wide-spread metabolic disorders such as obesity, hyperlipidemia, diabetes mellitus [28]. It is generally accepted that glucocorticoids at high doses are able to intensify gluconeogenesis [29] (the signs of such changes under the conditions used were clearly seen in our previous studies [11]), and this process maintenance is partially provided by pyruvate synthesis from alanine [27]. These data permit to associate the observed increment of ALT activity with the metabolic changes rather than with marked cytolysis. In addition, activity of γ-GT that is generally considered to be a sensitive marker of hepatocellular injury was not elevated (Figure 3), moreover, there was a decrease in its activity in the untreated group receiving dexamethasone ($p=0.06$ when compared with the intact control value). A positive interrelation between the activity of these enzymes, which might be expected in cytolysis, was not registered (Table 1), it even tended to be negative under the influence of dexamethasone.

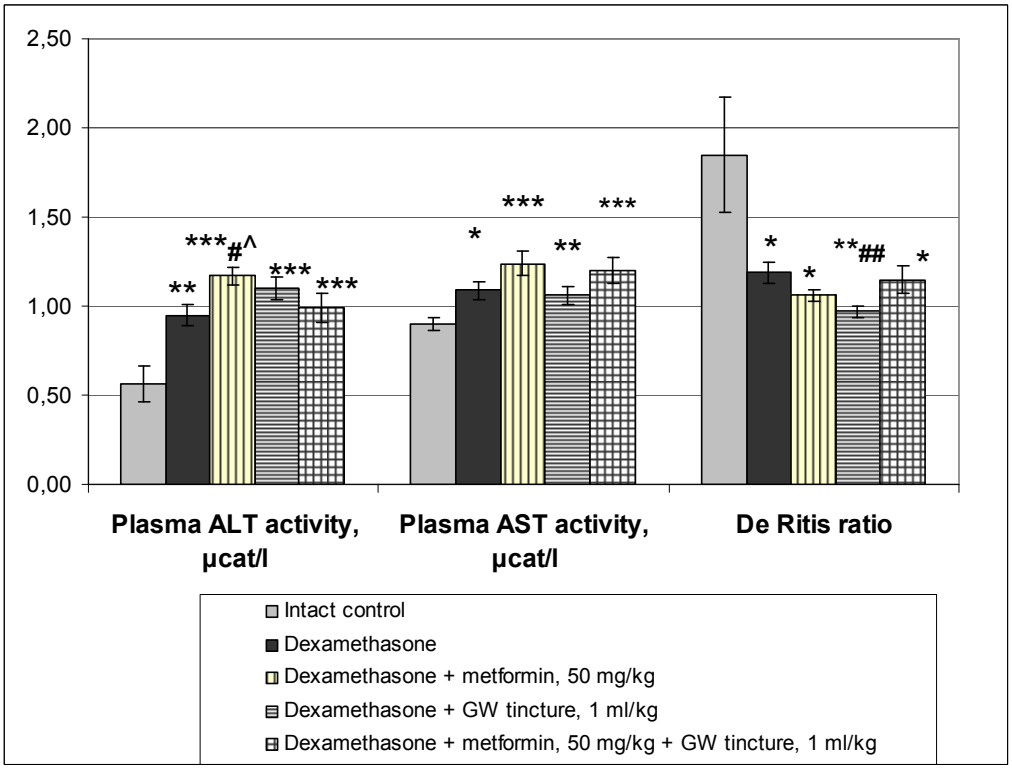


Figure 2. *The influence of goutweed tincture and metformin on plasma ALT activity, AST activity anf De Ritis ratio in rats receiving dexamethasone; Mean ± S.E.M.*

Notes. * – p<0.05 compared to intact control; ** – p<0.02 compared to intact control; *** – p<0.01 compared to intact control; # – p<0.05 compared to the untreated group; ## – p<0.02 compared to the untreated group; ^ – p<0.05 compared to the group receiving metformin and GW tincture.

