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A Comparative Pharmacological Study of the Effect of "Antidiabetic" Phyto-Complex and the Galenicals of White Mulberry Leaves and Sweet-Clover Grass on Clinical Course of the Alloxan-Induced Diabetic Model

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Abstract: The main purpose of the present research is to assess the efficacy of investigated galenicals on the functional and biochemical values of carbohydrate metabolism disorder using alloxan-induced diabetic model. The experiments were carried out on the pubescent male albino rats weighing 189- 313 grams. Alloxan-induced diabetic model was initiated by intraperitoneal injection of 200 mg/kg alloxan trihydrate. The animals were divided into some groups of 25 animals in each. The influence of the studied herbal products on a pathological alloxan-induced process was studied in placebo-controlled experiments. At that, lethality, change in weight, intake of food and water and behavioral performance modification of the animals were studied during 3 weeks. The animals were sacrificed on the 21st day and the blood was assayed for sugar, insulin and C-peptide. The conducted studies revealed that all tested herbal products, as opposed to synthetic hypoglycemic agents, have a positive effect on the course of alloxan-induced diabetes mellitus. However, the number of lethal cases in the groups of animals treated with studied galenicals, relative to the control group, decreases, whereas the weight of the animals increases. Back mutation of diabetes status is confirmed by the decrement of sugar content in blood plasma and increase of insulin and C-peptide concentrations. The herbal phyto-complex, proposed by authors, namely “Antidiabetic” phyto-complex surpasses "Mirfazin" phyto-complex used in medical practice for all the studied parameters.

Key words: Diabetes mellitus · Model · Alloxan · Sugar · Insulin · C-peptide

INTRODUCTION

Today herbal products are widely used in treatment of diabetes mellitus (DM) [1]. A number of investigations proved that phytotherapy slows down the progression of the disease and its late sequela [2-4]. Some authors believe that a number of biologically active substances of plants and in particular flavonoids exert beneficial pathogenic effect on diabetes [5-8]. However, for optimal use of herbal products in clinical practice, there are no sufficient experimental substantiations of their efficacy and mode of action.

Based on the foregoing, we found it useful to examine the effects of galenicals of white mulberry leaves, sweet-clover grass and "Antidiabetic" phyto-complex, prepared on account of the research results of hypoglycemic plants of Azerbaijan flora, using alloxan-induced diabetic model and compare the obtained results with the effectiveness of "Mirfazin" herbal medicinal product. To enable interpretation of these results, the data were compared with indices that were obtained with the preparations, whose sugar lowering mode is well studied (insulin, metformin, glibenclamide and tanakan) when used in the same conditions.

The alloxan-induced diabetic model is the most well-studied model of diabetes mellitus. It is characterized by an acute picture of the course of disease. At that, the severity of metabolic disorders, manifested by the fact of the animals’ mortality, change in their weight, intake of food and water and behavioral performance modification is of great importance for the evaluation of pathology progression along with an increase in the blood sugar concentration. Therefore, the study of these indices gives a realistic picture of the process clinic under

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the effect of the preparations studied and reflects the level of their performance efficiency [9]. In this case, the functional state of ß-cells of the pancreatic islet apparatus of the test animals and the effectiveness of the preparations can be evaluated by concentrations of insulin and C-peptide in the blood [9].

MATERIALS AND METHODS

The tests were carried out on mongrel pubescent male albino rats weighing from 189 to 313 grams. The rats were divided into two groups of 25 animals in each. After 24-hours of pre-starvation, blood glucose assay was conducted using test-method, then animals were weighed and underwent the intraperitoneal injection of alloxan trihydrate solution at a single dose of 200 mg/kg [9]. Given that a sharp increase in sugar concentration within the studied model begins on the second or third day after the administration of alloxan, all drugs were administered a day from the beginning of the experiment. Plant extracts for the experiments were prepared "extempore" in the ratio of 1:10 and administered into animals in appropriate doses using the catheter.

The animals of the 1st group (control) received physiological sodium chloride solution at a dose of 1ml/100g.

The animals of the 2nd group were administered "Antidiabetic" phyto-complex extract at a dose of 1ml/100g 3 times a day.

The animals of the 3rd group received the extract of mulberry leaves in a dose of 1ml/100g 3 times a day.

