

**“A COMPARISON OF MECHANISMS OF ACTION OF
HYPOGLYCEMIC PRINCIPLES OF MOMORDICA CYMBALARIA
AND SYNTHETIC ANTIDIABETIC DRUG”**

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ABSTRACT

Diabetes mellitus (DM) is one of the oldest known human disease currently affecting more than 200 million people worldwide. Diabetes mellitus is derived from two Greek words meaning siphon and sugar. In DM, patients have high blood level of glucose and this passes out with urine. This is because the endocrine pancreas does not produce either or not enough insulin or the insulin which is produced is not exerting its biochemical effect (or insulin resistance) effectively. Insulin is a major metabolic hormone which has numerous functions in the body and one main role is to stimulate glucose uptake into body's cells where it is utilized to provide energy. The disease is classified into type 1 and type 2 DM. Type 1 DM develops when the insulin producing β cells have been destroyed and are unable to produce

insulin. This is very Common in children and is treated with insulin. Type 2 DM (T2DM) develops when the body is unable to produce an adequate amount of insulin or the insulin which is provided does not work efficiently. This is due to life style habits including unhealthy diet, obesity, lack of exercise and hereditary and environmental factors. Some symptoms of DM include excess urination, constant thirst, lethargy, weight loss, itching, decreased digestive enzyme secretion; slow wound healing and other related symptoms. If let untreated, DM can result in severe long-term complications such as kidney and heart failure, stroke, blindness, nerve damage, exocrine glands insufficiency and other forms of complications. T2DM can be treated and controlled by prescribed drugs, regular exercise, diet (including some plant-based food) and general change in life style habits. This review is concerned with the role of plant-based medicine to treat DM. One such plant is Momordica

charantia which is grown in tropical countries worldwide and it has been used as a traditional herbal medicine for thousands of years although its origin is unknown. This review examines the medicinal chemistry and use(s) of *M. charantia* and its various extracts and compounds, their biochemical properties and how they act as anti-diabetic (Hypoglycemic) drugs and the various mechanisms by which they exert their beneficial effects in controlling and treating DM.

KEYWORDS: Diabetes mellitus, *Momordica cymbalaria*, hypoglycemic, insulin, pancreas.

INTRODUCTION

Diabetes or diabetes mellitus (DM) is chronic metabolic diseases characterized by high blood sugar levels caused either due to inadequate production of insulin or due to inability of body cell to respond to insulin. As per WHO, currently over 382 million people are affected globally and diabetes will emerge as 7th leading cause of death in 2030. There are many synthetic antidiabetic drug molecules available for the management of DM but these molecules are associated with numerous undesirable side effects. Hence there is an obvious need for search for safe and effective drug moieties for the treatment of DM. Herbal drugs are effective, cheap and are considered to be safe as they possess fewer side effects as compared to synthetic drugs. In traditional system of medicine, many medicinal plants have been identified for their hypoglycemic activity with potential use in DM. Important medicinal plants with hypoglycemic activity include *Azadirachta indica*, *Allium sativum*, *Ficus bengalensis*, *Lagerstroemia speciosa*, *Momordica charantia*, *Syzygium cumini*, etc. Principal leads have been identified which are responsible for hypoglycemic activity of these plants.

Diabetes Mellitus

Diabetes mellitus defines a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is one of the most common metabolic syndromes, since there are 200 million diabetic individuals in the world; this creates a need to understand the etiology of the disease and the factors influencing its onset. Several pathogenic processes are involved in the development of diabetes; these range from autoimmune destruction of the β -cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. Deficient action of insulin on target tissues and hyperglycemia are the basis of the abnormalities in carbohydrate, fat, and protein metabolism, causing diabetes' characteristic clinical features, micro and-macrovascular complications and increased risk of cardiovascular disease. The new

classification system (American Diabetes Association 2004) identifies four types of diabetes mellitus: type 1, type 2, “other specific types” and gestational diabetes.

Etiologic classification of diabetes mellitus

I. Type 1 diabetes (β -cell destruction, usually leading to absolute insulin deficiency).

A. Immune mediated

B. Idiopathic

II. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance).