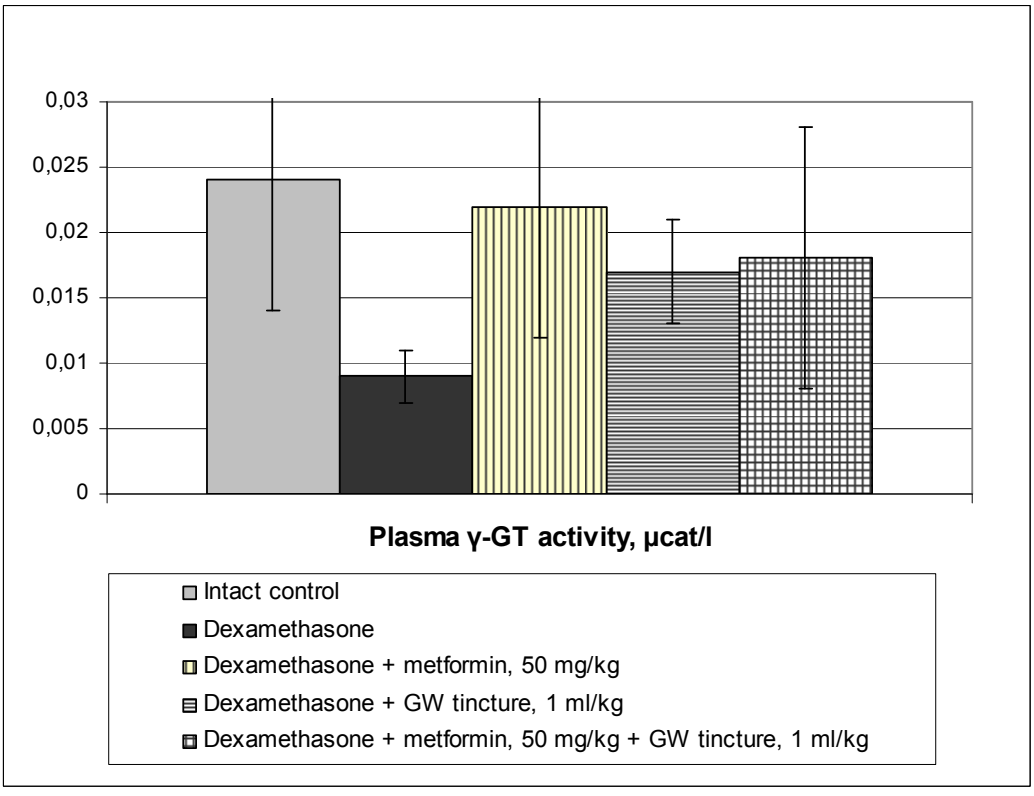


Figure 3. *The influence of goutweed tincture and metformin on plasma γ-GT activity in rats receiving dexamethasone; Mean ± S.E.M.*

Table 1. *Spearman's correlation coefficients of ρ between the individual biochemical parameters of rats rats receiving dexamethasone.*

Indices	Intact control	Dexamethasone	Dexamethasone + metformin, 50 mg/kg	Dexamethasone + GW tincture, 1 ml/kg	Dexamethasone+ metformin, 50 mg/kg + GW tincture, 1 ml/kg
ALT activity –liver glycogen content	+0,75	+0,40	–0,06	+0,31	+0,64
	p=0.08	NS	NS	NS	p=0.17
ALT activity – glycemia	+0,23	+0,60	+0,29	+0,20	+0,23
	NS	NS	NS	NS	NS
ALT activity –AST activity	+0,91	+0,36	+0,90	+0,49	+0,21
	p<0.02	NS	p<0.02	NS	NS
ALT activity –ALP activity	+0,63	+0,41	+0,21	+0,80	–0,97
	NS	NS	NS	NS	p<0.02
ALT activity –γ-GT activity	+0,67	–0,67	–0,50	–0,72	–0,50
	NS	NS	NS	NS	NS
γ-GT activity –de Ritis ratio	–0,77	+0,97	–0,10	+0,67	–1,00
	p=0.07	p<0.01	NS	NS	

Notes. GW – goutweed; NS – not significant.

