

**ANTIDIABETIC EFFECT OF BITTER GOURD,AN INTERVENTION STUDY AMONG
HYPERGLYCEMIA PATIENTS IN INDIA**

Submitted in partial fulfillment of the requirements for the award of

Master of Technology degree

BIOTECHNOLOGY

by

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**DEPARTMENT OF BIO AND CHEMICAL
SCHOOL OF BIO TECHNOLOGY**

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A" by NAAC

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APRIL- 2021



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DEPARTMENT OF BIO TECHNOLOGY

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of M.MARY SOUMIYA (39810003) who carried out the project entitled “**ANTIDIABETIC EFFECT OF BITTER GOURD, AN INTERVENTION STUDY AMONG HYPERGLYCEMIA PATIENTS IN INDIA**” done under my supervision from November 2020 to April 2021.

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I, M.MARY SOUMIYA hereby declare that the Project Report entitled “**ANTIDIABETIC EFFECT OF BITTER GOURD, AN INTERVENTION STUDY AMONG HYPERGLYCEMIA PATIENTS IN INDIA**” done by me under the guidance of Dr.UTHRA LAKSHMI is submitted in partial fulfillment of the requirements for the award of Master of Technology degree in BIO TECHNOLOGY.

DATE: 15/04/2021

PLACE:CHENNAI

**SIGNATURE OF THE
CANDIDATE**

AKNOWLEDGMENT

I am pleased to acknowledge my sincere thanks to Board of Management of SATHYABAMA for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to Dr WILSON ARUNI, pro vice chancellor and Dr RAMESH KUMAR, Head of the Department, Dept. of BIOTECHNOLOGY for providing me necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Project Guide Dr UTHRA LAKSHMI for her valuable guidance, suggestions and constant encouragement paved way for the successful completion of my project work.

I wish to express my thanks to all Teaching and Non-teaching staff members of the Department of BIOTECHNOLOGY who were helpful in many ways for the completion of the project.

ABSTRACT

Diabetes mellitus (hyperglycemia) commonly known as high blood sugar which causes due to either the production of insulin not enough or the body cells not responding properly to the insulin produced by the pancreas. From the report of WHO, About 422 million people worldwide have diabetes, the majority living in low-and middle-income countries, and 1.6 million deaths are directly attributed to diabetes each year. *Momordica charantia* (*M. charantia*), commonly referred to as bitter melon is well-known for its anti-diabetic amongst the indigenous populations of Asia, South America, India and East Africa. By using the *Momordica charantia* for diabetes, certain animal studies and human studies have shown a hypoglycemic effect. Bitter melon is rich in triterpene, protein, steroid, alkaloid, inorganic, lipid, and phenolic, which are responsible for the antidiabetic effects.

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CHAPTER 1

INTRODUCTION

1.1 BITTER GOURD;

Bitter gourd otherwise called as bitter melon, bitter apple, bitter squash, balsam-pear etc. Its botanical name is *Momordica charantia*. It grows widely in tropical and subtropical areas of Asia, Africa and Caribbean. Bitter gourd belongs to the Cucurbitaceae family (Bagchi, 2015). The origin of bitter melon is said to be India and it was introduced to China during the 14th century. It is used regularly in all Asian cuisines. Normally it is used to grow up to 5 meters (Grover, 2004). Male and female flowers bloom separately in each plant. Structure of fruit has a thin oblong shaped exterior and a thin layer of flesh surrounding a central seed cavity filled with large, flat seeds. Initially it will be in green color, once it starts ripening it will start to turn to yellowish red. Usually the outer skin is soft and tender, during ripening period of time fruit is more watery, but bitter. Pith and seed will look so white in unripe fruits. Usually seed is not edible; they will be removed before cooking. There are so many varieties of bitter melon that exist. They are differentiated based on various shapes and size and color. Bitter melon grown in India has a narrower shape with pointed ends. Some variants of fruit only grow 6-10cm, they are called miniature versions, they are popular in India, Pakistan, Bangladesh, Nepal and other Asian countries (Beloin, N, 2005).



Fig 1.1 Bittergourd

1.1.1 Nutritional value of bitter gourd

Nutritional component	volume	
Water	94.03	g
Energy	17	kcal
Energy	71	kJ
Protein	1	g
Total lipid (fat)	0.17	g
Ash	1.1	g
Carbohydrate, by difference	3.7	g
Fiber, total dietary	2.8	g
Calcium, Ca	19	mg
Iron, Fe	0.43	mg
Magnesium, Mg	17	mg
Phosphorus, P	31	mg
Potassium, K	296	mg
Sodium, Na	5	mg
Zinc, Zn	0.8	mg
Copper, Cu	0.034	mg
Manganese, Mn	0.089	mg
Selenium, Se	0.2	µg
Vitamin C, total ascorbic acid	84	mg
Thiamin	0.04	mg
Riboflavin	0.04	mg
Niacin	0.4	mg
Pantothenic acid	0.212	mg
Vitamin B-6	0.043	mg
Folate, total	72	µg
Folic acid	0	µg
Folate, food	72	µg

Folate, DFE	72	µg
Vitamin B-12	0	µg
Vitamin A, RAE	24	µg
Retinol	0	µg
Carotene, beta	190	µg
Carotene, alpha	185	µg
Vitamin A, IU	471	IU
Lutein + zeaxanthin	170	µg
Vitamin D (D2 + D3), International Units	0	IU
Vitamin D (D2 + D3)	0	µg
Fatty acids, total trans	0	g
Cholesterol	0	mg

1.1.2 Taxonomy;

kingdom	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Rosids
Order:	Cucurbitales
Family:	Cucurbitaceae
Genus:	Momordica
Species:	M. charantia

1.1.3 Anti diabetic property of bitter gourd;

From the beginning, There are so many verities of traditional and herbal remedies have been used to treat various types of diabetes in Asia and other developing countries (Malviya N,2010). Bitter gourd has been widely investigated thoroughly for the treatment of various type of diabetes (BALA,2012). With the traditional use supported by modern scientific evidence of the beneficial function of bitter gourd, it is one of the most promising plants for Type 1 diabetes today (Cefalu WT,2008). Investigation of the traditional uses of Bitter melon in India revealed that it is one of the most important plant for lowering blood glucose levels in patients with diabetes (Paul A 2010).

Bitter gourd is rich in nutrients and composed of many beneficial compounds which is very essential .Which include vitamins, Antioxident, bioactive chemicals, minerals which all plays an important role in treating a wide range of illnesses. The fruits contain high amounts of vitamin A, vitamin E, vitamin C, vitamins B1, B2 and B3, and vitamin B9. The caloric values for fruit, stem,leafe and seed were 213.26, 241.66 and 176.61 Kcal/100 g (Bakare RI,2010).