III. Other specific types

A. Genetic defects of β -cell function

1. Chromosome 12, HNF-1_β (MODY3)

2. Chromosome 7, glucokinase (MODY2)

3. Chromosome 20, HNF-4_β (MODY1)

4. Chromosome 13, insulin promoter factor-1 (IPF-1; MODY4)

5. Chromosome 17, HNF-1_α (MODY5)

6. Chromosome 2, NeuroD1 (MODY6)

7. Mitochondrial DNA

8. Others

B. Genetic defects in insulin action

1. Type A insulin resistance

2. Leprechaunism

3. Rabson-Mendenhall syndrome

4. Lipotrophic diabetes

C. Diseases of the exocrine pancreas

1. Pancreatitis

2. Trauma/pancreatectomy

3. Neoplasia

4. Cystic fibrosis

5. Hemochromatosis

6. Fibrocalculous pancreatopathy

D. Endocrinopathies

1. Acromegaly
2. Cushing's syndrome
3. Glucagonoma
4. Pheochromocytoma
5. Hyperthyroidism
6. Somatostatinoma
7. Aldosteronoma

E. Drug- or chemical-induced

1. Vacor
2. Pentamidine
3. Nicotinic acid
4. Glucocorticoids
5. Thyroid hormone
6. Diazoxide
7. b-adrenergic agonists
8. Thiazides
9. Dilantin
10. a-Interferon

F. Infections

1. Congenital rubella
2. Cytomegalovirus

G. Uncommon forms of immune-mediated diabetes

1. "Stiff-man" syndrome
2. Anti-insulin receptor antibodies

H. Other genetic syndromes sometimes associated with diabetes

1. Down's syndrome
2. Klinefelter's syndrome
3. Turner's syndrome
4. Wolfram's syndrome
5. Friedreich's ataxia
6. Huntington's chorea

7. Laurence-Moon-Biedl syndrome
8. Myotonic dystrophy
9. Porphyria
10. Prader-Willi syndrome

IV. Gestational diabetes mellitus (GDM) Patients with any form of diabetes may require insulin.

Type 1 diabetes mellitus

Type 1 diabetes mellitus (T1D) is characterized by β -cell destruction caused by an autoimmune process, usually leading to absolute insulin deficiency. This form of diabetes, which accounts for only 5–10% of all diabetes, is juvenile-onset diabetes; it results from a cellular-mediated autoimmune destruction of the β -cells of the pancreas by CD4 and CD8 T cells and macrophages infiltrating the islets. In this case insulin therapy is required for survival, to prevent the development of ketoacidosis, coma and death.

Gestational diabetes mellitus (GD) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. It is a common condition affecting about 7% of all pregnancies; its detection is important because of associated maternal and fetal complications. Pregnancy is a diabetogenic condition itself (placental secretion of hormones, such as progesterone, cortisol, placental lactogen, prolactin, and growth hormone), characterized by insulin resistance with a compensatory increase in β -cell response and hyperinsulinemia. Insulin resistance usually begins in the second trimester and progresses throughout the remainder of the pregnancy; insulin sensitivity is reduced by as much as 80%. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus recognized an intermediate group of subjects whose glucose levels, although not meeting criteria for diabetes, are nevertheless too high to be considered normal. This category includes the impaired glucose tolerance (IGT) and the impaired fasting glucose (IFG). Patients with IFG and/or IGT are now referred to as having “pre-diabetes” indicating the relatively high risk for development of diabetes in these patients. In the absence of pregnancy, IFG and IGT are not clinical entities in their own right but rather risk factors for future diabetes as well as cardiovascular disease.

Type 2 diabetes mellitus (t2d)

Type 2 Diabetes Mellitus (T2D) is a complex heterogeneous group of metabolic condition characterized by elevated levels of serum glucose; according to WHO, it is defined as resulting from a defect in both insulin secretion and in insulin sensitivity. β -cell dysfunction includes abnormalities in pulsatility and in kinetics of insulin secretion, quantitative and qualitative abnormalities of insulin, β -cell loss and its progression. T2D exerts a huge toll in human suffering and economy. A recent evaluation using a computerized generic formal disease model revealed that excess global mortality due to diabetes in the year 2000 was equivalent to 5.2% of all deaths and diabetes is likely to be the fifth leading cause of death, similar in magnitude to numbers reported for HIV/AIDS. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030, with India, China and USA being the top 3 countries estimated to have the highest numbers of people with diabetes.

Etiology of t2d

The recent global epidemic of T2D almost certainly indicates the importance of environmental triggers over last several decades. In all over the world, the diabetes epidemic is due to the increase in prevalence of obesity, linked to “westernized” lifestyle, namely changes in nutritional habits, with increased intake of saturated fats, refined sugars and alcohol, and reduced intake of fibres, and at the same time, reduction in physical activity. The comparison between Pima Indians from Arizona and Pima Indians from a remote area in Mexico, and native Mexicans showed the major role of environmental factors compared to genetic factors in the occurrence of diabetes. The role of environment has also been demonstrated from many years by urban–rural comparisons of diabetes prevalence, higher in the urban areas inside any ethnic group, in a lot of epidemiological studies all around the world. Nonetheless, T2D is among many complex diseases for which a genetic contribution is well accepted. Identification of the genetic components of type 2 diabetes is one of the most important areas of diabetes research because elucidation of the diabetes genes will influence all efforts toward a mechanistic understanding of the disease, its complications, and its treatment, cure, and prevention. Multiple lines of evidence support the view that genetic components plays an important role in the pathogenesis of T2D.