Previously it has been revealed that the positive correlation between the level of liver glycogen and plasma glucose (inherent in the intact animals) disappeares in dexamethasone-treated rats [11] possibly indicating the disorder of glycemia control mechanisms. It is well known that glucocorticoids at high doses promote gluconeogenesis in hepatic tissue inducing the expression of the rate-limiting enzymes for gluconeogenesis and increasing bioavailability of substrates for this process (partially due to the muscle wasting) [29]. The current calculation shows that the relationship between ALT activity and glycemia was intensified in the untreated group of rats receiving dexamethasone. Such changes may indicate the increased contribution of gluconeogenesis into glycemia maintaining. Moreover, the positive correlation between the level of liver glycogen and ALT activity was eliminated in the untreated group of rats receiving dexamethasone (Table 1), while this interrelation (as well as the interrelation discussed above) was partially restored in the group of animals receiving combination of metformin and GW tincture. These data possibly confirm the significant improvement of the carbohydrate metabolism values seen previously in these animals [11].

GW tincture per se did not influence on ALT activity (Figure 2), metformin caused its further increase, while their combined use led to the certain reduction in this value (statistically significant difference with the data of animals receiving metformin per se). In this context it should be noted that just in the group receiving combined treatment the lowest basal glycemia was seen [11]. The insufficiency of metformin hypoglycemic action was possibly connected with the respectively low dose used and in such dose the influence on gluconeogenesis might not be manifested [11]. The hypoglycemic action of GW tincture and its permissive effect on the action of metformin are also possibly mediated by gluconeogenesis suppression that can be attributed to such constituents of GW as hydroxycinnamic acids. Highly specific mechanisms of gluconeogenesis inhibition were established for chlorogenic acid [30], its metabolite ferulic acid [31], as well as p-coumaric acid [32]. So, these data are consistent with the minimal ALT activity in the group of rats receiving the investigated combination among the treated groups receiving dexamethasone as well as with the absence of its decrease against the background of metformin per se.

All of the investigated agents did not change AST activity (Figure 2), against the background of the tincture de Ritis ratio was slightly decreased that was mainly caused by the respectively low AST activity values. AST and ALT activity positively correlated in the intact animals, this interrelation was eliminated by dexamethasone and restored in the animals receiving metformin (Table 1). ALP activity was not significantly changed by dexamethasone (Figure 4). In the clinical study of bone biomarkers it was established that metformin is able to reduce alkaline phosphatase activity [33, 34], still its influence on alkaline phosphatase in dexamethasone-induced metabolism disorders is less investigated (so is the effect of the tincture). Combined treatment eliminated the decrease of ALP activity observed in rats treated with the tincture per se or metformin per se (statistically significant differences between the group receiving combination and the groups receiving both of its components) and a negative interrelation between ALP and ALT activity appeared in this group (Table 1).

As to γ-GT activity (partially discussed before), all of the studied agents showed a tendency towards its normalization, still the differences were not statistically significant because of rather high inter-individual variability of this value. It can be noted that γ-GT is known to participate in prooxidative/antioxidant processes and the decrease in its activity can be linked to glutathione depletion [35]. Glutathione depletion together with the increase of TBA-reactive substances was registered in rats under the influence of dexamethasone at a dose used in our study [36]. Furthermore, antioxidant mechanism of action was proven for metformin [37], and the ability of the tincture to reduce oxidative stress in liver injury was also established before [9]. These effects could possibly be manifested in dexamethasone-treated animals, still we have not addressed them in the current study. The negative interrelation between γ-GT activity and de Ritis ratio (inherent in tne intact animals) was restored in rats

