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NEW MOLECULAR SCREENING FROM A FOLK LORE MEDICINE GYMNEMA, USING ITS ACTIVE MOIETIES FOR LEAD OPTIMIZATION

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Introduction of plant:

Gymnema sylvestre (G. sylvestre) R. Br.The plant is native to central and western India, tropical Africa and Australia. Other names [38], Sanskrit: Meshashringi, madhunashini, Hindi: Gur-mar, merasingi, Marathi: Kavali, kalikardori, vakundi, Gujrathi: Dhuleti, mardashingi, Telugu: Podapatri, Tamil: Adigam, cherukurinja, Kannada: Sannagerasehambu Plant description G. sylvestre (Asclepiadaceae), a vulnerable species is a slow growing, perennial, medicinal woody climber found in central and peninsular India. Fig. 1 shows a 5-year old parent plant. It is a otent antidiabetic plant and used in folk, ayurvedic and homeopathic systems of medicine. It is also used in the treatment of asthma, eye complaints, inflammations, family planning and snakebite. In addition, it possesses antimicrobial, antihyper-cholesterolemic, hepatoprotective and sweet suppressing activities. It also acts as feeding deterrents to caterpillar, Prodenia eridania; prevent dental caries caused by Streptococcus mutans and in skin cosmetics [39]. G. sylvestre is a large, more or less pubescent, woody.

Chemical composition and pharmacological activity of *Gymnema sylvestre* are reviewed. Possible mechanisms of the hypoglycemic action are discussed. The physical and chemical properties, the proposed chemical nature, in addition to the role of triterpene saponins in the regulation of hyperglycemia and normalization of metabolic processes during diabetes mellitus are analyzed.

New capabilities for managing type 2 diabetes mellitus (DM) and reducing the frequency of vascular complications have recently emerged thanks to the search and introduction of new classes of drugs used for DM, including for the mechanism of action (secretogens, insulin sensitizers, drugs inhibiting the assimilation rate of carbohydrates in the bowel, rate inhibitors of glucose production by the liver, and selective inhibitors of PPAR-ã receptors) [1 – 7]. One contemporary research area is the modulation of glucose-dependent insulin secretion by gastrointestinal hormones (incretins) such as glucose-dependent insulin-releasing peptide (GIP) and glucagon-like peptide (GLP-1) [1, 8, 9], which regulate active insulin secretion by optimizing its biosynthesis, enhance proliferation of â-cells and neogenesis of Langerhans islets, and prevent cellular apoptosis, which is especially important for diabetes pathology. Therefore, it seemed interesting to review the existing literature on the pharmacological properties of the tropical liana Gymnema sylvestre (GS), the extract of which exhibits an incretin-mimetic mechanism of action[10, 11]. GS (synomyms Periploca sylvestris Willd., Gymnema melicida Edgew., Asclepias geminata Roxb.) is a climbing plant of the family Asclepiadaceae. It grows in open forest at elevation 100 – 1000 m in India, China, Indonesia, Japan, Malaysia, Sri Lanka, Viet Nam, and South Africa [12, 13].GS has been used in China and India for 2000 years to control sugar release in diabetes and as an antimicrobial agent[14 – 16].

Principal mechanisms of action of GS:

The literature review suggests that the pharmacological effects of administered GS extract occur through mechanisms such as modulation of incretin activity, stimulation of insulin secretion and release, regeneration of endocrinocyte Langerhans islets, activation of enzymes responsible for glucose utilization, reduction of glucose and fatty acid assimilation in the small intestine, and interference with the sensation of sweetness. It is known that hormones that regulate the incretin function, i.e., the formation and secretion of hormones by pancreas islets, are activated in response to the entry of food into the intestine. GLP-1 and GIP possess these properties [6, 8, 9]. Release of GIP into the portal vein in response to the intraduodenal administration of D-glucose in the presence of