- The spectrum of T2D prevalence in different ethnic groups’: The prevalence of T2D varies widely among populations, from 1% in Chile Mapuche Indian, 2% among Caucasians

in Europe to as high as 41% in the Nauru (Pacific Island) and 50% among Pima Indians in Arizona. Part of this observed ethnic variability can be attributed to non-genetic environmental and cultural factors; however, the observation that the disease prevalence varies substantially among ethnic groups that share a similar environment supports the idea that genetic factors contribute to disease predisposition.

- **Familial aggregation:** Other than genes, families share environments, culture and habits, yet familial aggregation of the disease is another source of evidence for a genetic contribution to the disease. Evidence for a genetic role includes the nearly 4- fold increased risk for T2D in siblings of a diabetic proband compared with the general population, the odds ratio (OR) of 3.4–3.5 with only a single affected parent, and the increase in the OR to 6.1 if both parents are affected.

- **Twin studies:** Multiple studies of twin concordance rates have been undertaken in T2D. Estimates for concordance rates have ranged from 0.29 to 1.00 in monozygotic (MZ) twins, while in dizygotic (DZ) twins the range was 0.10–0.43. Concordance among both MZ and DZ twins increases with the duration of follow up period. In spite of several caveats in twin studies, the high concordance in MZ twins and the 50% fall in DZ twins provides compelling evidence for a genetic component of T2D.

- **Heritability of intermediate phenotypes:** Insulin sensitivity and insulin secretion deteriorate in parallel in most human T2D. Both defects predicted subsequent T2D in several studies and both defects are shown to be present in nondiabetic but genetically identical co-twins of a diabetic proband. Data from multiple laboratories support a genetic basis for measures of both insulin sensitivity and insulin secretion. The relations between genetic and environmental factors in the development of T2D may be complex. Environmental factors may be responsible for the initiation of b-cell damage or other metabolic abnormalities, while genes may regulate the rate of progression to overt diabetes; indeed, in some cases genetic factors may be necessary for environmental factors even to start processes leading to the development of the disease.

Introduction to *Momordica Cymbalaria*

Momordica cymbalaria is one of the species of *cucurbitaceae* family. The synonyms are *Momordica tuberosa* Roxb or *Luffa tuberosa* Roxb. The plant is a perennial climber available only during the monsoon season and is found in the south Indian states of Andhra Pradesh,

Madhya Pradesh, Maharashtra and Tamil Nadu. The plant is traditionally used for the treatment of diabetes mellitus and also as a antiovolatory agent. MC also gives activity as Hepatoprotective, Cardioprotective, Antiulcer, Antimicrobial, Neuroprotective, Anticancer, Antidiarrhoeal and Anti implantation. Many plants have showed the role in introduction of new therapeutic agents, Instead of random search on plants if we search on traditional knowledge that is very beneficial to focussed and productive and certainly more economic.

Management of diabetes without any side effects is still a challenge to the medical system. This leads to increasing demand for natural products with antidiabetic activity and less side effect.

Diabetes mellitus is a chronic metabolic disorder affecting approximately 5% of the population. Currently available therapy for diabetes include insulin and various oral anti-diabetic agents such as sulfonylureas, metformin, a -glucosidase inhibitors, troglitazone, etc. These drugs are used as monotherapy or in combination to achieve better glycemic control. Each of the above oral agents suffers from a number of serious adverse effects.

Fruit powder of *M. cymbalaria* has been reported to have antihyperglycemic activity and antihyperlipidemic activity. Also have showed that the antihyperglycemic activity in rats was maximum with aqueous fraction of MC at the dose of 0.5kg The present investigation was, therefore, designed to confirm the effect of aqueous fraction of MC fruit on glycemic control and to study the effect on the lipid profile in normal and alloxan treated rats. An attempt was also made to elucidate the possible mechanism of the reported antidiabetic activity of MC fruit extract.

Chemical composition

Nutritional constituent

The calcium content of athalakkai is three times higher than that of the bitter gourd. Calcium is required for the growth of bones and teeth as well as for maintaining normal heart rhythm, blood coagulation, muscle contraction and nerve responses. The higher concentration of this nutrient in athalakkai may be exploited and used. Iron content in both the vegetables is almost the same. The ascorbic acid (Vitamin C) content of athalakkai is two times higher than that of bitter gourd. This is of interest, where there is shortage in vitamin C consumption. The content of potassium in athalakkai is also two times higher than in bitter gourd. The b

carotene content in athalakkai is negligible.[Parvathi S, Kumar VJF. Studies on chemical composition and utilization of the wild edible vegetable.