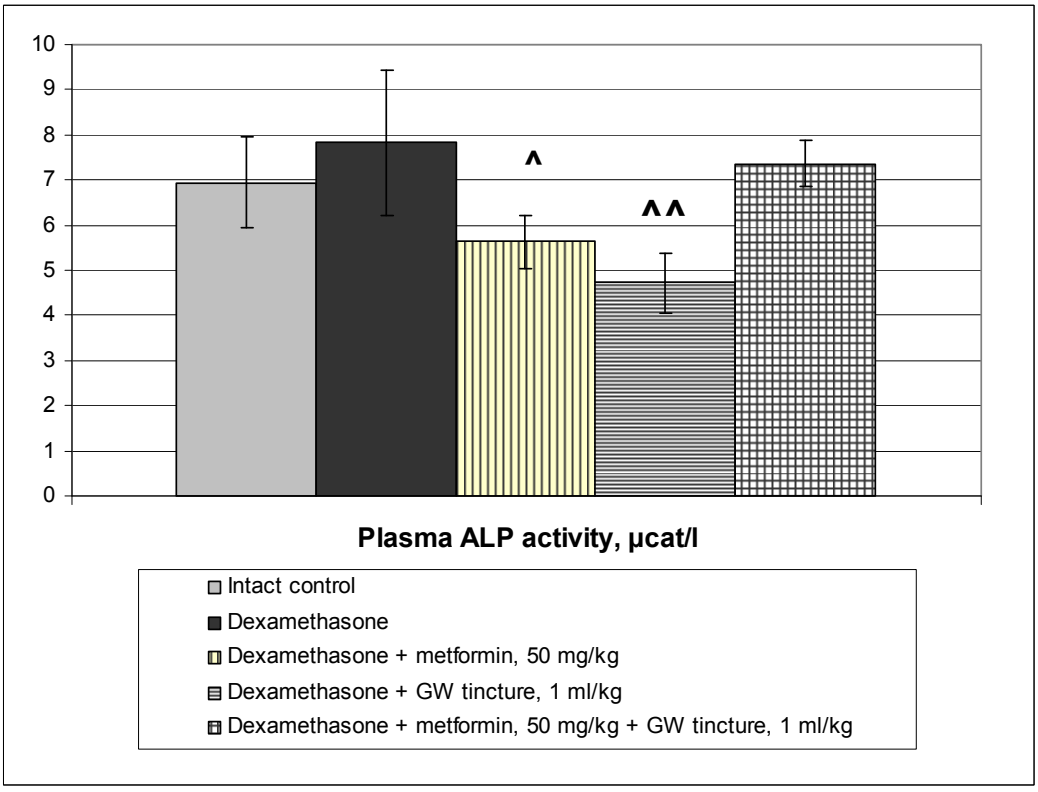
treated with the investigated combination while in the untreated group there was a statistically significant positive correlation indicating the decline in γ-GT activity in the animals with the most altered transferases activity (resulting in reduced de Ritis ratio, Table 1).

Total protein level in plasma remained practically unchanged in all of the experimental groups, but albumin concentration showed a significant increment in dexamethasone-treated rats (Table 2). The ability of dexamethasone to increase albumin synthesis is generally well known and has been confirmed in vitro [38]. Metformin did not influence on the dexamethasone-induced intensification of albumin synthesis that is consistent with the data [38] obtained on primary cultures of rat hepatocytes and on animals chronically pretreated with metformin. In contrast to metformin, GW tincture normalized albumin content in plasma ($p<0.01$ when compared with the untreated group), but after its simultaneous administration with metformin this effect dissapeared.