The animals of the 4th group were administered the extract of sweet-clover at a dose of 1ml/100g 3 times a day.

The animals of the 5th group received 1ml/100g of "Mirfazin" phyto-complex extract 3 times a day.

The 6th group received tanakan at a dose of 1mg/kg 3 times a day.

The 7th group received glibenclamide at a dose of 0.1 mg/kg once a day.

The 8th group received metformin (Siofor 500) at a dose of 5 mg/kg 2 times a day.

The animals of the 9th group were exposed to subdermal injections of insulin at a dose of 1 unit/kg 2 times a day.

All groups showed functional changes of animals. On the 21st day after 24-hour pre- starvation, the animals were weighed and then were decapitated to take blood samples for determination of sugar, insulin and C-peptide.

The sugar content in the blood was determined by the test analyzer using IME-DC test paper. Insulin and C-peptide levels were measured by ELISA method employing Chemwell enzyme immunodetector with a standard set of DEMENITECKILL-WELLSEE reagents, Cermany.

Statistical analysis of the obtained results was carried out by the Fischer-Student parametric method and by the Wilcoxon-Mann-Whitney non-parametric test (U criterion), Rosenbaum (Q criterion) and the lethality was determined by Fisher's exact test (FET) [10].

RESULTS AND DISCUSSION

When determining the effect of applied preparations on lethality of the animals, it was found that in the 1st group 17 out of 25 rats were died, which accounted for 68% (P □ 0.025). In the 2nd group, 1 rat died out of 25 that accounted for 4% (P □ 0.025), in the 3rd group 2 rats died out of 25 that accounted for 8% (P □ 0.025). The results obtained in the 2nd and 3rd groups were not statistically reliable for a given sample and the death of the animals in this case was random, independent of the use of preparations. In the 4th group 9 rats died out of 25, accounting for 36% (P □ 0.025), in the 5th group-12 out of 25 rats (48 %) (P □ 0.025). In the 6th, 7th, 8th and 9th groups the lethality was 40% (P <0.25), 60% (P <0.25), 80% (P <0.25) and 20% (P <0.25), respectively.

As is obvious from the results obtained, the lethality in the groups, treated by the herbal preparations, is far less than that in the control group, as well as in the groups treated by synthetic anti-diabetic agents. Moreover, the "Antidiabetic" phyto-complex and the infusion of white mulberry leaves have the most beneficial effect on the survival of animals with alloxan-induced diabetes, exceeding the effect of the "Mirfazin" phyto-complex by 32% and 28%, respectively. Synthetic antidiabetic agents of metformin and glibenclamide, as compared with herbal products, do not improve the animals' survival rate when injecting the alloxan at a dose of 200 mg/kg, while glibenclamide increases mortality even more because of its known additional toxicity [11].

Weight of the animals survived by the 21st day (Table 1) in the 1st group was 109.75±0.8 g at the initial weight of 296.64 ± 1.85 g, decreasing by 63.0%; in the 2nd group weight of the animals was 308.54 ± 4.45 g at the initial weight of 240.24±1.58 g, increasing by 48.7%; in the 3rd group the weight from 250.0 ± 3.31 g in the intact

Table 1: Changes in the animals weight when treated with the studied preparations on the background of an intraperitoneal injection of the alloxan trihydrate at a dose of 200 mg/kg

		Weight of the anymal in grams	
No of grour	Statistical value	Intact state	On the 21st day
1	M±m	296.64±1,85	109.75±0,8
	Min-Max	276-313	103-129
	P	-	U=0; P□0.01
2	M±m	240.24±1.58	308.54±4.45
	Min-Max	229-255	269-349
	P	-	Q =49; P<0,01
3	M±m	250.0±3,31	263.86±3.21
	Min-Max	234-281	245-295
	P	-	U=109; P□0,01
4	M±m	217.4±2.5	206.76±3.04
	Min-Max	203-253	189-240
	P	-	Q =49; P<0,01
5	M±m	282.48±3.92	280.8±4.15
	Min-Max	189-312	253-311
	P	-	Q =2; P>0.05
6	M±m	227.28±0,66	219,13±2.08
	Min-Max	218-231	205-232
	P	-	U=37; P<0.01
7	M±m	226.0±1.026	147.6±4.47
	Min-Max	217-233	136-156
	P	-	U=0; P<0.01
8	M±m	220.92±0.55	169.29±10.8
	Min-Max	217-227	124-220
	P	-	U=6; P<0,01
9	M±m	227/6±1.34	297.3±3.02
	Min-Max	218-241	265-315
	P	-	U=0; P<0.01