Medicinal values

The plant is traditionally used for the treatment of diabetes mellitus, rheumatism, ulcer, skin disease, and diarrhoea. The fruit of this plant have been reported to possess hypoglycaemic, hypolipidemic, cardio protective, hepatoprotective, nephroprotective and antioxidant properties.

Therapeutic uses

M.Cymbalaria fruit, leaf as well as root were considered as a tonic, stomatic stimulant, laxative, alternative and also having the nutritional composition for that it will be used properties (antidiabetic) in animal as well as human studies. The fruit juice and leaf tea of M. cymbalaria is employed for diabetes, malaria, colic, sores and wounds, infections, worms and parasites, as an emmenagogue, and for measles, hepatitis, and fevers. Fruit pulp, leaf juice and seeds possess antihelminthic activity. Root is astringent, abortifacient, aphrodisiac and also used to treat constipation, indigestion, diabetes, diarrhoea and rheumatism. Plants belonging to Momordica species have been used as therapeutic agents for the treatment of diabetes mellitus.

Following therapeutic uses of M.Cymbalaria are also reported

- 1) Hepatoprotective activity
- 2) Cardioprotective effect
- 3) Antidiarrhoeal activity
- 4) Antiulcer activity
- 5) Antimicrobial activity
- 6) Nephroprotective activity
- 7) Anticancer Activity
- 8) Anti implantion activity
- 9) Anti ovulatory activity(1).

Experiment on M.Cymbalaria as Antidiabetic Activity

There are two experiments that have been carried out on M.cymbalaria they are as follows.

- 1) Anti-diabetic Activity, Hypolipidemic Activity
- 2) Anti-hyperglycemic Activity in alloxan diabetic rats.

1) Anti-diabetic Activity, Hypolipidemic Activity

Fruit powder of *M. cymbalaria* showed Anti-diabetic effect and hypolipidemic effect as follows,

The treatment was given for 15 days. After the treatment, a significant reduction was observed in fasting blood glucose levels in the treated diabetic rats, but no hypoglycaemic activity in the treated normal rats. *M. cymbalaria* treatment showed considerable lowering of serum cholesterol and triglycerides in the treated diabetic group. There was a significant improvement in hepatic glycogen level in treated diabetic rats close to normal level after the treatment with *M. cymbalaria*. These results suggest that the *M. cymbalaria* fruit powder possesses antidiabetic and hypolipidemic effects in alloxan-induced diabetic rat.

2) Anti-hyperglycemic activity in alloxan diabetic rats

In this experiment aqueous, ethanol and hexane fraction of *M. cymbalaria* fruit were given to batches a rat. After and over night fast, the blood glucose levels were measured at 0, 1, 3, 5 and 7 h after the treatment. The aqueous extract of *M. cymbalaria* at a dosage of 0.5 g/kg b.w. is showed maximal blood glucose lowering effect in diabetic rats. The same dosage did not produce any hypoglycemic activity in normal rats. The antihyperglycemic activity of *M. cymbalaria* fruit was comparable with the treatment of glibenclamide, an oral hypoglycemic agent. Also having evaluation of anti-daibetic effect, The oral treatment with the aqueous extract of *M. cymbalaria* fruit (0.5 g/kg) for 6 weeks showed a significant antihyperglycemic as well as antihyperlipidemic effects in the alloxan-induced diabetic rats.

Description on insulin mimetic peptide

The aqueous extract of *M. cymbalaria* fruits has showed a potent anti hyperglycemic activity. The extract at a dose of 0.5 g/kg of body weight is effective for reducing blood glucose levels to near normal in the diabetic rats. A 17 k Da protein with an isoelectric point of 5.0 was identified as the active principle of antidiabetic action present in the aqueous extract of fruits of *M. cymbalaria*. It is named as M.Cy protein and found to be a novel protein by comparing its N-terminal amino acid sequence with those in the protein data bank. It did not produce hypoglycemia in either normal or diabetic rats. The results suggest that 'M.Cy protein', present in the fruits of *M. cymbalaria* is an effective antihyperglycemic active principle in STZ induced diabetic rats at a dose of 2.5 mg/kg of body weight(11) A comparison between the N-terminal sequence of M.Cy protein and α chain of human insulin was made since both are anti hyperglycemic proteins.

Insulin α chain:- Gly Ile Val Glu Gln Cys Cys Thr Ser Leu Tyr-

M.Cy protein:- Gly Leu Glu Pro Thr Thr Thr-

Similar such insulin mimetic peptide was reported in its related counterpart, *M. cymbalaria*. And also in other plant species namely *Canavalia ensiformis*, *Vigna unguiculata* and *Bauhinia*. the presence of insulin like peptide have been reported and their amino acid sequence were compared with bovin insulin as in table, Amino acid sequences of bovine insulin and insulin isolated from *Canavalia ensiformis*, *Vigna unguiculata* and *Bauhinia variegata*.

