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THE MECHANISMS OF CURCUMA LONGA RHIZOME ACTION ON GLUCOSE METABOLISM IN ALLOXAN - INDUCED DIABETIC RATS

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ABSTRACT

The effects of the *Curcuma longa* rhizome as a food additive were studied on different processes of glucose metabolism: glucose concentration in blood, rate of glucose absorption in the gut, concentration of hormones - insulin and C-peptide in plasma, content of glycogen in the liver, structural and functional organization of the islet apparatus of the pancreas in Alloxan-induced diabetic rats. Turmeric was selected due to the fact that this phytopreparation has a wide spectrum of action and, according to American and Korean Studies, caused a decrease of glucose concentration in blood [1,2]

Under the influence of the turmeric rhizome intake in rats with diabetes, as compared with diabetic animals on a standard diet, a lower increase of the glucose concentration in blood, the tendency of decrease of glucose absorption in the gut, higher concentration of the insulin and C-peptide in plasma and the significant increase of glycogen content in the liver were found. The microstructure of pancreatic tissue samples of experimental animals using turmeric intake, was characterized by the better preservation of the islet apparatus in comparison with a group of animals on a standard diet.

The results indicate the positive effect of the *Curcuma longa* rhizome on the homeostatic mechanisms of the glucose metabolism regulation in the Alloxan-induced diabetic rats.

KEY WORDS: diabetic rats, glucose metabolism, turmeric, Curcuma longa rhizome

INTRODUCTION:

Increased interest to use phytopreparations for the correction of various pathological conditions[3;4] makes it necessary to study the mechanisms of their action. Data has appeared in recent years on the biological effects of plant *Curcuma longa*, such as antimicrobial[5], anti-inflammatory[6], antioxidant[7], antiproliferative[8] etc., making it promising to study the mechanism of its action at different pathological states, and, in particular, at diabetes mellitus (DM)[9]. The

specific action of *Curcuma longa* on various organs and tissues has been found: skin, the gastrointestinal tract, liver, respiratory system. In acute and long lasting studies in mice, rats, guinea pigs and monkeys, it has been shown that turmeric rhizome powder is not toxic to the organism[10].

Curcuma longa contains different components such as: carbohydrates (4,7-8,2%), essential oils (2.44%), fatty acids (1,7-3,3%), curcuminoids (curcumin, and demetoksikurkumin/ bisdemetoksikurkumin) the total content of which is about 2%, although it may reach 2.5-5.0% of dry weight. Curcumin is a characteristic substance of the turmeric rhizome and one of the most important biologically active substances of this plant[11].

To study the effect of turmeric and mechanisms of its action we took into account, that diabetes, as according to WHO statistics[12], is one of the most common diseases in the world: there are now 347 million diabetic patients in the world and their growth rate is 609,000 cases per year. Therefore, the use of this phytopreparation in the complex therapy for the correction of glucose metabolism in diabetes mellitus patients is of great theoretical and practical value[13].

Therefore, the purpose of the present study was to elucidate the mechanisms of Curcuma longa rhizome action on the glucose metabolism in rats with an experimental model of diabetes mellitus.

MATERIAL AND METHODS: To achieve the goal mentioned above experiments on adult male 9-10-week-old Wistar rats (weight: 190-210 g) were performed. All animals were divided into four groups. The first (n = 19) and the second (n = 19) groups were intact healthy animals. Rats of the third (n = 22) and the fourth (n = 21) groups were injected with 10 % Alloxan solution in dose 0.1 ml/100 g body weight into the interscapular region to get the DM model, while animals of the first and the second groups received the same volume of saline solution. All animals were fed on a commercially available diet and allowed free access to drink water. Animals of the second and the fourth groups were fed additionally with turmeric rhizome powder at the weight of 2% of the food.

In all rats 0.2 ml of blood samples from the tail notch were collected for glucose measurement on the 1-st, 3-rd and 6-th days after injection. The glucose concentration in the blood was determined with the picric acid by Krecelius-Seifert method (1928, 1942), cited by Kiskun AA [14] on the spectrophotometer "Spekol" (Germany) at a wavelength of 560±5 nm.

The amount of glucose absorption in the intestine was determined through *in vivo* experiments on rats by filling the immobilized segment of the small intestine of length ~ 20 cm with 2 ml of 30 % glucose solution for an hour, with preservation of its innervation and blood supply, under anesthesia by barbital sodium (0.1 ml/100 g body weight injected intramuscularly) according [15]. The difference between the infused amount of glucose and that left in the intestine after one hour was indicated as the absorbed amount.