increased by 9.22% up to 263.86 ± 3.21; in the 4th group the weight in intact decreased from 217.4 ± 2.5 by 4.9% and amounted to 206.76 ± 3.04 g, in the 5th group the weight of intact animals amounting 282.48 ± 3.92 g virtually remained unchanged (it was less than the average weight of the animals in the intact condition by 0.59% at Q=2, P □ 0.05, that is not confirmed statistically and is of random character). In the 6th group the original weight of the animals 227.28 ± 0.66 g decreased by 3.6% and amounted to 219.13 ± 2.08 g; in the 7th group weight of 226.0 ± 1.026 g decreased by 34.7% to 147.6 ± 4.47 g; in the 8th group the weight of intact rats equal to 220.92 ± 0.55 g decreased by 23.4% and amounted to 169.2 ± 10.8 g; whereas in the 9th group the weight by the end of the experiment was 297.3 ± 3.02 g at initial value of 227.6 ± 1.34 g, increasing by 30.6%.

Comparing these results, we come up to a conclusion that in terms of considered index, the preparations of “Antidiabetic” phyto-complex and white mulberry leaves surpass "Mirfazin" phyto-complex and other studied preparations.

No deviations on organ weights, taken at necropsy in the groups, were observed, though significant pathological changes in the biochemical and histological studies were found.

Monitoring of general condition of the animals showed that compared with the control group and groups of animals treated with synthetic hypoglycemic agents in the group administered with insulin and herbal products, the behavioral activity of animals has increased, aggression has decreased, ulcers on the skin surface healed faster and water requirement has sharply decreased.

Table 2 presents the blood plasma test results to determine the sugar content. As is obvious, the concentration of sugar in the blood of intact animals was determined within the range 111.8±1.46 mg/dl. In the 1st group sugar concentration increased by 370.7% and reached 526.2±3.76 mg/dl. In the 2nd group, in comparison with a control group, sugar concentration decreased by 76.9%, in the 3rd group-by 75.5%, in the 4th group-by 44.5%, in the 5th group-by 75.4% and in the 6th group-by

Table 2: Changes in blood sugar, insulin and C-peptide in blood plasma of the animals when applying the studied drugs at the background of the alloxan-induced diabetic model (n=5)

No of group	Statistical values	Sugarmg/ml	InsulinmcED/ml	C-peptideng/ml
Intact	M±m	111.8±1.46	3.8±0.12	0.15±0.01
	Min-Max	108-115	3.4-4.1	0.12-0.18
	P	-	-	-
1	M±m	526.2±3.76	0.16±0.09	0.006±0.0015
	Min-Max	518-539	0.0-0.5	0.00-0.01
	Pintact	P<0.01	P<0.01	<0.01
2	M±m	121.8±3.5	1.16±0.11	0.046±0.0044
	Min-Max	113-131	0.9-1.5	0.03-0.05
	Pcontrol	P<0.01	P<0.01	P<0.01
3	M±m	129±2.68	1.120±0.038	0.044±0.005
	Min-Max	123-139	1.0-1.2	0.03-0.06
	Pcontrol	P<0.01	P<0.01	P<0.01
4	M±m	292.2±8.24	0.72±0.13	0.03±0.0045
	Min-Max	261-307	0.4-1.1	0.02-0.04
	Pcontrol	P<0.01	P<0.01	P<0.01
5	M±m	129.2±9.6	0.48 ±0.07	0.02±0.0032
	Min-Max	119- 136	0.3-0.7	0.01-0.03
	Pcontrol	P<0.01	P<0.05	P<0.01
6	M±m	371.8±13.83	0.2±0.032	0.008±0.002
	Min-Max	317-388	0.1-0.3	0.00-0.01
	Pcontrol	P<0,01	P<0,05	P<0,05
7	M±m	445.4±11.23	0.2±0.07	0.008±0.001
	Min-Max	421-483	0.0-0.4	0.00-0.01
	Pcontrol	P<0.01	P<0.05	P<0.05
8	M±m	216.6±3.12	0.24±0.05	0.01±0.005
	Min-Max	207-225	0.0-0.3	0.0-0.03
	Pcontrol	P<0.01	P<0.05	P<0.05
9	M±m	118±3.7	2.94±0.17	0.004±0.002
	Min-Max	107-127	2.5-3.5	0.0-0.02
	Pcontrol	P<0.01	P<0.01	P>0.05

29.3%. In the 7th group the sugar content in the blood plasma decreased by 8.3%, in the 8th group-by 58.8% and finally in the 9th-by 77.6%.

