THE STUDY OF THE SPECIFIC ANTIDIABETIC ACTIVITY OF MOMORDICA CHARANTIA CULTIVATED IN THE BUKHARA REGION OF THE REPUBLIC OF UZBEKISTAN

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Annotation. *Diabetes mellitus (DM) is a chronic disease characterized by impaired glucose processing in the body. Researchers are actively looking for new ways to treat diabetes, including the search for natural substances that can inhibit enzymes responsible for glucose processing, such as α-amylase and α-glucosidase. In this study, we conducted a virtual screening and a molecular dynamics study to identify potential natural inhibitors of these enzymes. We evaluated the binding of 12 biologically active compounds from the Momordica charantia L. plant to α-amylase and α-glucosidase. Our results showed that the phyto compound contained in plants has the highest affinity for binding to these enzymes. We also performed molecular dynamics simulations to evaluate the stability of enzyme-ligand complexes. Finally, we concluded that the plant's BAS can be an effective drug for the treatment of diabetes due to its ability to bind to α-amylase and α-glucosidase. However, additional animal and human studies are needed to confirm the effectiveness of this compound.*

Keywords. *momordica charantia, Momordica charantia L., fruits, juice, pulp, BAS, charantin, momordin, momordicin, momordenol, momordicillin.*

Materials and methods of research. A literary review of the results of a study by foreign scientists on the hypoglycemic activity of the medicinal plant Momordica charantia L. cultivated in the Bukhara region of the Republic of Uzbekistan.

Introduction. Phytotherapy, or the use of medicinal plants, has long been used to treat diabetes mellitus in various countries of the world [1,31]. Traditional medicine has considerable experience in the use of plants in the treatment of this disease [2,32]. Scientific medicine also studies and approves some of them. For example, metformin, widely used for the treatment of type 2 diabetes, is produced from the galega officinalis plant [3,33]. Antidiabetic compounds have also been found in green curry leaves, fenugreek seeds, potatoes and harantia momordica [4.34]. In 1980, the World Health Organization recognized the study of herbal antidiabetic drugs and the study of their mechanism of action as important [5,35]. More than 800 plants with antidiabetic properties are currently known [6,36]. However, many of them are not included in clinical practice due to insufficient experimental and clinical data and insufficiently studied mechanisms of action [7,37]. Most clinical studies of herbal preparations are conducted in combination with insulin or synthetic hypoglycemic agents [8,38]. However, the design of such studies often does not meet the requirements of evidence-based medicine due to the small sample size and free interpretation of the results [9,39]. Many clinical studies have insufficient evidence base and are evaluated at the C level (non-randomized studies on a limited number of patients) [10,40]. Over the past 15 years, the results of experimental and clinical studies have been published concerning the use of plants,

including the plant “Momordica charantia L” studied by us, for the treatment of diabetes mellitus [11,41]. They are included in this review.

The main part. Momordica charantia, also known as Chinese bitter melon (Momordica charantia L), is a food and medicinal plant that is widely used for the treatment of diabetes mellitus [13,43]. It contains more than 200 compounds, including peptides, triterpenoids: momordicin, momordenol, momordicilin, steroid saponins like charantin and momordin, lignan honokiol, polyenic fatty acids, amino acids, vitamins and trace elements. Some of these compounds, such as charantin and momordicin, have similar structures to synthetic hypoglycemic agents as sulfonylurea derivatives: glibenclamide, gliclazide, glipizide [14,44].

Studies have shown that the juice of momordica fruits can reduce blood glucose levels and increase the concentration of insulin in plasma [15,45]. It can also increase the number of betta cells and restore the normal structure of peripheral nerves in the streptozotocyon model of diabetes mellitus in rats [16,46]. Glucose absorption from the jejunum with the participation of Na+-dependent transport decreased, glucose intake into skeletal muscles, on the contrary, increased [17,47]. In addition, fruit juice stimulates the utilization of glucose and amino acids by skeletal muscles of rats in the L6 line [18,48]. Extracts with charantin and isolates from momordica fruits also showed antidiabetic effects [19,49]. They can reduce blood glucose levels, increase glucose tolerance and insulin sensitivity of tissues [20,50]. They can also restore the size of pancreatic islets, the number of betta cells and the insulin content in granules [21,51]. Some of these compounds can also inhibit the activity of a-glucosidase, an enzyme that breaks down glucose in the intestine and increases its level in the blood [22,52]. In the liver of obese KK/HIJ mice, it increased the expression of the IRS-1 insulin receptor, and activated the GLUT4 transporter in skeletal muscles [23,58]. Aqueous and methanol extracts from momordica fruits also have an antidiabetic effect [24,53].