At the end of the experiment (on the 6-th day after the Alloxan or saline injections) the blood samples in a volume of 5 mL from inferior vena cava were taken from animals under ether anesthesia in chilled tubes for determining the concentration of the hormones - insulin

and C-peptide by ELISA, using standard kits for the tablet spectrophotometer "Power Wave" (USA). For morphological study samples of pancreatic tissue were collected. The glycogen was determined in liver tissue using a PAS- reaction according to McManus method and measuring the intensity of colouring on the spectrophotometer "Spekol" (Germany) at a wavelength of 430±5 nm[14].

Statistical analysis of the results was carried out by determining the arithmetic mean (M) and their errors (±m). Differences between obtained results were evaluated by methods of variation statistics using a non-parametric Wilcoxon-Mann-Whitney test for independent samples and were considered significant at $p \leq 0,05$. Calculations were made by conventional formulas using the standard software package Statistica 7.0.

All experiments were performed in accordance with international guidelines for biomedical research involving animals, adopted by the International Council of Scientific Societies (CIOMS) in 1985, with Article XI of the Helsinki Declaration of the World Medical Association (1964) and laboratory practice regulations in the Russian Federation (Ministry of Health Order № 267 19.06.2003).

RESULTS: Previously, it has been shown[16] that after Alloxan injection the blood glucose concentration in animals on the 1-st day of observation was already significantly higher than in controls, indicating the development of diabetes (Table 1). However, during the entire period of observation the blood glucose concentration in animals of the 4-th group was significantly lower compared with those in the Wistar rats of the 3-rd group, fed a standard diet, while a little higher than in the control animals. It should be noted that in intact rats receiving powder rhizome of *Curcuma longa* (group 2) blood glucose concentration at days 3 and 6 of the experiment was also significantly lower than in animals of the 1-st group.

Consequently, the intake of *Curcuma longa* rhizome powder resulted in the decrease of blood glucose concentration in control rats (group 2), while in animals with Alloxan-induced diabetes there occurred a less significant increase and more rapid normalization of blood glucose.

Table 1. Glucose concentration in the blood of rats of different groups (mmol /L) (M ± m)

Number of group	Group of animals	Background sample	Days of experiment		
			1	3	6
1	Control	4,4 ± 0,34	3,6 ± 0,07	4,0 ± 0,20	4,4 ± 0,07
2	Control + turmeric	4,0±0,34	3,5±0,42	2,8±0,25*	2,2±0,15*
3	Alloxan	5,0 ± 0,12	18,3 ± 0,18*	21,0 ± 0,10*	16,1 ± 0,30*
4	Alloxan + turmeric	4,2 ± 0,30	12,2 ± 0,05*Δ	6,9 ± 0,17*Δ	5,3 ± 0,15*Δ

Note: In this and the following tables:
* - Significant differences from control rats (group 1);
Δ - significant differences between experimental groups (3 and 4).

One of the reasons for decreasing blood glucose after ingestion of turmeric may be a reduction of the absorption rate of carbohydrates in the gastrointestinal tract due to inhibition of the Na^+-glucose cotransporter [17,18]. To test this hypothesis,

the experiment with perfusion of immobilized small intestine with 30 % glucose solution was performed. It can be seen that in the diabetic rats the rate of glucose absorption was more than 2-fold higher than in control ones (Table 2), which may be due to the activation of the Na^+-glucose cotransporter. Rhizome of *Curcuma longa* powder even in control animals contributed to reducing the absorbed amount of glucose, while in diabetic rats of the 4-th group receiving turmeric there was observed only the tendency toward the reduction of the absorption rate. Turmeric, probably, inhibits the Na^+- glucose cotransporter (SGLT), as similarly synthesized blockers[13], but under the conditions of diabetes this effect was less pronounced.

Table 2. Glucose absorption in the gut of rats of different groups (M ± m)

Number of group	Group of animals	The amount of glucose in the intestinal perfusate at 1 hour after infusion of the solution, µmol	The amount of glucose absorption, µmol	% of glucose absorption
1	Control	37,6±4,3	25,1±2,9	40,2±8,8
2	Control + turmeric	46,5±5,0	16,0±1,7*	25,6±7,1*
3	Alloxan	6,4±1,5*	57,8±1,7*	92,5±2,4*
4	Alloxan + turmeric	10,5±2,6*	53,9±2,4*	86,2±4,1*

Note: * - significant differences from control rats;

The amount of glucose in the perfusate - 62,5 ± 0,97 µmol.

The glycogenesis process may play an important role in maintaining the glucose concentration in plasma.