The fruit is very rich in minerals including zinc, potassium, calcium, magnesium, iron and phosphorus. On addition to that, it is a good source of dietary fiber. Bitter melon is widely known for its high antioxidant properties due in part to phenols, flavonoids, isoflavones, terpenes, anthroquinones, and glucosinolates, all of these responsible for the bitter taste(Snee LS 2011)

Various types animal studies have proven hypoglycaemic effects of the fruit, leave, pulp, seed and whole plant of Bitter melon in normal animals(Jeong J,2008),(Ismail WFE,2012). Most importantly, Bitter gourd improves glucose tolerance and suppresses postprandial hyperglycaemia in rats(Abdollah M,2010), and Bitter melon extract can enhance lipolysis and insulin sensitivity(Chen Q,2003). Some studies shows that the hypoglycaemic effect of *Bitter melon* was comparable with oral administrated medications such as chlorpropamide tolbutamide and glibenclamide(Ojewole JA,2006). So many biochemical data have shown the possible mechanisms of the anti-diabetic

actions of Bitter gourd with the theme being activation of the AMP-activated protein kinase system(Miura T ,2001)(Cheng HL,2008).

1.2 DIABETES

Diabetes is a condition that results occur due to lack of the insulin in a person's blood, or the body has a problem using the insulin which has been produced which is otherwise called as insulin resistance. The name "diabetes", usually mean diabetes mellitus. People with diabetes Mellitus are called "diabetics".

The glucose level in the blood is controlled and maintained by several hormones. Pancreas secretes the insulin. When food is consumed, the pancreas makes insulin to send a message to other cells in the body. This insulin transfer the signal to the cells to take up glucose from the blood. For energy glucose will be used as a energy source. Extra glucose which is not needed right away is stored in some cells as glycogen. When food is not consumed, to generate energy, cells break down the stored glycogen into glucose.

Symptoms include increased thirst and increased appetite frequent urination. When it remain untreated, diabetes may lead to many complication (Saedi, E 2016). Serious long-term complications may include cardiovascular diseases, eye damage, chronic kidney disease, stroke, foot ulcers, damage to the nerves, and cognitive impairment, (Fisher JN, July 2009).

According to 2019 survey, nearly 463 million people had diabetes worldwide with type 2 diabetes making up nearly 90% of the cases.(Idf diabetes atlas, 2019).Rates are similar in both men and woman (Vos T,2010). Each year rates will continue to rise. Diabetes may doubles a person's risk of early death in life span. According to survey diabetes resulted in approximately 4.2 million deaths in 2019, (Irwin JM, eds 2010). It secure 7th rank to lead cause of death globally.

1.2.1 Complications

All types of diabetes may lead to the risk of long-term complications. These may develop after many years more or less 10-20 years. But most of the cases people may not received a diagnosis before that time. Long-term complications may lead to damage to blood vessels. According to research diabetes will doubles the risk of cardiovascular disease (Sarwar N,2010) and about 75% of death happens in people with diabetes are due to coronary artery disease (KushnerFG,2013) Other diseases may include stroke, and peripheral artery disease.

The major complications of diabetes due to damage in small blood vessels include damage to the nerves eye and kidney. Diabetes also increases the chance of having glaucoma, cataracts, and other eye problems. Most common recommendation for people with diabetes to visit an eye doctor atleast once a year. The kidney damage, known as diabetic nephropathy, can lead to urine protein loss, tissue scarring, and finally chronic kidney disease, may requiring dialysis or kidney transplantation(Cukierman,2005). Diabetic neuropathy, is the most common complication of diabetes which leads to the damage of nerves. The symptoms can are tingling, pain numbness, and altered pain sensation, which may lead to damage to the skin. Diabetes-related foot problems, such as diabetic foot ulcers may occur, and it is very difficult to treat. (Yang Y,2015).

1.2.2 Types of diabetes:

1.2.2.1 Type 1 diabetes mellitus

In case of type 1 diabetes the pancreas does not make insulin. Type 1 diabetes Mellitus happens when the part of the pancreas that makes insulin is gt by that person's own immune system. In order to live, a person with type 1 diabetes must take insulin for the rest of their life directly through intra dermal insulin delivery. They need to check their blood sugar level on a regular basis, often, many times each day.

Type 1 diabetes happens quite often in younger people, however, it can occur in adults, although this is much less common. About 1 out of every 10 people with diabetes have type 1 diabetes.

1.2.2.2 Type 2 diabetes mellitus:

Type 2 diabetes Mellitus is very different from type 1 diabetes. In type 2 diabetes, the person makes enough insulin, but either the insulin may not work properly in that person's body as it should, or they do not make enough insulin to process the glucose. When insulin does not work as it should, glucose in the blood cannot get into the parts of the body that need sugar.

1.2.2.3 Gestational diabetes:

Gestational diabetes mellitus is mostly like type 2 diabetes. It happens to some women during their pregnancy.

1.2.2.4 Other types of diabetes:

Other types of diabetes include but are not limited to:

- Maturity onset diabetes of the young (MODY)
- Latent autoimmune diabetes of adults (LADA)

Type 2 diabetes happens most of the time in an older person who is obese.

Symptoms in type1 diabetes usually happen suddenly. But In type 2 diabetes, there may be mild symptoms or there will be no symptoms at all which makes it much harder to detect.(Sarwar N,2010)

1.2.3 Ways of treating diabetes;

The physical activity and exercise are one of the basic strategies in the treatment of diabetes. Promoting exercise, within a specific plan, provides in general terms multiple benefits: Increased insulin sensitivity in tissues, improvement of glycaemic control[44], benefits in lipid profile and blood pressure, maintenance or weight loss, cardiovascular benefits, better quality of life, psychological well-being and improvement of depression(*Phielix E,2010*).

TZD are currently available in United States: Rosiglitazone and pioglitazone. In Europe, since 2010, rosiglitazone was suspended by the European Medicines Agency, based on the overall risks of rosiglitazone exceed their benefits. French and Germany Medicines Agencies also discontinued pioglitazone in 2011.

Sitagliptin, which is approved for the treatment of T2DM in many countries, can be employed alone or dual therapy with sulfonylurea, metformin or TZD or third therapy. The normal dose of sitagliptin is 100 mg once daily; half dose is utilized in patients with an eGFR 30-50 mL/min, and quarter dose in those with an eGFR < 30 mL/min(*Bergman AJ,2007*).

From the day when diabetes were found the chemical method of oral administrated were started. For a prolog time chemical method is wildy used as a best option to control the blood sugar level or increase the insulin secretion.

1.2.4 Side Effects;

There are several long and short term side effects are lead by drug administration of diabetes, some of them are listed below;

- Sulfonylureas: lowering blood sugar, stomach upset, skin rashes and itching, weight gain
- Biguanides/Metformin: sickness with alcohol, kidney complication, stomach upset, tiredness and dizziness.
- Alpha-glucosidase inhibitors: diarrhea, gas, bloating

- Thiazolidinediones: , liver disease risk, anaemia risk, swelling of legs or ankles, weight gain
- Meglitinides: weight gain, low blood sugar

1.3.TRANSDERMAL DRUG DELIVERY

Trans dermal drug delivery is a painless method of delivering drugs by applying a drug onto intact and healthy skin (Han T, Das DB,2015). The drug first penetrates through the stratum corneum and then passes through the deep epidermis and dermis without drug accumulation in dermal layer. When the drug reaches the dermal layer, it will be available for absorption through the dermal microcirculation (Donnelly R.F., Singh T.R.R.,2012). Trans dermal drug delivery has many advantages over other traditional routes of drug delivery (Donnelly RF,2012). It can provide a non-invasive alternative to parenteral routes, thus issues such as needle phobia (Han T, Das DB,2015).the most important advantage of TDD is, large surface area of the skin and ease of access allows many placement options on the skin for transdermal absorption(Schoellhammer CM,2015).