As is seen from the results obtained, lowering of the sugar content in the blood of alloxan-induced animals treated by herbal products is represented by statistically-valid values lower than those obtained for the animals treated with synthetic drags. Exogenous insulin, as expected, reduces sugar in the blood of animals, compensating for shortage of endogenous insulin.

In terms of blood sugar decay, "Antidiabetic" phyto-complex infusion surpasses "Mirfazin" phyto-complex, white mulberry leaves and sweet-clover extract. High efficiency of used herbal products may be associated with the presence of various biologically active substances, including flavonoids, which provide multiple-factor action. Tanakan is a Ginkgo Biloba extract. It did not show practically significant hypoglycemic effect despite the content of flavonoids providing anti-oxidant action as it is mentioned in the literature. Metformin, much more likely, reduces hepatic glucose production by an average of 30%, that leads to a decrease in the level of fasting blood glucose [11, 12]. This is due to its known mode of action, which is associated primarily with the suppression of gluconeogenesis and increase of glycogen synthesis in the liver. Glibenclamide, with due regard to the natural reversion to the diabetic status in alloxan-induced rats virtually does not reduce plasma concentrations of glucose.

When determining the content of insulin and C-peptide, we have obtained the following results, shown in Table 2. Insulin concentration in plasma of intact animals was 3.8±0.12 mcED/ml and the C-peptide-0.15±0.01 ng/ml. In the 1st group these figures were decreased by 95.8% and 96.0%, respectively. In the 2nd group, compared with the control group, the figures increased by 626.0% and 666.6 %, respectively. In the 3rd group they increased by 837.5% and 633.3%; in the 4th group-by 350,0% and 400.0%; in the 5th group-by 200.0%

and 233.3%; in the 6th group-by 25.0% and 33.3%; in the 7th group-by 25.0% and 33.3%; in the 8th group-by 50.0% and 66.7%; and in the 9th group the concentration of insulin was determined in an amount of 2.94±0.17 mcED/ml, though the content of C-peptide was decreased by 33.3%.

As it is obvious from the obtained results, the increase in the concentration of insulin and C-peptide in the blood of alloxan-induced animals treated by herbal products have statistically-valid values higher than those obtained for the animals treated with synthetic drugs. Exogenous insulin is determined in the blood within acceptable limits, though the lack of C-peptide suggests that endogenous insulin is not produced. “Antidiabetic” phyto-complex and white mulberry extract increase the content of insulin and c-peptide in the blood in much larger quantities than the "Mirfazin" phyto-complex. Metformin and glibenclamide virtually do not change these indices.

Thus, in contrast to the synthetic drugs and insulin, studied herbal products, except of Tanakan, increase concentrations of insulin and C-peptide in the blood to varying degrees.

The analysis of the obtained results suggests that the hypoglycemic action mode of studied galenicals is primarily based on specific antioxidant action of their flavonoids. Flavonoids, being traps for free radicals, as well as directly entering into a redox reaction with alloxan, neutralize its toxic effect; saturate the organism with exogenous antioxidants, increasing thereby the efficiency of the antioxidant protection system of the organism and protect cells against the damage. Therefore, the use of herbal products containing flavonoids at diabetes mellitus can be considered as a pathogenic treatment of this disease.

Summarizing the overall results of the conducted research, we can conclude that the herbal products of the "Antidiabetic" phyto-complex, white mulberry leaves and sweet-clover grass in the blood plasma of alloxan-induced animals reduce the amount of sugar and increase the level of insulin and C-peptide, thereby having a positive effect on the course of experimental diabetes mellitus. The proposed “Antidiabetic” phyto-complex surpasses the "Mirfazin" phyto-complex for all the studied parameters.

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