Momordica charantia L, as well as dimers and trimers of lignan honokiol isolated from the plant, significantly inhibited the activity of a-glucosidase [59, 60]. The most active was the honokiol trimer, which reversibly inhibited the action of the enzyme due to conformational changes in the secondary structure of the protein [55, 60]. An alcoholic extract from the pulp of fruits on a streptozotocin-induced DM model restored the size of pancreatic islets, the total area and number of betta cells, and the insulin content in granules, but did not affect the number of alpha cells [26,61]. In vitro studies, saponin-enriched alcoholic extract from dried momordica fruits, as well as momordicin II and cu gua glycoside G isolated from it, increased insulin secretion by betta cells of the MIN6 line [56, 62]. Triterpenoid saponins in vitro activated 5'AMP-dependent protein kinase, contributing to the formation of an active enzyme complex with calmodulin-dependent betta protein kinase [63]. In the study of cellular permeability using the MultiScreen Caco-2 test system simulating the intestinal barrier, it was shown that cucurbitane triterpenoids are able to penetrate the epithelial cell monolayer [25,54]. This suggests the ability of triterpenoids to be absorbed from the digestive tract and have an antidiabetic effect [27,64]. At the same time, not all triterpene derivatives have a hypoglycemic effect [57,65]. The aqueous extract from fruits, as well as the n-butanol fraction of aqueous and methanol extracts, reduced the increased content of glucose, glycated hemoglobin, insulin, leptin, free fatty acids in plasma, and the calculated HOMA-IR insulin resistance index [66,

67]. A cascade of intracellular reactions was activated in skeletal muscles: the expression of the insulin receptor IRS-2, the GLUT4 transporter, and the activity of phosphatidylinositol-3 kinase, which catalyzes the phosphorylation of phosphoinositides involved in intracellular signal transduction during activation of insulin receptors, increased [28,29]. An aqueous extract from fruits containing momordicosides A, F2, K and L increased phosphorylation of the IRS-1 receptor and P-serine/threonine protein kinase, which plays a key role in signal transduction from the insulin receptor [12, 67]. Seed oil with a high content of cis-9, trans-11, trans-13 isomers of conjugated linoleic acid dose-dependent weakened fat accumulation, increased phosphorylation of acetyl-CoA carboxylase, protein kinase A and transcription activator-3 [42].

Immunohistochemical examination of white adipose tissue revealed an increased number of markers of apoptosis - TUNEL - positive nuclei. The ability to inhibit fat accumulation is associated with the activation of protein kinase A and the programmed death of white adipose tissue cells, however, the safety of this effect should be carefully studied [68]. It is possible that some of the effects of momordica are realized with the participation of PPAR receptors: in C57BL/6J mice with obesity, the extract increased the expression of PPAR-g genes in white adipose tissue and PPAR-a in the liver, reduced the expression of NFkB [66, 69]. Momordica a-Eleostearic acid had pronounced antioxidant and anti-inflammatory properties [70]. ADMc1 protein isolated from momordica seed extract had a significant and prolonged (up to 8 hours) hypoglycemic effect in rats with type 1 diabetes and in NOD mice with spontaneous type 1 diabetes [71]. Polypeptide-P also has an insulin-like effect, which reduced blood glucose levels in gerbils, langur monkeys and in the clinic in patients with type 1 diabetes. Fetal lectin activated dimerization of the insulin receptor with the development of a hypoglycemic effect [72]. In M. charantia discovered a trypsin inhibitor, the mclRBP protein, capable of binding to insulin receptor sites other than hormone binding sites. Mass spectrometry has shown that 3 sites of this protein interact in the insulin receptor with a leucine-enriched domain and ligand-binding repeats containing a large amount of cysteine. As a result, the effects of insulin are potentiated, the activity of 5'AMP-dependent protein kinase increases fivefold, and glucose utilization by 3T3-L1 adipocyte progenitor cells increases. Intraperitoneal administration of the mcIRBP protein significantly reduced blood glucose levels in intact animals [73, 74].