Trans dermal drug delivery avoids pre-systemic metabolism, thus improving bio availability(Das DB,2015).

1.3.1 Skin Structure;

Skin is the largest organ of the body with the surface area of 1.7 m², which is 16% of the total body mass of an average person (Menon GK,2002). To provide a protection barrier between the body and the external environment against the permeation of ultraviolet (UV) radiation, chemicals, allergens, the loss of water and microorganisms is the main function of the skin (Benson H.A., Watkinson A.C.,2012). Skin can be divided into three main regions, they are given below

- (1) the outermost layer, the epidermis, which contains the stratum corneum;

- (2) the middle layer, the dermis and
- (3) the inner layer, the hypodermis

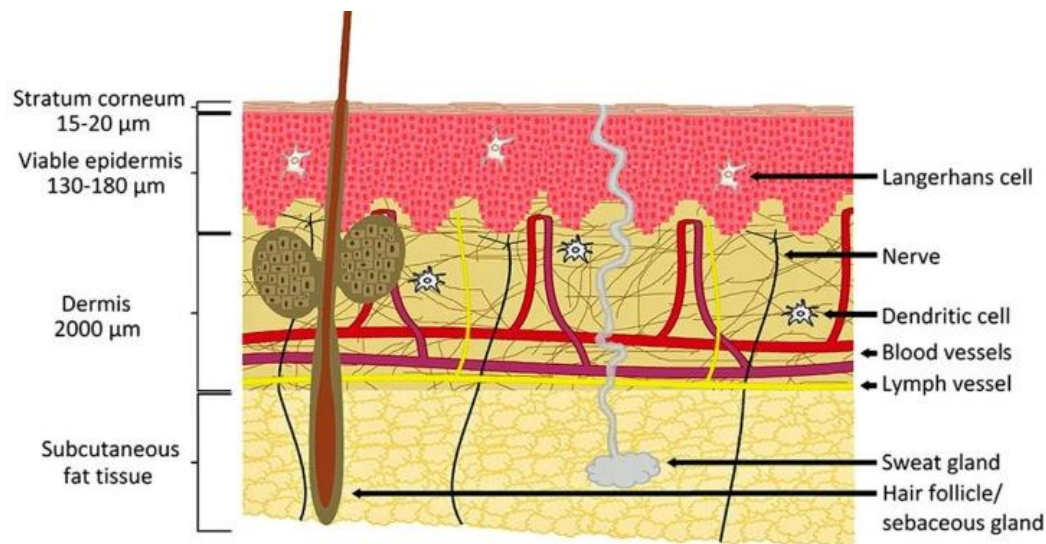


Fig 1.2 Skin Structure

(K, Jiskoot W, Bouwstra JJ. 2012 Jul 20)

1.3.1.1 Epidermis

The epidermis is the outermost part of the skin and differ in thickness with approximately 0.8 mm on the soles of the feet and palms of the hands (Benson H.A., Watkinson A.C,2012). It consists of multi-layered epithelial cells and the epidermis is often indicated to as the epidermal layers below the stratum corneum (Donnelly R.F,2012). The stratum corneum is the most superficial layer of the epidermis (Domínguez-Delgado C.L., Rodríguez-Cruz I.M,2010). It will direct contact with the external environment and its barrier properties may be partly related to its very high density and its low hydration of 15%–20% (Walters K.A,2002)

1.3.1.2 Dermis

Thickness of the dermis is approximately 2–3 mm and consists of collagenous (70%) and elastin fibres which gives strength and elasticity to skin (*Liu X,2002*). Blood vessels

found in the dermis which provide nutrients for both the dermis and epidermis. Macrophages, lymphatic vessels and Nerves, also present in the dermis layer.

1.3.1.3 Hypodermis

The hypodermis is the deepest layer of the skin and consists of a network of fat/lipid cells (*Liu X, 2002*). It is the middle layer between the skin and the underneath tissues of the body, such as muscles and bone. Hypodermis-resident fat cells account for approximately 50% of the body's fat with the other predominant cells of the hypodermis.

1.3.2 Steps involved in TDD

PENETRATION

The entry of a substance into a particular layer of the skin;



PARTIONING

Partitioning from the stratum corneum into the aqueous viable epidermis;



DIFFUSION

Diffusion through the viable epidermis and into the upper dermis;



PERMIATION

The penetration of molecules from one layer into another, which is different both functionally and structurally from the first layer;



ABSORPTION

Absorption: The uptake of a substance into the systemic circulation.