Table 2. *Influence of goutweed tincture and metformin on plasma biochemical markers in rats receiving dexamethasone; Mean ± S.E.M; Q_{50} (Q_{25}–Q_{75}), n=5–7 in each group.*

	Intact control	Dexamethasone (untreated)	Dexamethasone + metformin, 50 mg/kg	Dexamethasone + GW tincture, 1 ml/kg	Dexamethasone + metformin, 50 mg/kg + GW tincture, 1 ml/kg
Total protein, g/l	68.6±1.67	67.9±1.17	73.3±2.90	66.2±2.76	69.9±1.90
	70.2	**66.7**	**71.3**	**68.9**	**70.6**
	(67.3–71.3)	(66.0–70.0)	(69.4–74.2)	(61.9–70.5)	(67.9–73.4)
Albumin, g/l	36.9±1.31	44.7±0.70***	42.3±1.50*	37.5±1.95###	40.4±2.06
	36.6	**44.6**	**42.4**	**38.7**	**40.4**
	(35.5–38.1)	(43.5–46.1)	(39.9–45.3)	(33.3–40.6)	(38.7–44.2)
Urea, mM/l	3.06±0.32	5.39±0.55**	5.58±0.51**	5.91±0.79*	5.07±0.32***
	3.04	**5.20**	**5.63**	**6.19**	**5.04**
	(2.59–3.30)	(4.96–5.40)	(5.09–6.37)	(4.95–7.36)	(4.67–5.11)
Uric acid, mM/l	0.079±0.008	0.080±0.006	0.079±0.010	0.086±0.009	0.072±0.004
	0.071	**0.073**	**0.078**	**0.077**	**0.071**
	(0.066–0.093)	(0.070–0.085)	(0.065–0.084)	(0.076–0.084)	(0.064–0.080)

Notes. * – $p<0.05$ compared to intact control; ** – $p<0.02$ compared to intact control; *** – $p<0.01$ compared to intact control; ### – $p<0.01$ compared to the untreated group. GW – goutweed. Medians are highlighted in bold.



Notes. ^ – $p<0.05$ compared to the group receiving metformin and GW tincture; ^^ – $p<0.02$ compared to the group receiving metformin and GW tincture.

Figure 4. *The influence of goutweed tincture and metformin on plasma ALP activity in rats receiving dexamethasone; Mean ± S.E.M.*

Catabolic action of the high dose of dexamethasone was accompanied with the increase in plasma urea concentration by 70% ($p<0.01$ when compared with the intact control, Table 2). Renal origin of this phenomenon is not beleivable since urea clearance did not change significantly (it even tended toward the increase), and urea excretion showed a statistically significant two-fold increment (Figure 5). All of the investigated agents did not lead to the reduction of plasma urea level. From the previous studies it is known that anabolic effect is not inherent in GW tincture, while its renal effects are dose-dependent and also depend on the conditions of the experiment [7, 8]. At some models (for example, in alloxan-induced diabetes in mice) the decrease in urea clearance was seen under the influence of the tincture [7]. Still, in dexamethasone-treated animals receiving GW tincture such effects were not pronounced, and the differences of the investigated markers of urea exchange with the data of the untreated group did not reach a level of statistical significance (Table 2, Figure 5). Since in the group receiving metformin the tendency was seen towards the relative increase in urea clearance and excretion, the differences between this group and the group treated with the tincture per se appeared to be statistically significant (Figure 5). Even more interesting fact is that combining metformin with the tincture allowed to maintain urea clearance ($p<0.05$ when compared with the value of animals receiving the tincture per se). Therefore, metformin is able to eliminate the potentially unfavourable influence of GW tincture on renal excretion of urea, the mechanism of this interaction needs further research. Recently attention has been attached to metformin pleiotropic effects including influence on urea cycle (associated with the changes in interlocked nitric oxide cycle) that was seen in patients with type 2 diabetes [39]. In the experiments of Owolabi O. J., Omogbai E. K. (2012) metformin reduced the level of urea in blood plasma of diabetic rats (favourable renal effects were also established, but there were no data about urea clearance) [40].