The physiological regulation of insulin action is determined by the balance of phosphorylation and dephosphorylation of the insulin receptor. Protein tyrosine phosphatase-1B dephosphorylates the receptor and is a negative regulator of the insulin signaling pathway. In mice deprived of this enzyme, the phosphorylation of the insulin receptor and the sensitivity of skeletal muscles and liver to the hormone increased, and resistance to the development of diabetes and obesity appeared [75, 87]. Protein tyrosine phosphatase-1B is an optimal target in the search for drugs for the treatment of type 2 diabetes and obesity. Lipid and saponins fractions from momordica fruits inhibited the enzyme in skeletal muscles in insulin-resistant db/db mice. The concentration of glycated hemoglobin in the blood, lipoperoxidation products in adipose tissue and body weight of animals decreased significantly [76]. A number of cucurbitacins from M. charantia fruits inhibited the activity of protein tyrosine phosphatase-1B more strongly than sodium orthovanadate, a well—known inhibitor of this enzyme [77, 88].

One of the key enzymes regulating carbohydrate metabolism is glycogen synthase-kinase-3. This enzyme phosphorylates and inactivates glycogen synthase. Glycogen synthase-kinase-3 inhibitors, preventing phosphorylation of glycogen synthase, activate glycogen synthesis, which maintains normal plasma glucose levels. Isolated from momordica, charantin, momordenol and, in particular, momordicilin inhibited the activity of this enzyme, which was established using the molecular docking method [72, 78].

It is known that delayed wound healing in DM leads to high mortality and reduces the quality of life of patients. Ointment with M. charantia fruit extract accelerated wound healing in rats in the streptozotocin-induced model C, while the expression of transforming growth factor-b and the amount of total protein in the wound significantly increased [79]. When casein hydrolysate isolated from an aqueous extract of M. charantia seeds was injected into a vein, the bleeding time was significantly prolonged, and the partially activated thromboplastin time increased. The extract accelerated fibrinogen hydrolysis and lysis of a stabilized fibrin clot [80], and also reduced blood viscosity in healthy people and patients with DM [81].

The juice of immature fruits of M. charantia, when administered for a long time to C57BL/6 mice receiving a fat-enriched diet, had a neuroprotective effect. It prevented the growth of the permeability of the blood-brain barrier, migration of lymphocytes to the brain, inflammatory activation of astrocytes and microglia, inhibited the activity of interleukins-16 and -22, activated the enzymes of antioxidant protection superoxide dismutase, catalase and glutathione peroxidase [82]. After cerebral ischemia caused by occlusion of both carotid arteries in mice with the DM model, lyophilized fruit juice limited the size of the cerebral infarction zone, production of malondialdehyde, and improved short-term memory and motor functions [83]. The pulp of fruits as part of a diet based on a model of diabetes caused by streptozotocin reduced the rate of glomerular filtration in the kidneys of rats, the activity of the enzyme synthesis of glycosaminoglycans 1-glutamine-fructose-6-phosphate-aminotransferase, as well as enzymes of their degradation N-acetyl-B-glucosaminidase and P-glucuronidase, increased in the basement membrane of the glomeruli the content of heparin sulfate [84]. Thus, extracts of the medicinal plant under study can prevent the development of thrombotic, neurological complications and nephropathy in DM. Clinical trials of juice, pulp and extracts from momordica have confirmed their antidiabetic effects in patients suffering from diabetes, however, the evidence of clinical studies is insufficient [85, 86].

In general, Momordica charantia contains many biologically active compounds that can help lower blood glucose levels and improve pancreatic and skeletal muscle function. However, more in-depth research is needed to fully understand the mechanisms and effectiveness of its action in the treatment of diabetes mellitus.

Conclusions. Medicinal plants “Momordica charantia L” have versatile therapeutic effects and can complement or even replace insulin and synthetic drugs for the treatment of diabetes. Biologically active components of plants help to normalize the metabolism of carbohydrates and fats, and also have antioxidant, anti-atherosclerotic, anti-inflammatory, vasoprotective and renal protective effects. They promote the formation of new β-cells in the pancreas, slow down gluconeogenesis and glucose absorption in the intestine as alpha-

glucosidase inhibitors, have the properties of incretinomimetics, activate glucose transport through GLUT4 and increase insulin sensitivity at receptors.

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