Table 1.

Plant species		Insulin sequence
Bovine insulin α -chain	1	GIVEQCCASVCSLYQLENYCN 21
Bovine insulin β -chain	1	FVNQHLCGSHLVEALYLVCGERGFFYTPKA 30
<i>Canavalia ensiformis</i> I-SC	1	GIVEQCCASVCSLYQLENYCN 21
<i>Canavalia ensiformis</i> I-LC	1	FVNQHLCGSHLVEALYLVCGERGFFYTPKA 30
<i>Vigna unguiculata</i> I-SC	1	GIVEQXXASVXSLYQLENYXN 21
<i>Vigna unguiculata</i> I-LC	1	FVNQHLCGSHLVEALYLVCGERGFFYTPKA 30
<i>Bauhinia variegata</i> a	1	GIVEQ 5
<i>Bauhinia variegata</i> b	1	FVNQH 5

cardioprotective effect of *m.cymbalaria*
 Koneri R (13) has reported that *M. cymbalaria* (500 mg/kg of body weight) prevented the alterations in marker enzymes of myocardial infarction, and oxidative stress along with uric acid. Myofibrillar alterations such as myocytosis and myofibrillar degeneration are reported in isoproterenol treated rats. Cardiac sections of the isoproterenol treated animals showed infiltration of inflammatory cells and continuity in the muscle fiber was lacking suggesting an irreversible cell injury. Rats pretreated with *M. cymbalaria* showed normal myofibrillar structures with striations and revealed a marked protection by the extract against myocardial necrotic damage. Administration of isoproterenol raised LDL cholesterol and decreased HDL cholesterol level in the serum. An increase in concentration of total cholesterol and LDL cholesterol, and a decrease in HDL cholesterol are associated with raised risk of myocardial infarction. High level of circulating cholesterol and its accumulation in heart tissue is accompanied with cardiovascular damage. *M. cymbalaria* elevated the HDL level and decreased the LDL cholesterol level. There is a growing body of evidence from epidemiologic, clinical, and laboratory data indicating that elevated triglyceride levels are an independent risk factor for cardiovascular disease. Hypertriglyceridemic patients are at a risk for cardiovascular disease often develops a lipoprotein profile characterized by elevated triglyceride, dense LDL, and low HDL cholesterol which causes myocardial membrane damage. Hypertriglyceridemia observed in isoproterenol treated rats is clinically reported in ischemic heart disease. Pretreatment with *M. cymbalaria* prevented the elevation of triglycerides cholesterol and LDL in serum, signifying that the myocardial membrane is intact and not damaged. Antihyperlipidemic, antioxidant and antidiabetic activity along with cardioprotective properties of *M. cymbalaria* adds to the accumulating evidence for therapeutic potential of this plant.

Other activities of *m.cymbalaria***1) Anti-Cancer**

The methanol extract of aerial parts of *M. cymbalaria* Hook f (200 mg/kg of body weight) has showed significant anticancer activity as compared to standard cyclophosphamide against ehrlich ascites carcinoma induced cancer in mice.

2) Anti-Ulcer

The aqueous extract of *Momordica tuberosa* is proved to have anti ulcer property. The reduction in non protein sulfhydryls concentration, gastric content, haemorrhage and ulceration in the ulcer induced Wistar rats suggested that the anti ulcer activity of the aqueous extract of *Momordica tuberosa* is due to the presence of polyphenolic constituents.

3) Anti-Diarrhoeal Activity

Rushabendra Swamy BM (15), have reported that the methanol extract of fruit of *Momordica cymbalaria* exhibited significant anti- diarrhoeal activity against castor oil induced diarrhoea in rats. The extract had a similar activity as that of antidiarrhoeal drug diphenoxylate, when tested at 200, 400 & 600 mg/kg and statistically significant reduction in the frequency of defecation and the wetness of the fecal droppings when compared to untreated control rats.

The methanol extract of *M. cymbalaria* (MEMC) significantly inhibited the prostaglandin E₂ (PGE₂) induced intestinal fluid accumulation (enter pooling). It has been shown that E type of prostaglandin cause diarrhoea in experimental animals as well as human beings. Their mechanism has been associated with dual effects on gastrointestinal motility as well as on water and electrolyte transport. PGE₂ also inhibit the absorption of glucose a major stimulus to intestinal adsorption of water and electrolytes. These observations tend to suggest that MEMC reduced diarrhoea by inhibiting PGE₂ induced intestinal accumulation of fluid.