Catabolic effect of dexamethasone also caused the decline in body weight (Figure 6) and all of the investigated preparations led to its further decrease. It is consistent with the well-known ability of metformin to reduce body weight (or, at least, not to promote weight gain) and with the experimental data concerning the absence of GW tincture influence on body weight of diabetic animals [7].

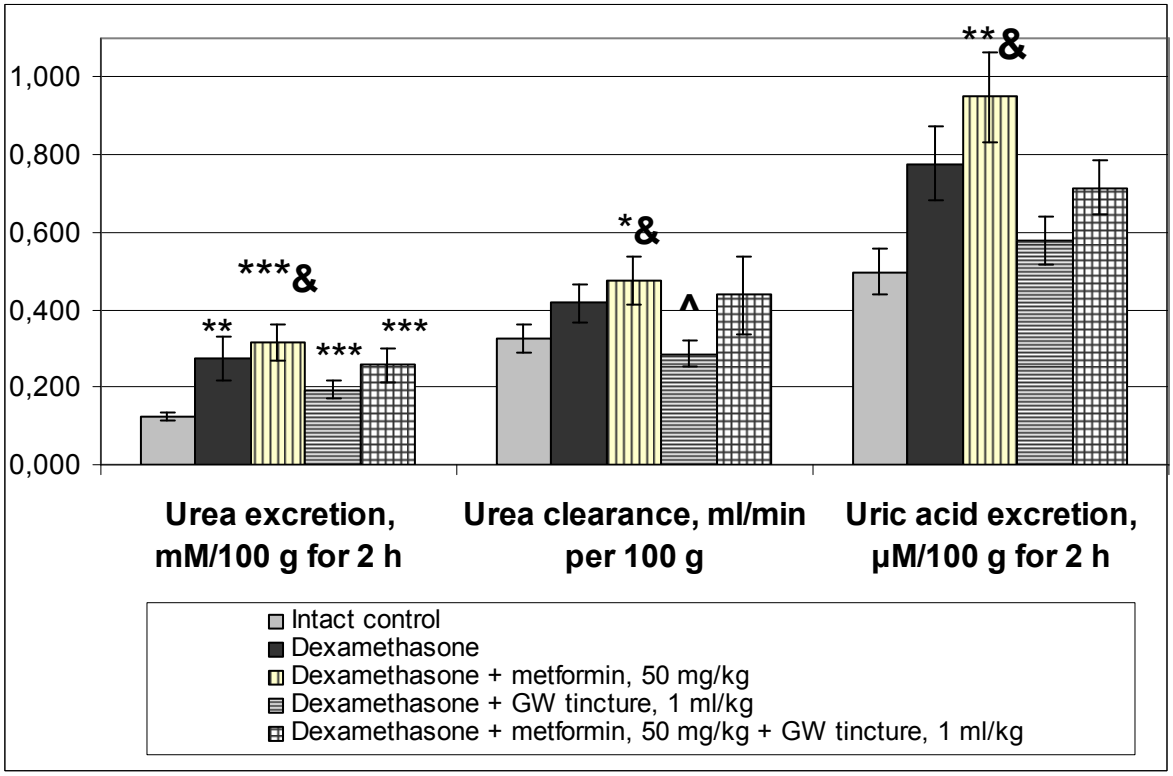
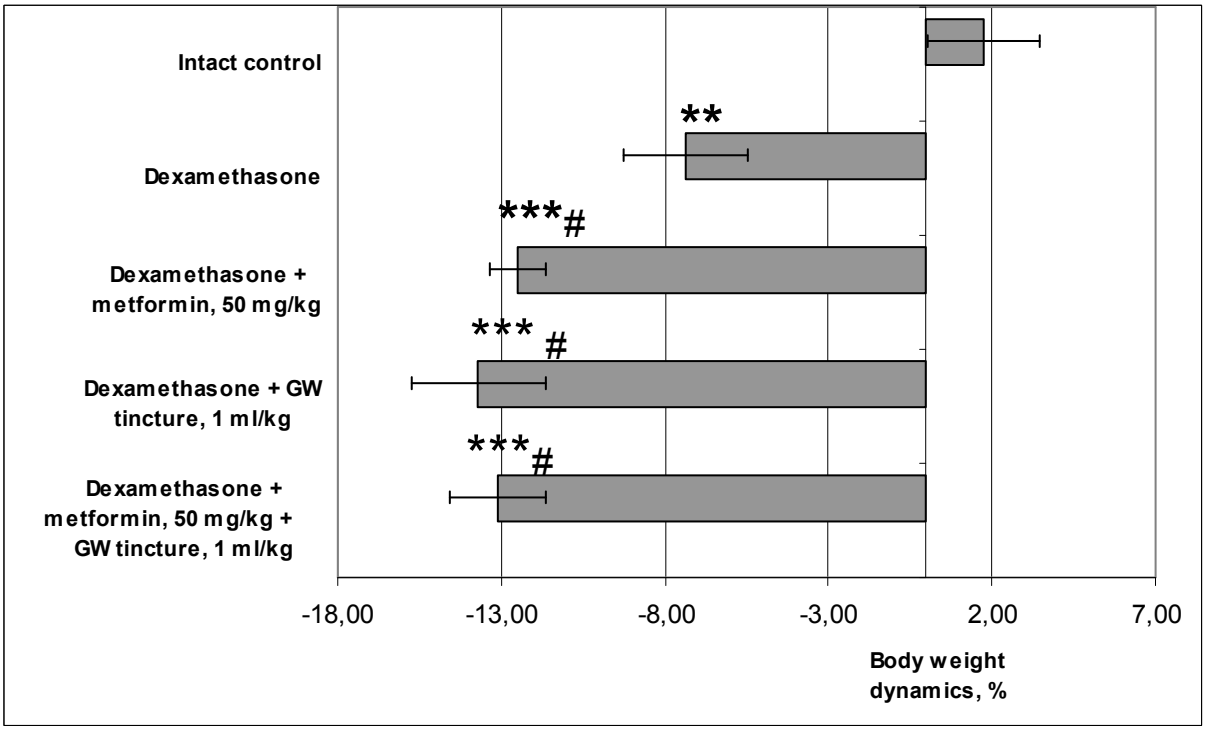