GS extract enriched in GA by inhibitors of certain proposed glucose sensors and transporters in the intestinal lumen has been studied experimentally[12]. Intraduodenal adminisration of D-glucose caused a dose dependent increase of the concentration of portal immunoreactive GIP. This suggested that the extract of GS leaves increases GIP secretion by endocrine k-cells of the small intestine [22]. The literature suggests that the hypoglycemic activity of GS is due to stimulated release of insulin (and possibly regeneration of Langerhans islet cells) and enzymes responsible for glucose utilization and inhibition of glucose absorption in the bowel [11, 23 – 25]. The mechanism of the hypoglycemic action of GS is currently linked to the increase of insulin release from insulin-producing cells [25].Microscopy studies of a group of animals receiving GS extract showed that nuclei of endocrinocytes were significantly enlarged in all sections of the organ with the same volume fraction and area of pancreatic islets [26]. The results agreed completely with investigations in which it was proposed that use of GS increases the endogenous level of insulin, possibly due to regeneration of pancreas cells [17]. This means that the hypertrophy of endocrinocytes occurs probably due to the effect of GS on the increased secretion of GIP [19].An autogenic hormone in blood that was connected with the hypoglycemic effect was observed in experimental animals with an experimental form of immunodependent DM during a study of GS activity. This was direct evidence of the insulinotropic effect of the drug that was based on stimulation of functionally capable cells of pancreas Langerhans islets [2]. Furthermore, morphological signatures of apoptosis were absent in a group of animals with this pathology that received GS extract. This was confirmed by the observed low level of expression of p53 (a key enzyme of the cascade cycle involved in proteolysis of proteins in cytoplasm and the cell nucleus) and cascade 3 (marker of external apoptosis activation). A statistically significant increase of the percent ratio of PCNA:positive cells was also noted. Considering that PCNA is a proliferation marker, a high level of its experssion is most probably confirmation of repair in pancreas tissue with DM. This was also confirmed by moderateexpression of Ki-67 [11, 26, 27].Extrapancreatic effects of GS extract are evident in the prolonged hypoglycemic action of exogenous insulin administered to dogs without a pancreas, the intensification of its effect,

and an extended time during which a reduced level of glucose was observed [11].Use of GS extract to treat alloxan diabetes in rats can significantly increase their life expectancy. The activities of phosphorylase, gluconeogenesis enzymes, and sorbitoldehydrogenase were increased in experiments with rabbits with alloxan diabetes after use of GS [23, 28]. The amount of insulin in the blood plasma was incrased in mice with streptozotocin-induced DM upon administration of the saponin fraction and triterpene glycosides (gymnemosaponin V and GA I – IV) isolated from the methanol extract of GS leaves [29]. Use of GS extract for 21 days after streptozotocin intoxication reduced reliably in blood plasma the level of glucose and the amount of Hb Alc, increased the insulin content, and normalized the concentration of high-densitylipoproteins (HDL) [26].The level of glycemia and insulinemia was restored to normal after 60 days in rats with diabetes after oral administration of the alcohol fraction of GS (GS3 and GS4). The number of Langerhans islets and cells in the pancreas doubled [16, 24]. Dynamic results of a glucose-tolerance test were also positive. Hypoglycemic activity for GS extract was indicated by an increase of cell-membrane permeability [13].This conclusion was confirmed in later investigations of theinfluence of fraction GSon the mechanism of *in vivo* insulin secretion [21]. In particular, its influence on insulin secretion in linear animals was studied. It was found that GS4 stimulated hormone secretion in HIT-T15, MIN6, and RINm5F cell lines and in Langerhans islets in the absence of other stimuli regardless of the incubation temperature. The results confirmed that use of GS 4 increased insulin secretion, most probably due to an increased membrane permeability and not from stimulation of exocytosis by other pathways [25].The change of blood lipid spectrum and hyperglycemia with DM increases the risk of developing atherosclerosis [2].Therefore, it is significant that GS possesses not only hypoglcemic properties but also can correct disrupted lipid exchange [29]. The effect of GS extract on lipid metabolism has been investigated [30]. Oral administration of GS extract for two weeks to rats with experimentally induced hyperglycemia reduced in blood serum an elevated level of triglycerides (TG), total cholesterol (CS), and the content of very low-density lipoproteins (VLDL) and low-densitylipoproteins (LDL). The ability of GS extract (at a dose of 100 mg/kg) to reduce TG and CS in blood serum and its antiatherosclerotic