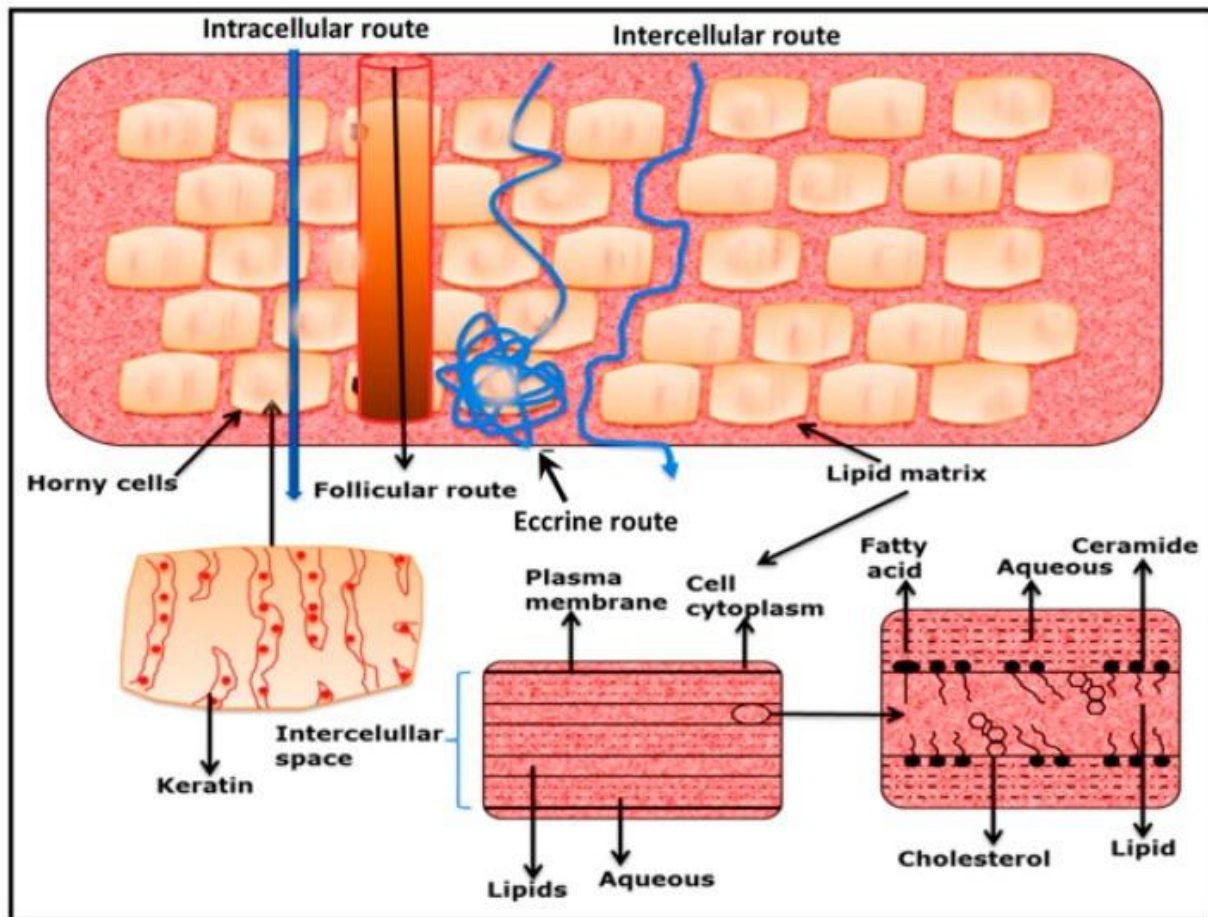


Fig 1.3 Route of TDD in skin

(Shahzad Y, Louw R, Gerber M, du Plessis J J, 2015 MAR 28)

1.3.4 Advantage of TDD;

- TDD are a painless, noninvasive way to deliver substances directly into the body.
- Trans dermal drug discovery are a better way to deliver substances that are broken down by the stomach acids, not well-absorbed from the gut, or extensively degraded by the liver.
- Topical patches over a controlled, steady delivery of medication over long periods of time.

- TDD have minimal side effects when compared to oral medications or supplements.
- TDD are easier to use.
- TDD are an alternative to people who cannot, or don't like to take medications or supplements orally.
- TDD are cost-effective.
- It's a better option for people who don't prefer injection pain . (Sunita A. Chaudhary1,2012)

CHAPTER 2

LITERATURE SURVEY

Diabetes mellitus (hyperglycemia) commonly known as high blood suger which cause due to either the production of insulin not enough or the the body cells not responding properly to the insulin produced by the pancreas.From the report of WHO, About 422 million people worldwide have diabetes, the majority living in low-and middle-income countries, and 1.6 million deaths are directly attributed to diabetes each year.Momordica charantia (M. charantia), commonly referred to as bitter gourd is well-known for its anti-diabetic amongst the indigenous populations of Asia, South America, India and East Africa By using the Momordica charantia for diabetes,certain animal studies and human studies have shown a hypoglycemic effect.Bitter gourd is rich in triterpene, proteid, steroid, alkaloid, inorganic, lipid, and phenolic, which are responsible for the antidiabetic effects(*Raman B.V 2012*)

Herbal remedies have been used cheaply around the world to treat diabetes. In fact, in many parts of the world, especially in poor countries, this may be the only available treatment for diabetics. There are several reviews by various authors about herbal remedies for diabetes (Joseph 2015)

The main components of the bitter melon that counteract the effects of diabetes are triterpene, proteid, steroids, alkaloids, inorganic, lipid, and phenolic compounds (*Saeed MK, Shahzadi I, Ahmad I2010*). Several glycosides are separated from the M stem. Charantia fruit also collected under the category of cucurbitane-type triterpenoids(*Chang CI, Chen CR, Liao YW, Cheng HL,2008*) . In particular, the four triterpenoids have a function modified by AMP-protein kinase which is a hypoglycaemic-mediated mechanism of action of bitter melon(*Tan MJ, Ye JM, Turner N, Hohnen-Behrens C, 2008*)

Skin absorption (lateral, skin) carries chemicals from the skin surface to the skin and blood circulation. Skin absorption is related to the level of exposure and the potential effect of an organism through the skin. Human skin encounters many agents intentionally and unintentionally. Skin absorption may be the result of exposure to the skin at work, in

the environment or at the consumer's exposure to chemicals, cosmetics, or pharmaceutical products. Some chemicals can be absorbed in sufficient quantities to create adverse effects on the system. Dermatitis is considered one of the most common occupational diseases. To test whether the chemical can be dangerous it can cause dermatitis or other more systematic effect (*Dang, 1996*) there are several anti diabetic components present in the bitter gourd, for this study we have taken syrinjaldehyde, pendadecanoic acid, Ursolic acid. Because only these 3 components have molecular weight which is less than 500Dal, so that it can able to penetrate throught the skin easily.

CHAPTER 3

AIM AND SCOPE

The aim of this work is to reduction of blood glucose level of the people with hyper glycemia, Reduction of blood glucose level by increasing the insulin secretion, From the components from the bitter melon and the route of administration is Trans dermal drug delivery system through foot sole. Without oral administration or insulin injection the insulin sensitivity will be increases. As the result reduced glucose level will be observed. And the pathway of the insulin secretion should be analyzed. Ligand from the bittergourd and the receptor protein and docking study should be analyzed.

OBJECTIVE

- To identify the route of administration.
- To done clinical trial for hyperglycemia and analyze the reduction of glucose level in particular time interval.
- Insulin secretion pathway analysis using reactome pathway analyzer.
- To identify specific receptor protein in the insulin pathway which is responsible for the insulin secretion.
- Compound analysis of bitter gourd and selection of ligand.
- Receptor-lignd docking study to evaluate the binding and simulation.

SCOPE

This study and clinical trial will reveal the anti diabetic components present in the bitter gourd and effectiveness of trans dermal drug delivery system, Mechanism of the route of administration.

CHAPTER 4

MATERIALS AND METHOD

4.1 TOOLS USED;

4.1.1 Auto dock;

AutoDock is molecular modeling simulation software. It is effective for protein-ligand docking. AutoDock 4 is available under the GNU General Public License. AutoDock is one of the most cited docking software applications in the research community

AutoDock Vina is a successor of AutoDock, significantly improved in terms of accuracy and performance. It is available under the Apache license.

Both AutoDock and Vina are maintained by Scripps Research, specifically the Center for Computational Structural Biology (CCSB) led by Dr. Arthur J. Olson.

AutoDock consists of two major programs:

- AutoDock for docking of the ligand to a set of grids describing the target protein;
- AutoGrid for pre-calculating these grids.

Usage of AutoDock has contributed to the discovery of many drugs, including HIV1 integrase inhibitors.

4.1.2 kegg

Abbreviation of keg is Kyoto Encyclopedia of Genes and Genomes, it is a collection of databases dealing with drugs, chemical substances, genomes, biological pathways, diseases KEGG is utilized for bioinformatics research and education purpose, In genomics, metagenomics and other omics studies, modeling and simulation in systems biology, and translational research in drug development.

Following information can be retrieved from the keg database

Systems information

- Pathway - pathway maps for cellular and organismal functions
- Module - modules or functional units of genes
- Brite - hierarchical classifications of biological entities

Genomic information

- Genome - complete genomes
- Gene - genes and proteins in the complete genomes
- Orthology - ortholog groups of genes in the complete genomes

Chemical information

- Compound glycon - chemical compounds and glycans
- Reaction, Rpair, Rclass - chemical reactions
- Enzyme - enzyme nomenclature

Health information

- Disease-human diseases
- Drug- approved drugs
- Environ - crude drugs and health-related substances

4.2.3 Reactome;

Reactome is a free online database of biological pathways. There are many type of Reactomes that concentrate on particular organisms, the most of these is focused on human biology, Expert biologists have developed the reactome, in collaboration with Reactome editorial staff who are all PhD level biologists. Content is cross-referenced to many bioinformatics databases. The rationale behind Reactome is to visually represent biological pathways in full mechanistic detail, while making the source data available in a computationally accessible format.