To elucidate the mechanisms causing the significantly lower hyperglycemia in rats treated with turmeric, the glycogen content in the liver tissue was analyzed. In Alloxan-induced diabetic rats the glycogen content in the liver was significantly lower than in the control group (Table 3). However, in rats of the 4-th group, the glycogen content in the liver was increased almost to the control level and significantly differed from that of animals of the 3-rd group. Note, that in healthy rats, while inducing turmeric, glycogen content in the liver was also significantly lower than in the 1-st control group.

Table 3. Glycogen content in the liver of rats (mg/100 g wet weight) (M ± m)

Number of group	Group of animals	Glycogen content
1	Control	888,6 ± 45,17
2	Control + turmeric	728,0 ± 64,4*
3	Alloxan	457,6 ± 33,93*
4	Alloxan + turmeric	748,7 ± 56,36 Δ

Note: see Table 1

Since at Alloxan-induced diabetes the endocrine function of the pancreas is impaired, it was important to evaluate changes in the concentration of basic glucose regulating hormones.

Analysis of the results showed that in diabetic rats (group 3) plasma insulin

concentration was significantly lower than in control animals, that coincides with data obtained previously and confirms the development of diabetes type I [16]. A similar trend was observed concerning the level of C-peptide. However, in animals receiving turmeric(group 4) the concentration of these hormones in plasma increased (Table 4).

Table 4. Concentration of hormones in the blood of rats with Alloxan diabetes (M ± m)

Number of group	Group of animals	Insulin, IU / mL	C – peptide, ng / ml
1	Control	3,1 ± 0,23	3,2 ± 0,33
2	Control + turmeric	2,9 ± 0,23	2,6 ± 0,15
3	Alloxan	1,9 ± 0,18*	2,0 ± 0,30*
4	Alloxan+ turmeric	2,3 ± 0,50	2,3 ± 0,26*

Note: see Table 1

As increasing the endocrine activity of the pancreas under the influence of *Curcuma longa* may be due to structural changes in the islet apparatus of the gland, the morphology of the pancreas in rats with Alloxan-induced diabetes has been investigated.

The morphometric analysis of samples from the pancreas of animals has been demonstrated that the area of the islets in rats with Alloxan-induced diabetes was almost 2 times larger than similar samples from the control group (Table 5).

Table 5. Area of the Langerhans' islets, μm ²

Number of group	Group of animals	S, μm ²
1	Control	1575,0 ± 100,6
3	Alloxan	3110,6 ± 230,7 *
4	Alloxan+ turmeric	1391,4 ± 104,8 Δ

Note: see Table 1

These results do not agree with the data of other researchers, showing reduction of the area of the pancreatic islets in the model of Alloxan diabetes[19]. To resolve this contradiction we applied a method of morphological analysis, which allowed for visualizing the morphology of the pancreatic islets.

Preparations at the light level of the pancreas had marked differences in the group of rats with an Alloxan diabetes model from similar samples of the control group (Fig. 1A, 1B). Figure 1B shows that intercellular substance of gland stroma both in the periphery of islets of Langerhans, and in endocrine part of the gland have symptoms of disorder. On histologic sections pays attention plethora of blood vessels and perivascular edema of the intercellular substance of space. A typical feature of rat's pancreas samples of this group was the sludge phenomenon of erythrocytes in the lumen of the capillaries. In the cytoplasm of endocrine cells there are numerous small and large optically clear vacuoles (Fig. 1B). At the periphery of the islets visible cells with signs of lethal damage are found. Thus, increase of the islets of Langerhans area was resulted from tissue edema.

Morphologic analysis of the pancreas samples of rats treated with turmeric demonstrated reduction of

structural damage compared to the samples in the second group of rats (Fig. 1G). Signs of edema of the intercellular substance of connective tissue stroma was visible only in insular part of the gland and was absent in the intercellular substance that separated the exocrine part from the endocrine, as was the case in similar samples of gland in rats of the second group. Therefore, the insula part of the gland had significantly less area compared with those in the rats of the 2-nd group and did not differ from the intact animals. In samples of rat's pancreas in the 3-d group multicellularity in periphery of endocrine part of the gland was observed (Fig. 1D). The cells in this compartment had various morphology. Among the identified cells there were cells with low and high level of differentiation as well as with sublethal damage. The signs of water - salt homeostasis disorders in the structures of the gland had been revealed.