Figure 5. *The influence of goutweed tincture and metformin on urea and uric acid renal excretion in rats receiving dexamethasone; Mean ± S.E.M.*

Notes. * – $p<0.05$ compared to intact control; ** – $p<0.02$ compared to intact control; *** – $p<0.01$ compared to intact control; ^ – $p<0.05$ compared to the group receiving metformin and GW tincture; & – $p<0.05$ compared to the group receiving GW tincture. GW – goutweed.



Notes. ** – p<0.02 compared to intact control; *** – p<0.01 compared to intact control; # – p<0.05 compared to the untreated group. GW – goutweed.

Figure 6. *The influence of goutweed tincture and metformin on body weight dynamics in rats receiving dexamethasone; Mean ± S.E.M.*

Determination of plasma uric acid concentration led to the quite similar results in all of the studied groups (Table 2). It is not surprising given that the presence of the active uricase metabolizing uric acid into allantoin is the prerequisite of the low basal uricemia in rodents as well as its stability even under the conditions of different metabolic shifts. Still in the untreated group the clear tendency towards the increment of uric acid excretion was registered, indicating the partial involvement of the kidney into normouricemia maintaining. Metformin led to the further increase in this value and there were statistically significant differences with the data of the intact control group as well as the with group receiving GW tincture. Normouricemic effect of metformin was established in patients with gout, still it was unrelated to the changes in renal uric acid excretion [41]. At the same time, uric acid excretion in animals receiving GW tincture was statistically significantly lower than the value of metformin-treated group. This is satisfactorily explained when considering the significant inhibitory influence of GW tincture on xanthine oxidase activity [9], whereas such effect is not inherent in metformin. Although the decline in plasma xanthine oxidase activity was seen in patients with type 2 diabetes mellitus receiving metformin [42], this change was considered to be secondary phenomenon linked to the normoglycemic action with the subsequent limitation of oxidative stress. The absence of the direct suppressive influence of metformin on xanthine oxidase activity was confirmed in HUVEC cells: after 2-fold increase in glucose level the activity of this enzyme increased 3.3-fold and was not changed after metformin treatment [37]. Therefore, combining metformin with xanthine oxidase inhibitors, such as phytochemical constituents of GW, seems to be promising and it is expedient to study such combinations using other experimental models with the primary disorders of purine metabolism.

The question about mechanisms of action and the active components of GW tincture was partially discussed before allowing to suppose the primary role of hydroxycinnamic acids and flavonoids (macroelements, especially potassium and magnesium, also may take part in the tincture activity) [9, 11]. The inhibitory effect of hydroxycinnamic acids on gluconeogenesis was mentioned above. Besides, quercetin is among the flavonoids of GW tincture and it is under extensive research now as a highly promising antidiabetic agent (able to downregulate the key gluconeogenesis enzymes as well as to exert hepatoprotective effect) [43]. In addition to the numerous data concerning quercetin efficacy on the models of diabetes and metabolic syndrome, including those induced with glucocorticoids [44], there are experimental results proving the additional beneficial effects of this flavonoid in dexamethasone-treated animals, such as neuroprotective activity [45] as well as the ability to counteract glucocorticoid-induced osteoporosis [46].

The synergistic mechanisms of the herbal drugs constituents are generally recognized and with high probability are involved into GW tincture activity. Synergism is possible in regard to the different hydroxycinnamic acids and flavonoids. For example, the favourable metabolic effects of coffee are believed to be the result of the synergistic polyphenols action with the significant contribution of hydroxycinnamic acids. In the context of the obtained results, it is not without interest that

consumption of coffee was associated with lower risk of elevated ALT activity [47, 48]. Synergistic action of hydroxycinnamic acids is supposed to directly mediate the hepatoprotective activity of the medicinal plants from the different families [49].

Still there is not enough data concerning the mechanisms of interaction of hydroxycinnamic acids (as well as flavonoids and other GW constituents) with the conventional antidiabetic drugs including metformin. In-depth study of GW tincture interaction with metformin with the analysis of liver enzymes activity should be addressed in future and the current results substantiate the expedience of such research.

4. Conclusions

In conclusion, it has been shown that in dexamethasone-treated rats goutweed tincture (1 ml/kg intragastrically) combined with the respectively low dose of metformin (50 mg/kg intragastrically) causes several favourable effects: reduction in plasma ALT activity (statistically significant change when compared to the group receiving metformin per se) and increase in urea clearance (statistically significant change when compared to the group receiving goutweed tincture per se) as well as normalization of ALP activity up to the values of the intact control. Extrarenal mechanisms are possible in the influence of the tincture on purine metabolism, while metformin tends to enhance renal uric acid excretion (uricemia remains unchanged in all of the groups).

The data obtained, together with the previous results concerning the normalization of carbohydrate and lipid metabolism, evidence that it is possible to decrease the effective dose of metformin by combining it with goutweed tincture. Such decrease is important in the aspect of safety (and the current study shows that the investigated combination is not hepatotoxic, furthermore, its effects are partially beneficial, namely the ability to decrease ALT activity) as well as from the point of view of reducing treatment costs. Besides, combination of goutweed tincture with metformin is rational as it allows maintaining renal excretion of urea and normalizing purine metabolism with the possible contribution of goutweed components that, in contrast to metformin, inhibit xanthine oxidase. In-depth study of GW preparations interaction with metformin at the level of the liver is expedient.

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