The website can be used to browse pathways. The whole data is fully available for download in following formats PDF, SBML and BioPAX.

4.2.4 *protein plus*;

Protein plus is a common software to create computational tools for structure based molecular model design. The primary aim of *ProteinsPlus* is to support life scientists who working with protein structures. Protein structures are major element for understanding the function of protein. By using the protein plus we can able to find the binding pocket the protein and protein ligand interaction and stability.

. They are an important resource in many biotechnological application areas from pharmaceutical research to biocatalysis. *ProteinsPlus* has its focus on protein-ligand interactions..

4.2.5 *pymol*;

Pymol is an easy-to-imagine software. It is an open source cellular system and was developed by Warren Lyford DeLano. It was sold by Schrodinger. PyMOL can produce high-quality 3D images of small molecules and biological macromolecules, for example proteins. According to the original author, nearly a quarter of all published images of 3D protein structures in science textbooks were made using PyMOL. PyMOL is one of the few open source modeling tools available for use in structural biology.

4.2.6 *Babel*;

Open Babel is a chemical toolbox designed to translate multiple languages of chemical data. It is an open project, which allows anyone to search, modify, analyze, or store data from molecular models, chemicals, solid state materials, biochemistry, or related fields. Table allows us to convert any type of file into pdb or pdbq, which can be used to work with auto dock or auto dock vina.

4.3 METHOD

4.3.1 Clinical trial method



Fig 4.1Thirty hyperglycemia patients were selected randomly in all age group

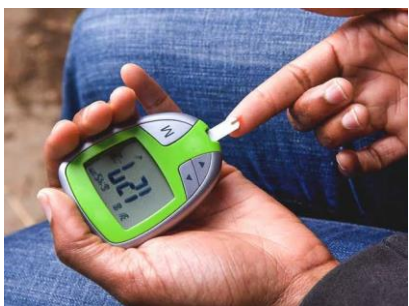


Fig 4.2 Day 1 blood glucose level test in fasting



Fig 4.3 Organic healthy bitter gourd were chosen and seeds were removed and chopped into pieces



Fig 4.4 Grind using motor and pistol until it gets slurry(No water should be added)



Fig 4.5 Take 300 ml of slurry and add it into 5 liter of warm water(98-105°F)



Fig 4.6 soak the patients foot into the warm water with bitter gourd slurry for 30 min



Fig 4.7 After the treatment over let the foot air dry.



Fig 4.8 Repeat the same for 2nd and 3rd day.



Fig 4.9 3rd day 30 min of post treatment check the blood glucose level.

4.3.2 Docking

Docking is a method which evaluates the position of inhibitor compounds with the receptor for prediction of binding energy of complex or the affinity of ligands towards the protein. Docking analysis has been done with the help of Discovery studio. Before that a grid box has to be set up around the binding site or the active site of the protein. It is an essential step to perform molecular docking of ligand and receptor. The two protein files were in .pdb format and placed in a folder named by the respective ligand. 30 compounds have been used for each protein, so folders have been created with giving the ligand names. Then the protein molecule was saved in .pdbqt format and so was for the ligand names also. A conf.txt file is also included in the folder. It has a certain format to give the input text. Now it was all set for docking. The docking is used to get information about protein ligand interactions, PLP (Piecewise Linear Potential) value and PMF (Potential of Mean Force) value. Retrieval of protein sequence from protein database. The proteins were collected from uniprot. These proteins are responsible to cause pneumonia. The proteins are collected along with their protein code, locus. Determination of the structure for the proteins. Homology modeling is also known as comparative modeling of the proteins which is a technique of constructing an unknown atomic resolution model of the target protein from its amino acid sequence and an experimental 3D structure of a related homologous protein (the template) which is done using an online tool swiss model.

4.3.2.1 Protein structure preparation;

Once the structure of the proteins is modeled it is further prepared for docking studies.

4.3.2.2 Energy minimization:

Energy minimization is done for the modeled proteins in order to obtain a stable conformer of the molecule using the tool smart minimize. The bad steric clashes are removed with 1000 steps at RMS gradient 0.1 and 0.03 respectively. Before that, the suitable force field CHARMM available with Accelrys life science software was applied.

4.3.2.3 Validation:

The validation and evaluation of the predicted protein structures is done using tool Mol probity and rampage. Here the structures are validated based on the ramachandran plot in which if the favored and the allowed regions are above ninety percent then the model is said to be a correct model which was modeled.

4.3.2.4 Ligand preparation;

The ligands are selected based on the anti diabetic activity towards their targets from Drug bank database and their analogs are collected from Pubchem. These ligands are used for docking studies and analyzed whether there are bounded to the active sites of their targets.

4.3.2.5 Autodock ;

Auto dock is a method which evaluates the position of inhibitor Compounds with the receptor for prediction of binding energy of complex or the affinity of liands towards the protein. Docking analysis has been done with the help of auto dock vina. First removal of water molecule should be taken place. And remove other inhibitor present in the protein, inorder to that add hetro atom and delete inhibitor, add hydrogen atom and add colmen charges and protein molecule is ready to dock and save it in PDBQT format the same folder where autodock.exe present. After this add target ligand and add hydrogen bonds and colmen charges. After this steps that a grid box has to be set up around the binding site or the active site of the protein. It is essential step to perform molecular docking of ligand and receptor. Generate GPF file and save it in the same folder. And run the grid. Result will be stored in the same folder. After running auto grid, run auto dock. And the result will be stored in the same folder. And analyze the result. And result can able to visualize through pymol. And by using the protein plus tool ligand interaction and binding pocket analyzed.

CHAPTER 5

RESULT

5.1 OUTPUT OF THE CLINICAL TRIAL

No	Patient	Age and sex	Glucose level DAY 1	Glucose level DAY2	Glucose levels DAY 3	Remark If any
1	Patient 1	49/M	178	151	<u>134</u>	Nil
2	Patient 2	53/F	289	261	<u>203</u>	Felt bitterness in throat
3	Patient 3	84/F	348	322	<u>257</u>	Felt bitterness in throat
4	Patient 4	55/M	234	224	<u>176</u>	Nil
5	Patient 5	42/F	203	175	<u>138</u>	Nil
6	Patient 6	24/M	186	153	105	Felt burning sensation in foot
7	Patient 7	58/M	219	185	132	Nil

8	Patient 8	33/F	151	122	97	Felt bitterness in throat
9	Patient 9	72/M	295	247	212	Felt bitterness in throat
10	Patient 10	29/M	149	112	86	Felt dizziness
11	Patient 11	65/F	246	197	146	NILL
12	Patient 12	33/M	198	166	123	NILL
13	Patient 13	37/M	231	213	171	NILL
14	Patient 14	52/M	188	157	132	Felt bitterness in throat
15	Patient 15	46/F	322	281	228	Felt burning in foot sole
16	Patient 16	26/M	224	194	153	NILL
17	Patient 17	34/F	145	119	98	Felt dizziness