4) Antiimplantation and antiovolatory Activity

Koneri Raju, have reported that anti implantation activity of ethanolic root extract of *Momordica tuberosa* in rats and it may not be due to estrogenic or progestrogenic activities. reported the antiovolatory and abortifacient potential of the ethanolic extract of root of *M. cymbalaria* on rats. The estrous cycle in the rats treated with extract (250 and 500 mg/kg) showed a decrease in the duration of estrous and the metestrous phases. It was also characterised by a prolongation of the proestrous phase. The prolongation of the proestrous phase indicates that maturation of the follicle in the preovulatory phase was delayed, leading

to non-maturation of graffian follicle. Non-availability of matured graffian follicle was indicated by reduction in theestrous and the metastrous phases. Therefore, ovulation was inhibited. Ethanolic extract at 500 mg/kg showed 100% abortifacient activity, while 250 mg/kg dose did not show abortifacient activity.

Mechanism of action saponins in diabetics

Its various mechanisms of action have been reported in diabetic rats. Antiovolatory, abortifacient, anti-implantation and cardio-protective activities have also been reported. Fruits of MC are also reported to have antimicrobial activity. The antidiabetic activity of saponins of *Momordica cymbalaria* may be due to reversing of the atrophy of the pancreatic islets of β cells, as a result of which there may be increased insulin secretion and increase in the hepatic glycogen level and these may attenuate hyperinsulinaemia. The α adrenergic blocking effect might contribute to their insulin secretion and sensitizing effects. In the present study, an active phyto molecule- an oleanane-type triterpenoid saponin has been isolated and studied for antidiabetic activity.

Mechanism of action of *Momordica cymbalaria*

An currently available therapy for diabetes include insulin and various oral anti-diabetic agent such as sulphonylureas, metformin, α -glucosidase inhibitor, torglitazone, etc. these drugs are used as monotherapy or in combination to achieve better glycemic control, these drug suffer from various side effects the mechanism of actions are below.

Mechanism of action of sulphonylureas

Various mechanism of action of sulphonylureas as follows,

1. Pancreatic mechanism

All sulphonylurea hypoglycemics inhibit the efflux of K^+ (K^+ channel blockers) from pancreatic β -cells via a sulphonylurea receptor which may be closely linked to an ATP-sensitive K^+ -channel. The inhibition of efflux of K^+ leads to depolarization of the β - cell membrane and, as a consequence, voltage-dependent Ca^{++} -channels on the β -cell membrane then open to permit entry of Ca^{++} . The resultant increased binding of Ca^{++} to calmodulin results in activation of kinases associated with endocrine secretory granules thereby promoting the exocytosis of insulin-containing secretory granules.

2. Extra-pancreatic mechanisms

The sulfonylureas also reduce serum glucagon levels possibly contributing to its hypoglycemic effects. The precise mechanism by which this occurs remains unclear but may result from indirect (secondary) inhibition due to enhanced release of both somatostatin and insulin. Sulfonylureas may also potentiate insulin action at target tissues (drug-dependent characteristic).

Sulfonylureas(k_{atp} channel blocker)

Sulphonylureas provoke a brisk release of insulin from pancreas, the mechanism of which is detail in fig. The rate of insulin secretion at any glucose concentration is increased, i.e. insulin release is provoked even at low glucose concentration risking production of severe and unpredictable hypoglycaemia. in type 2 DM the kinetics of insulin release in response to glucose or meals is delayed and subdued. the SUs primarily augment the 2nd phase insulin secretion with little effect on the 1st phase. That they do not cause hypoglycaemia in pancreatectomised animals and in type I diabetics (presence of atleast 30% functional beta cell is essential for their action), confirms their indirect action through pancreas. A minor action reducing glucone secretion, probably by increasing insulin and somatostatin release has been demonstrated. hepatic degradation of insulin is also slowed.