potential are similar to clofibrate in effectiveness. In 1990, a group of researchers published results from treatment with GS extract of type 1 and 2 DM patients [23]. The preparation ProBeta based on the GS4 fraction was patented and clinical trials were conducted in the USA [4, 23, 24, 31]. Treatment with GS extract (400 mg/d.l) of 27 patients with type 1 DM who were on insulin therapy reduced thelevel of fasting glucose (up to 35%) and the amount of Hb Alc and also normalized lipid concentrations in blood serum. The requirement for exogenous insulin decreased (up to 50%) and the level of endogenous insulin increased in the patients 12 months after the start of treatment. It was proposed that use of GS increased the endogenous level of insulin and C-peptide, possibly due to pancreatic regeneration.In Russia, tests of compositions consisting of GS extracts of various purity (certified preparations of GS dry extracts) in various concentrations in combination with extracts intensifying the antioxidant effect (grape stem) and possessing immunomodulating and regenerative properties (*Inula helenium,* licorice) are underway. More than 30 different combinations that were investigated in detail under *in vitro* and *in vivo* conditions were studied. The most effective of these, Di b, was studied with experimental diabetes. It was found that use of the preparation reduced glycemia, the amount of Hb Alc , the CS level, the content of LDL and VLDL, and the index of atherogenesis [7]; activated repair processes in endocrinocytes, and prevented the development of apoptosis [27]. The preparation is currently in clinical trials [3, 7, 26, 27]. Clinical tirals conducted in the USA confirmed that GS has hypoglycemic activity. The level of fasting glucose decreased by 18%; its postprandial level, by 28%; the amount of Hb Alc by 10% in 65 patients with poorly managed hyperglycemia who were administered GS preparations over 90 d. Use of GS could reduce the dose of peroral hypoglycemic preparations in 16% of the test participants [4]. Several reports suggest that the reliable toxic dose of GS has not been found. The LD50 in mice and rats is greater than 5 g/kg [7, 32]. Administration of extract produced by aqueous alcohol extraction of GS (19.5:1) at a dose of 250 – 8,000 mg/kg orally to mice did not produce any behaviorial or neurological effects. The toxicity of GS powder was tested for two weeks in Wistar rats. Intermediate hematological and biochemical blood parameters were measured after 26 weeks; final ones, after 52

weeks. No side effects were found upon administration of GS at doses of 504 – 563 mg/kg/d in man [25]. Considering these facts, it is obvious that GS is a source of biologically active substances. The very broad spectrum of pharmacological activity indicates that use of the extract at various doses and in various combinations improves the condition of latent forms of DM (prediabetes) and the complex treatment of insulin-independent DM, prolongs the action of hypoglycemic preparations, and regenerates cells for insulin-dependent and insulin-independent DM.

Structural aspects:

Chemical structure of biologically active components of GS The pharmacological properties of GS are due to a group of more than 20 triterpene saponins (TS), known as gymnemic acids (GA), that are pentacyclic and more rarely tetracyclic compounds of general formula C30H48 GS leaves contain 4 – 10% saponins including GA I – XVIII and gymnemosaponins I – V. Some researchers think that just GA V – VII possess antidiabetic activity [8, 14, 17, 18]. GA include GA I-VII themselves, gymnemosides A-F, and gymnemosaponins [19]. All GA are based on gymnemogenin (3_, 16_, 21_, 22_, 23, 28-hexahydroxyolean-12-ene).GA I, II, III, and IV were isolated from GS leaves and can supress the sensation of sweetness. They all contain glucuronic acid and the aglycon gymnemogenin esterified at the 21- and 28-positions Esterified saponins in the structures of the second series of GA (V – VII and VIII – IX) have an oxyglycoside moiety bonded to glucuronic acid. Their structures were determined as GA III and IV 3-O-D-arabino-2-hexulopyranoside [10,20]. New TS, the aglycons of which are tetracyclic triterpenes (dammoran derivatives) and which were called ginenosides I-VII in addition to saponins, gymnemasins A,B, C, and D, were isolated from GS leaves. The aglycon in the last ones was gymnemanol, 3_,16_,22_,23,28-pentahydroxyolean-12-ene. Some GA contain an acyl group whereas gymnemosaponins are unacylated [19]. Thus, pure saponins could be isolated from the total saponin fraction and their structures could be solved by interpreting results of NMR(DQF-COSY, HMQC, HMBC) and FABMS experiments [21].The hydrocarbon part of TS contains monosaccharides usually encountered in plants such as D-glucose, D-galactose,