18	Patient 18	37/F	193	185	153	NILL
19	Patient 19	78/M	221	198	164	NILL
20	Patient 20	44/M	182	167	131	NILL
21	Patient 21	68/F	258	231	197	Felt bitterness in throat
22	Patient 22	81/M	214	190	152	NILL
23	Patient 23	38/M	173	162	144	NILL
24	Patient 24	43/M	289	245	206	NILL
25	Patient 25	34/F	131	107	83	NILL
26	Patient 26	39/F	156	139	113	NILL
27	Patient 27	56/M	190	176	142	NILL
28	Patient 28	49/M	158	133	121	NILL

29	Patient 30	43/F	385	269	284	NILL
30	Patient 31	53/F	215	191	167	NILL

5.2. COMPONENTS IN BITTER GOURD

Many bioactive chemicals of *M. charantia* fruit carved in books, are classified as carbohydrates, proteins, lipids and more . *M. charantia* contains triterpenoids , saponins, polypeptides , flavonoids , alkaloids and sterols . Previous phytochemical studies have identified organisms and their related functions.

Though there is lots of components present in the bitter gourd and which can able to analysis using gas chromatography technique. But all the components present in the bitter gourd can't able to penetrate through the skin by trans dermal method. Skin allows the components which weights below 500 Dal. Thermally stable and small molecule which measures less than 500 Dal, and has anti diabetic property has been analyzed for further literature survey and docking study

- 1.Ursodeoxycholic acid-392.6 dalton
- 2.Syringaldehyde-182.17 dalton
- 3.Pentadecanoic acid-272.38 dalton

5.2.1 Ursodeoxycholic acid-392.6 dalton ;

- UDCA increases GLP-1 response to nutrient via provoking bile acid excretion from liver to intestine.
- UDCA improves glycemic control in people with type 2 diabetes.

5.2.1.1 Research

- Clinical trial strength-20 people

- Drug: UDCA
- UDCA for 12 weeks, then Sitagliptin add-on therapy for
- additional 12 weeks.
- UDCA dosage: dosing from 600 mg for initial 4 weeks. Then, if
- there is no adverse effect, UDCA is escalated to 900 mg.

5.2.1.2 Outcome;

- 1. Change from Baseline in Glucagon-like peptide-1 (GLP-1) response to lipid meal test (fat 55%) [Time Frame: 6 months]
- 2. Change from Baseline in energy expenditure [Time Frame: 6months]
- 3. Change from Baseline in fasting plasma glucose level

5.2.2 SYRINGALDEHYDE;

- Decrease of hyperglycemia by syringaldehyde in diabetic rats
- Diabetes induced rats were treated with syringaldehyde injection, and the result was observed that syringaldehyde has the ability to lower postprandial glucose, without the change of insulin level.
- However, the potential mechanisms for this action of syringaldehyde remain obscure

5.2.3 PENTADECANOIC ACID (C₁₅H₂₈O₄);

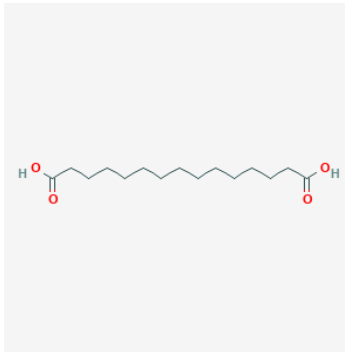


Fig 5.1 pentadecanoic acid structure

- Pentadecanoic acid is one of the free fatty acid ligand and will bind with FFAR1 which place a major role in the insulin secretion

pentadecanoic acid predominantly promotes glucose uptake and GLUT4 translocation to the plasma membrane. But pentadecanoic acid shows no effect on the insulin dependent pathway involving insulin receptor substrate Tyr632 and Akt, but it also increases the phosphorelation of AMPK and Akt of 160 of kDa (AS160).