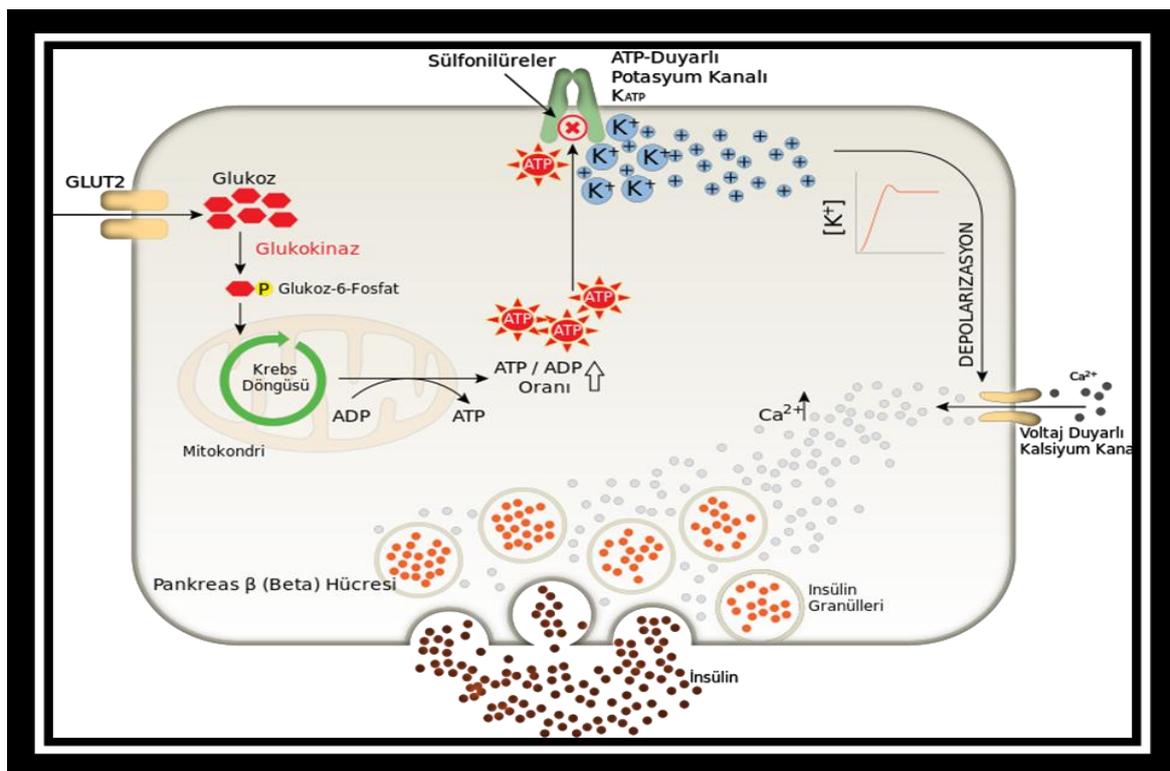


Figure 1.

Mechanism of action of metformin

Biguanides do not cause insulin release but presence of insulin is essential for their action. metformin is not effective in pancreatectomized animals and in type1 diabetics. though the details are not clear, recent studies have recognized activation of AMP-dependent protein kinase (AMPK) to play crucial role in mediating the action of metformin, the key features of which are :

- 1) Suppresses hepatic gluconeogenesis and glucose output from liver .this is the major action responsible for lowering of blood glucose in diabetics.
- 2) Enhances insulin-mediated glucose uptake and disposal in skeletal muscle and fat. insulin resistance exhibited by type2 diabetics is thus overcome.this translates into-
 - Glycogen storage in skeletal muscle
 - Reduced lipogenesis in adipose tissue and enhanced fatty acid oxidation.
- 3) Interferes with mitochondrial respiratory chain and promotes peripheral glucose utilisation through anaerobic glycolysis.

AMPK activation by metformin appears to be an indirect consequence of interference with cellular respiration and lowering of intracellular ATP and other energy sources.

Metformin also retards intestinal absorption of glucose other hexoses, amino acid and vit.B₁₂.

M. cymbalaria fruit extract and insulin secretion status

This plant material was dried in shade, powdered and the powder was used for the extraction of antidiabetic principle/s into different solvents.

Preparation of extract- The active principle/s of *Momordica cymbalaria* fruits were extracted into three different solvents, water, 95% ethanol and hexane. *Momordica cymbalaria* fruit powder was soaked in the above individual solvents in different glass jars for 2 days at room temperature and the solvent was filtered. This was repeated three to four times until the extract gave no coloration. The extracts were distilled and concentrated under reduced pressure in the Buchi, rotavapour R-114 and finally freeze dried. These extracts were used for further studies. The yield of the aqueous, ethanolic and hexane extracts were 9.4, 5.2 and 2.0% respectively (w/w in terms of dried starting material).

Insulin secretion status**M.Cy protein Introduction****Isolation of M.Cy protein**

Aqueous extract of M.Cymbalaria as above, then further step.

Isolation and Purification of the M.Cy Protein**1) Ammonium sulphate fractionation of the aqueous extract**

All purification procedure were conducted at 4°C unless otherwise noted. Aqueous extract of MC was subjected of ammonium sulphate fractionation to precipitate protein (20% -fraction, 40%-fraction and 60%-fraction) using different conc. (20,40 and 60%) of ammonium sulphate. The protein precipitate were collected by centrifugation and dissolve in 50mM acetate buffer, pH4. Further this fraction were dialyzed with dialysis membrane (sigma, 10kDa cut-off) in the same buffer to eliminated the traces of ammonium sulphate. protein conc. were determined in these fractions and antihyperglycemic activity was verified. Electrophoresis of the active fraction was carried out on 10% SDS polyacrylamide gel along with standard marker proteins and stained with silver nitrate.