D-xylose; D-gluconic, D-galacturonic acids; arabinose, rhamnose, and fucose. The carbohydrate chain includes from 1 to 10 different monosaccharides that differ in the attachment site and binding mode [16, 19].

Gymnemosides also were found in GS extract. Six triterpene glycosides, gymnemosides a, b, c, d, e, and f were isolated from the glycoside fraction of GS .The physicochemical properties of TS vary over wide ranges. They are mostly amorphous substances without a characteristic melting point (usually with decomposition). Only individual representatives that contained less than four monosaccharides were obtained in a crystalline form. The solubility in water and other polar solvents increases with increasing number of monosaccharides. Saponins with 1 – 4 monosaccharide units are poorly soluble in water. TS can be neutral and acidic compounds. The acidic nature is due to the presence of carboxylic groups located both in the sapogenin molecule and in the uronic acids, if the last occur in the carbohydrate part of the saponin. Strong acids cleave the glycoside bonds in all saponins. Saponins with an *o*-acetylglycoside bond are unstable toward bases. Acidic saponins form salts that are soluble with monovalent and insoluble with divalent and polyvalent metals. Many saponins form molecular complexes with proteins, lipids, sterols, and tannins.The fundamental ability of TS to destroy erythrocytes and release hemoglobin is due to the formation of complexes of saponin with cholesterol of erythrocyte membranes. However, peroral administration of the saponins destroys the ability to cause hemolysis [10, 21].

Efficiency of fractions:

Gymnemic fractions also inhibit glucose uptake in the intestine (Shimizu et al., 1997). Alcoholic extract also stimulate insulin secretion from the rat islets of Langerhans and several pancreatic beta cell lines in absence of other stimulus (33). However, triterpene glycosides exhibited little or no inhibitory activity against glucose absorption in OGTT conducted in rats. Gymnemic acid I and gymnemasaponin V lacked anti-hyperglycemic effect (37). Oral administration of aqueous leaf extract to normal and STZ diabetic rats showed dose-dependent decrease in blood glucose level (35). In another study, water-soluble fraction of alcoholic extract of the plant significantly lowered the hepatic glycogen content of the glucose fed rats (17). Oral administration of a water-soluble leaves

extract of *G. sylvestre* (400 mg/day) to 27 IDDM patients on insulin therapy lowered fasting blood glucose, glycosylated haemoglobin (HbA1c), glycosylated plasma protein and insulin requirements but it remained higher than controls. In addition, it reduced serum lipid level to near normal levels (24). In a clinical observation of aqueous decoction of *G. sylvestre* leaves (2 gm thrice daily) to 10 healthy persons (10 days) and 6 diabetic patients (15 days) significantly reduced the fasting and OGTT glucose level in all the groups except OGTT in healthy group (36). Gymnemic acid IV at 13.4 mg/kg increased plasma insulin levels in STZ-diabetic mice. Gymnemic acid IV (1 mg/mL) did not inhibit alpha-glycosidase activity in the brush border membrane vesicles of normal rat small intestines (34).

All The Possible Research orientations of gymnema screened but, there is an essential need of utilization of active parent structures are in need to get possible number of hits by using different bio informatics tools. so that new molecules with a new mechanisms may be possible for to control of DM.

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