5.2.3.1 Reactome pathway;

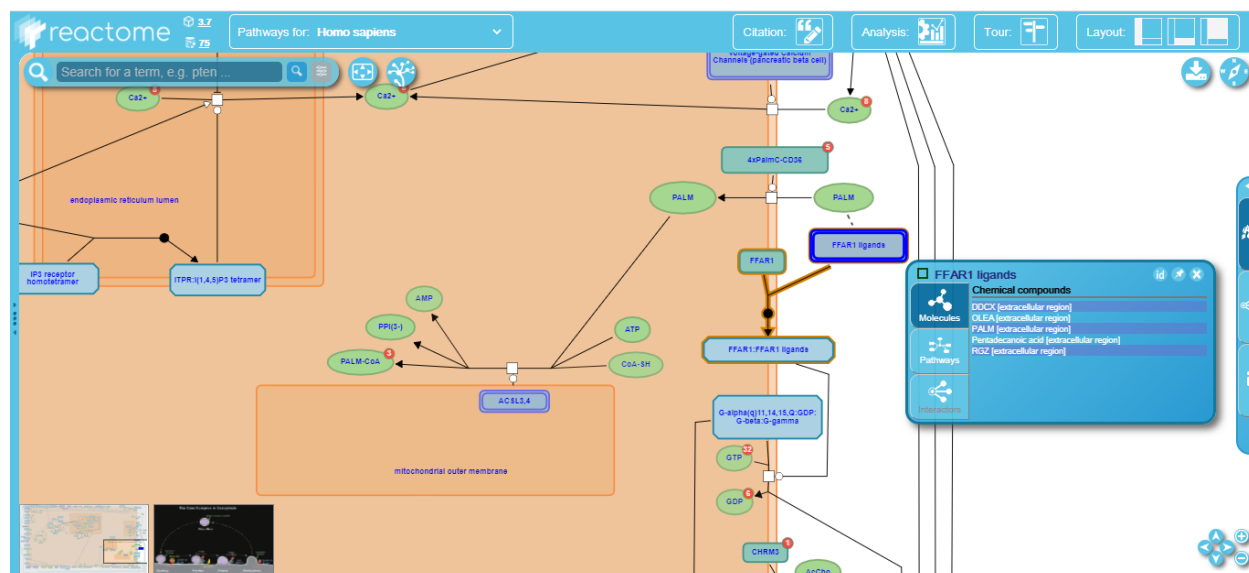


Fig 5.2 Insulin secretion reactom pathway

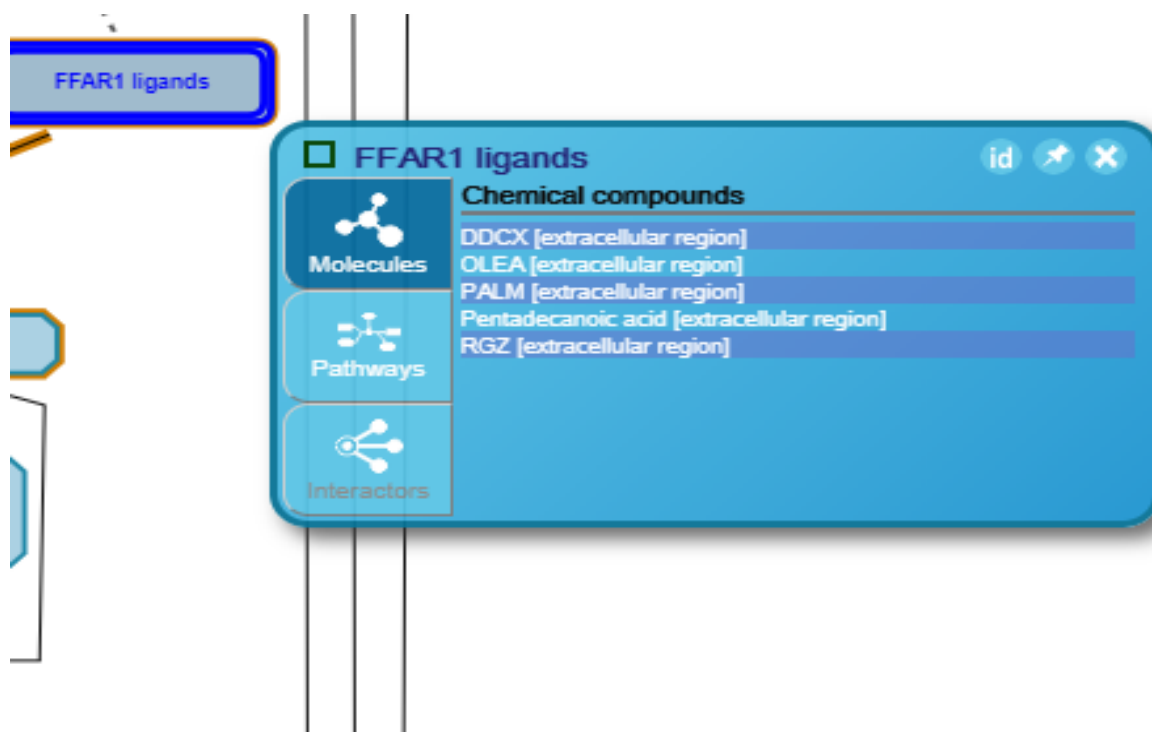


Fig 5.3 Pentadecanoic acid

5.2.3.2 Mol property of pentadecanoic acid

miLogP	4.90
TPSA	74.60
natoms	19
MW	272.38
nON	4
nOHNH	2
nviolations	0
nrotb	14
volume	285.06

5.2.3.3 Docking result;

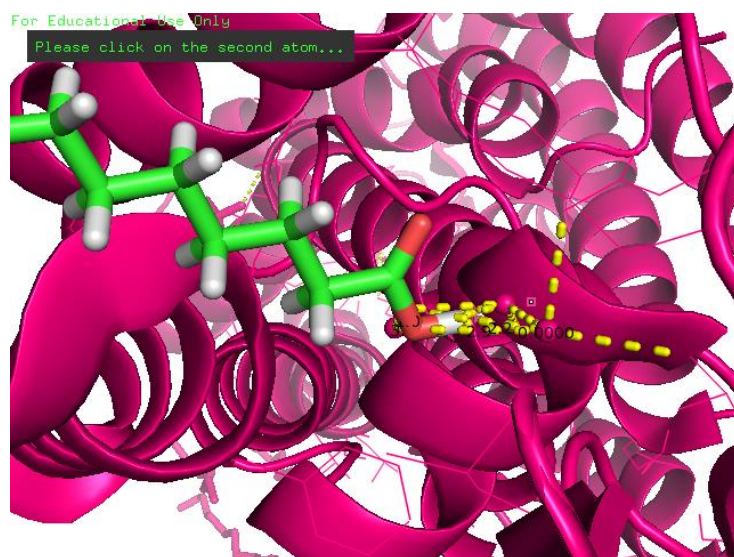


Fig 5.4 Docking result of 4phu protein and pentadecanoic acid

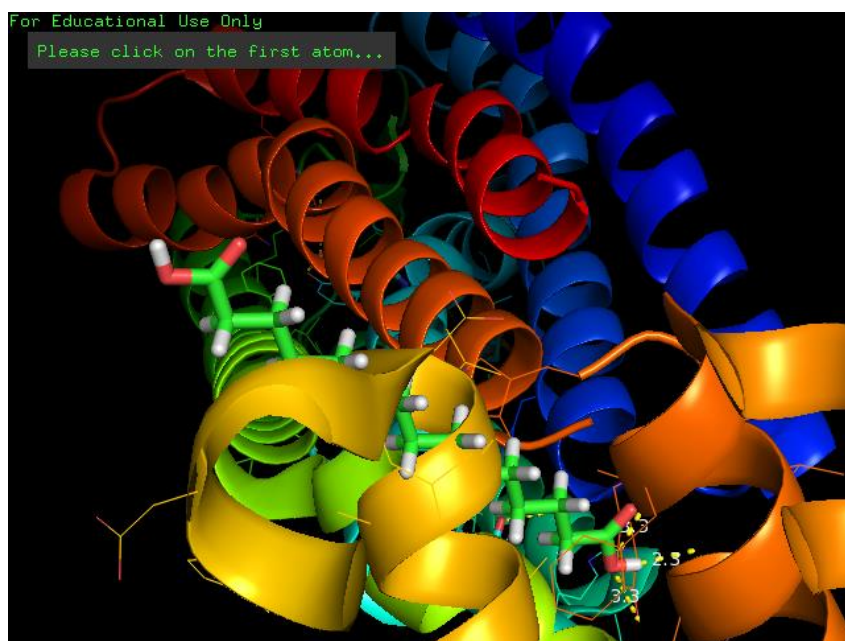
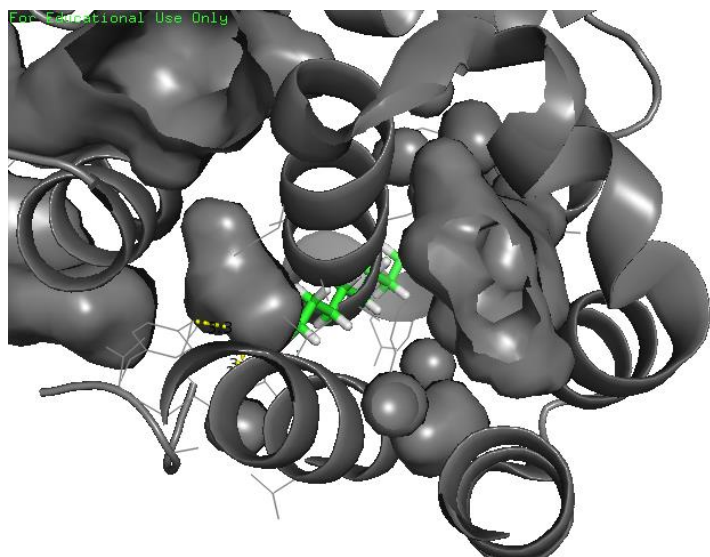