DISCUSSION:

One of the reasons for decreasing blood glucose after ingestion of turmeric may be a reduction of the absorption rate of glucose in the gastrointestinal tract due to inhibition of the Na^+-glucose cotransporter [17,18]. Really, we demonstrated the lower level of glucose absorption rate in intact and diabetic rats using powder of *Curcuma longa* rhizome in comparison with animals on standard diet. A lower concentration of glycogen in the liver at diabetes mellitus is probably due to an impaired glycogen synthesis as a result of reduced activity of glycogensintetase and the decrease of glucose oxidation processes due to a defect in the pyruvate dehydrogenase complex[20,21]. Intake of *Curcuma longa* rhizome resulted in significant increase of glycogen content in the liver. One can suggest that turmeric can activate liver enzymes involved in glycogenesis. A slight decrease of glycogen content in the organ of the 2-nd group rats, probably, was due to a decrease in the blood glucose concentration in animals of this group (N 2), owing to that there was a redistribution of carbohydrates between the organ-depot (liver) and the blood for maintenance of the glucose concentration in plasma at normal values.

One of the most important findings is that *Curcuma longa* rhizome intake can stimulate secretion of pancreatic glucose regulating hormones – insulin and C-peptide. To explain this result we have studied the morphology of pancreas.

At Alloxan-induced diabetic rats we have observed the signs of structural and functional disorders of the gland, as known, that can reflect one of the pathogenetic mechanisms of diabetes - free radical damage. Swelling of the intercellular substance of the stroma, numerous optically clear vacuoles in the cytoplasm of endocrine cells are probably the result of damage in membrane organelles and their protein carriers that maintain water and electrolyte homeostasis[22,23].

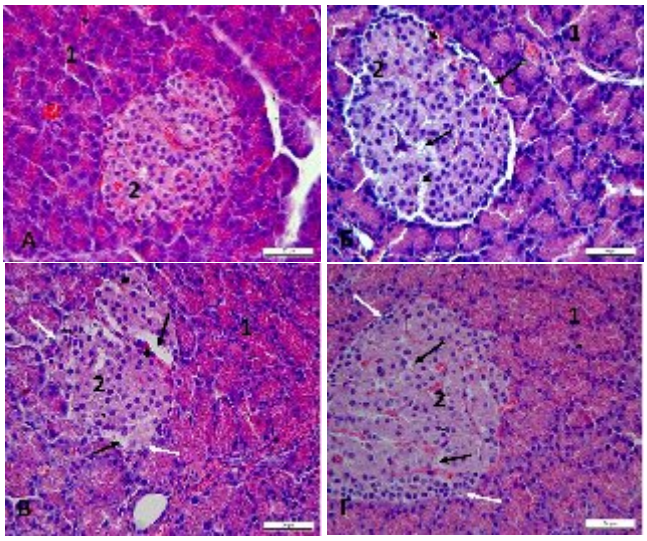
In the group of animals consuming turmeric, stabilization of structural and functional disorders of the pancreas manifested itself in the absence of signs of stromal edema and hypercellularity at the periphery of the islets of Langerhans. From our point of view, it reflects the positive impact of the active ingredients of turmeric, in particular curcuminoids [6-9], on erythrocytes, capillaries, and endocrinocytes. The absence of sludge phenomenon of erythrocytes proof this

point. Increased levels of low-differentiated cells at the periphery of the gland can be a sign of the migration of stem cells from stem niches to this part of the gland by hematogenous way[22], which is necessary for the formation of new endocrine cells.

These effects could be due to the weakening of the Alloxan action on the insular apparatus of the pancreas under the influence of rhizome of turmeric powder, and (or) the influence of turmeric on homeostatic mechanisms of regulation of glucose metabolism. We suggest that the first effect hardly occurs because the turmeric reduced glucose absorption from the gastrointestinal tract and its concentration in blood not only in rats with Alloxan -induced diabetes mellitus, but also in the control animals. In addition, we have clinical observations that turmeric causes decreased glycosylated hemoglobin and plasma glucose in patients with type 2 diabetes who were on combination therapy with sulfonylurea and biguanides (unpublished data). Therefore it is difficult to assume that the observed effects described in this paper due to the direct effect of turmeric on Alloxan.

CONCLUSION: Thus, intake of *Curcuma longa* provides the hypoglycemic effect in Alloxan -induced diabetic rats as a result of partially regeneration of β - islet cells, reducing the structural damage of cells and the intercellular substances, improving blood supply, resulting in stimulation of insulin and C - peptide secretion, the activation process of glycogenesis in the liver, and reducing the rate of glucose absorption in the small intestine. These data gives good impact for clinical use of *Curcuma longa* rhizome powder in complex therapy of patients with diabetes mellitus.

Figure 1 - pancreas of control group rat (A), with Alloxan diabetes model (B, C), and after intake of the rhizome turmeric



powder (D). 1 - exocrine glands; 2 - pancreatic islet.
Dark arrows indicate swelling of the intercellular substance; light - endocrinocytes with signs of degradation; arrow head - sludge phenomenon.
Colouring: hematoxylin + eosin.

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