2) Purification of the 20%-fraction by gel filtration

The active 20% -fraction, ammonium sulphate precipitated fraction, was further purified by gel filtration using Sephadex column (32cm×2cm). for this, 5g of Sephadex G-50 (Sigma, St. Louis, MO) was swollen overnight in 500ml of 50mM acetate buffer pH4.8 at 37°C. The column was then packed ensuring that no air bubbles were trapped. 1ml of the 20%-fraction containing 32mg of precipitate protein was passed through Sephadex G-50 column and different fraction were eluted at flow rate of 0.5ml/min, using 50mM acetate buffer pH4.8. The protein conc. of the collected peak fractions (PF-1, PF-2 and Pf-3) was estimated and antihyperglycemic activity was evaluated.

3) reverse phase HPLC

The peak fraction 2 (PF2) obtained from gel filtration, which showed maximum hyperglycemic activity, was further purified on C-18 reverse phase HPLC (column 4.6µm×150µm) (shimadzu). The c-18 column was equilibrated initially with 0.1% trifluoroacetic acid (TFA) in water for 30min. The active PF2 was dialyzed against degasified 50mM acetate buffer pH4.8 and 1ml (12µg) of these PF2 was injected into the column. Different fraction were eluted using linear gradient of acetonitrile (0-80%, v/v) in 0.1% TFA (0.1ml TFA in 99.9ml of water). The elution pattern was monitored by UV detector at 280nm. The reverse phase

HPLC fraction (RPF) containing the purified protein was dialyzed against 50mM aceted buffer pH 4.8 and used for further stadies. These step were repeated to get enough active principle used for the preent studies. The yield of the purified protein was 0.012%(w/w).

Characterization of M.Cy Protein

Molecular weight was found to be 17 kDa. SDS-PAGE analysis of RPF confirmed the purity & homogeneity of the active principle (protein). We named this pure active principle as M.cy protein. This protein on isoelectric focusing gave a single band with an isoelectric point (pI) of 5.0 indicating its acidic nature (data not shown). Single band in SDS-PAGE & in IEF indicates that the protein is pure & monomeric in nature. Our preliminary studies on the structure of M.cy protein showed 26% of α –helical conformation & 27% of β -pleated conformation. The M.Cy protein lost its antidiabetic activity at higher temperature (100⁰c) & pH higher than 6(data not shown). And description of insulin mimetic petide as above.

Effect of different dose of m. cy protein

The effect of different doses of M.Cy protein on fasting blood glucose level of both normal and diabetic rats is given in table no 1. The glucose levels of diabetic untreated rats (group 6) were significantly higher than those of normal untreated rats (Group 1). Intravenous administration of different doses of M.cy protein in normal rats (Group 2-5) did not produce any hypoglycemic activity indicating that M.cy protein will not cause hypoglycemia. The M.cy protein at a dose of 2.5 mg/kg. b.w showed a maximum decrease (68.7%,*p* <0.001) in the blood glucose levels in the diabetic rats after 6h of treatment. Reduced action at a lower dose may be because of insufficient concentration of M.cy protein to produce antihyperglycemic action. Decreased antihyperglycemic activity was observed with higher concentration following typical sigmoid curve of dose dependence (Spinhour, 2005) as most of the drugs show. Treatment with glibenclamide, an insulin secretogogue, at a dose of 0.02 g/kg b.w. resulted in 29.8% of reduction in blood glucose after 5 h of treatment indicating the presence of functional pancreatic β cells. Subcutaneous & intraperitoneal administration of m.cy protein showed similar percentage (66%, *p*<0.001 & 69%, *p*<0.0001 resp.) of reduction in blood glucose. However, oral administration of the protein did not show any antihyperglycemic activity. No hypoglycemic condition was observed & blood glucose levels reached near normal in our studies with M.cy protein. An emphasis is laid on glucose homeostasis as a severe hypoglycemic can result in life-threatening situation. Therefore, absence of hypoglycemia with M.cy protein is more promising. In conclusion, we have

isolated a novel 17kDa protein from the aqueous extract of the fruits *Momordica cymbalaria* & named it as M.Cy protein/kg b.w. is the effective & optimum dose for reducing blood glucose level to near normal in the diabetic rats. The result also indicated that the protein can produce rapid & consistent decrease in blood glucose levels by subcutaneous or intravenous or intraperitoneal routes. Though there was a delayed onset of action of M.Cy protein with subcutaneous administration, antihyperglycemic activity similar to that with intravenous administration was expressed within 7 h of treatment. Unlike insulin, insulin secretagogues or small protein or peptide isolated from *Momordica charantia*, the treatment with M.Cy protein did not produce hypoglycemia in either diabetic or normal rats. The results of the present study suggest that M.cy protein can be considered as a promising antihyperglycemic agent.

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