Fig 5.5 Docking result of 5tzy protein and pentadecanoic acid

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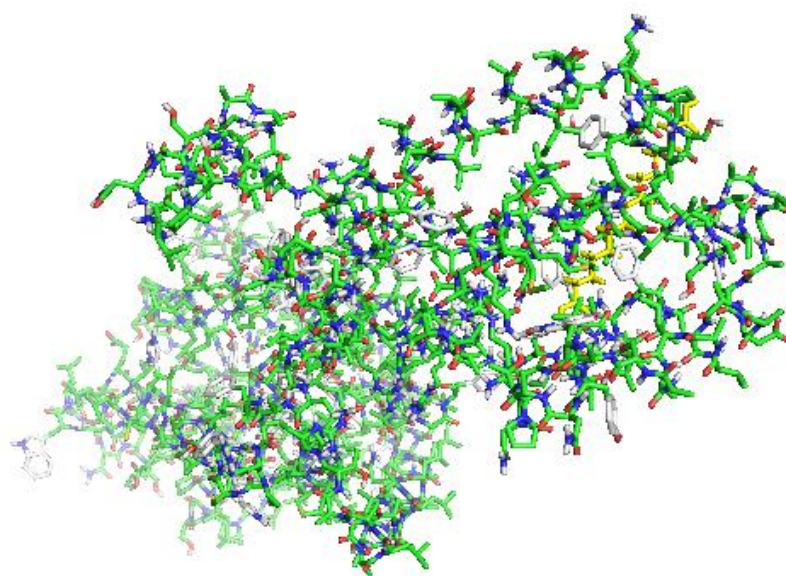


Fig 5.6 pymol result 4phu

5.2.3.4 protein plus result

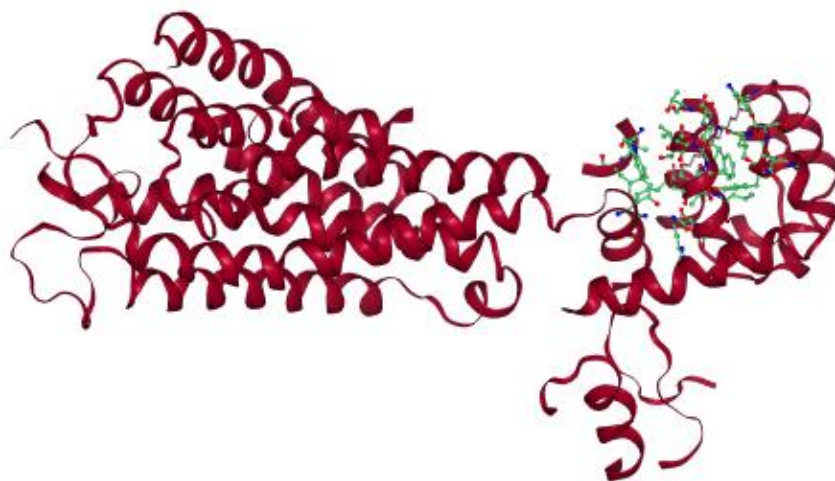


Fig 5.7 Protein plus result of 4phu and pendadecanoic acid and its binding pocket

DISCUSSION

Diabetes mellitus (hyperglycemia) commonly known as high blood sugar which cause due to either the production of insulin not enough or the the body cells not responding properly to the insulin produced by the pancreas. From the report of WHO, About 422 million people worldwide have diabetes, the majority living in low-and middle-income countries, and 1.6 million deaths are directly attributed to diabetes each year. *Momordica charantia* (*M. charantia*), commonly referred to as bitter melon is well-known for its anti-diabetic amongst the indigenous populations of Asia, South America, India and East Africa. By using the *Momordica charantia* for diabetes, certain animal studies and human studies have shown a hypoglycemic effect. Bitter melon is rich in triterpene, proteid, steroid, alkaloid, inorganic, lipid, and phenolic, which are responsible for the antidiabetic effects (*Raman B.V 2012*)

Herbal remedies have been used cheaply around the world to treat diabetes. In fact, in many parts of the world, especially in poor countries, this may be the only available treatment for diabetics. There are several reviews by various authors about herbal remedies for diabetes (Joseph 2015)

The main components of the bitter melon that counteract the effects of diabetes are triterpene, proteid, steroids, alkaloids, inorganic, lipid, and phenolic compounds (*Saeed MK, Shahzadi I, Ahmad I 2010*). Several glycosides are separated from the M stem. *Charantia* fruit also collected under the category of cucurbitane-type triterpenoids (*Chang CI, Chen CR, Liao YW, Cheng HL, 2008*) . In particular, the four triterpenoids have a function modified by AMP-protein kinase which is a hypoglycaemic-mediated mechanism of action of bitter melon (*Tan MJ, Ye JM, Turner N, Hohnen-Behrens C, 2008*)

Skin absorption (lateral, skin) carries chemicals from the skin surface to the skin and blood circulation. Skin absorption is related to the level of exposure and the potential effect of an organism through the skin. Human skin encounters many agents intentionally and unintentionally. Skin absorption may be the result of exposure to the skin at work, in the environment or at the consumer's exposure to chemicals, cosmetics, or pharmaceutical products. Some chemicals can be absorbed in sufficient quantities to

create adverse effects on the system. Dermatitis is considered one of the most common occupational diseases. To test whether the chemical can be dangerous it can cause dermatitis or other more systematic effect (*Dang, 1996*) there are several anti diabetic components present in the bitter gourd, for this study we have taken syrinjaldehyde, pendadecanoic acid, Ursolic acid. Because only these 3 components have molecular weight which is less than 500Dal, so that it can able to penetrate throught the skin easily. Pendadecanoic acid were given to diabetes patients through trans dermal drug delivery system. This extracellular acid goes and binds to the free fatty acid receptor and induce the insulin secretion, due to excess insulin the blood glucose level will be normalize in hyperglycemia patients.

CHAPTER 6

CONCLUSION

Outcome of the clinical trial was positive and reduced glucose level were observed in all the patients, but in various level. It showed better result in the elder people who is above 50. The continuous usage of the bittergourd treatment may lead to hypoglycemia. It is recommended to use 3 days with at least 7 days gap in between. The pathway of the insulin secretion mechanism have been proven through protein ligand docking and binding pocket were analyzed by protein plus software. The final product were obtained in the powdered form, followed by sundry and grinding.

SUMMARY

Majority of world population, nearly one in ten were affected by diabetes. There is two type of diabetes, hyperglycemia and hypoglycemia. Hyperglycemia is further divided into type 1 and 2 diabetes. In this research we concentrate on type 1 diabetes, which is inability to secrete insulin by organ. By using the trans dermal drug delivery system drug will be delivered, the traditional way of delivering the drug will be orally or taking insulin directly under the skin. The new way of trans dermal treatment will be very efficient and painless approach to treat diabetes patients. Bitter gourd is wildlly known for its anti diabetes property. When it was exposed to patients foot sole, the anti diabetic component (pentadecanoic acid) which has lesser molecular weight travels thought the blood circulation, goes and binds to the free fatty acid receptor. And this complex induce the insulin secretion in patient's body. The protein ligand bonding were analyzed using auto dock viana and protein plus. Clinical trial was taken place for 30 hyperglycemic patients, result shows the gradual decrease in the blood glucose level. The final product were obtained in the powdered form by drying and grinding the bitter gourd. And it shows significant result in the reduction of blood glucose level in patients.

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