ISOLATION, CHARACTERIZATION AND EVALUATION OF ANTIDIABETIC ACTIVITY ON LEAVES OF *Merremia hederacea* (BURM. F.) HALLIER F.

A dissertation submitted to

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Submitted by

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COLLEGE OF PHARMACY

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TAMIL NADU

CERTIFICATE

This is to certify that the dissertation entitled **"ISOLATION, CHARACTERIZATION AND EVALUATION OF ANTIDIABETIC ACTIVITY ON LEAVES OF** *MERREMIA HEDERACEA* **(BURM. F.) HALLIER F."** submitted by **YUVARAJ.B, Reg. No: 261715709** to The Tamil Nadu Dr. M.G.R. Medical University, examination is evaluated.

EXAMINERS

1.

2.

Place: Chennai-03

Date

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INDEX

INTRODUCTION

DIABETES MELLITUS

Diabetes mellitus is a disorder that affects the body's ability to make or use insulin. Insulin is a hormone produced in the pancreas that helps transport glucose (blood sugar) from the bloodstream into the cells so they can break it down and use it for fuel. People cannot live without insulin. Diabetes results in abnormal levels of glucose in the bloodstream. This can cause severe short-term and long- term consequences ranging from brain damage to amputations and heart disease.

Diabetes mellitus is a clinical syndrome characterized by inappropriate hyperglycaemia caused by a relative or absolute deficiency of insulin or by a resistance to the action of insulin at the cellular level. It is the most common endocrine disorder, affecting 16 million individuals in the United States and as many as 200 million worldwide. The primary defect in fuel metabolism results in widespread, multi-organ complications that ultimately encompass virtually every system of the body and every specialty of medicine. It has been said that to know diabetes is to know medicine and health care. Although from a clinical standpoint this may be true, our increasing knowledge of the pathophysiology of the syndrome, together with the mechanisms of long- term complications, has placed diabetes research at the frontier of immunology and molecular biology.[1]

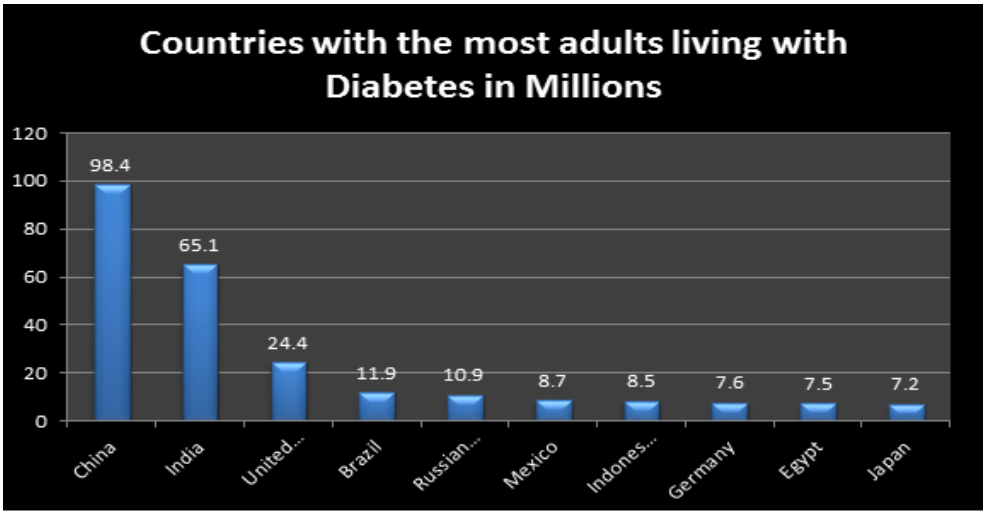


Fig. no: 1 Statistics of different countries living with diabetes

ROOT CAUSES OF DIABETES MELLITUS (DM)

The root causes of diabetes are complex. Most cases begin with one of two processes:

Metabolic: Unhealthy lifestyle factors such as over- eating, physical inactivity and obesity can impair the body's ability to use insulin. This is called insulin resistance. Uncontrollable risk factors including genetics, family history and age can also be involved. Metabolic forms of diabetes include:

Type 2 diabetes: This accounts for 90 - 95% of diabetic cases, according to the U.S. National Institutes of Health (NIH). Some of these patients have had prediabetes that went uncontrolled. Once considered a disease of middle and old age, type 2 is also becoming more common in youths as the incidence of childhood obesity grows.

Gestational diabetes: Hormonal changes contribute to this condition which can develop in any previously non- diabetic woman during pregnancy, especially those who are overweight.

AUTOIMMUNE

The body's immune system can mistakenly destroy the insulin-producing beta cells of the pancreas. The causes of autoimmune diabetes are poorly understood, but genetics and family history play a role, and viruses or other environmental factors are believed to figure in. Autoimmune forms of diabetes include:

Type 1 diabetes: Formerly known as juvenile diabetes, this form generally develops in children and young adults.

Latent autoimmune diabetes of adulthood: This variation of type 1 can occur later in life. Individuals with auto- immune diabetes who overeat, are sedentary, gain weight or have certain genes can, like people with metabolic forms of diabetes, develop insulin resistance. This state is known as double diabetes.

Diabetes can also result from another disease, such as pancreatitis, or even from a medical treatment, including pancreatectomy (surgical removal of the pancreas) or certain medications. This is known as secondary diabetes. In addition, there are uncommon inherited disorders that cause diabetes, such as maturity-onset diabetes of the young and Wolfram syndrome. Most cases of diabetes last the rest of a person's life. However, gestational diabetes generally ends when the pregnancy does, and some cases of secondary diabetes are also temporary [2].

FACTORS CONTRIBUTE IN DIABETES

Diabetes involves chronic levels of abnormally high glucose (hyperglycemia). Many patients, especially those with type 2 diabetes, also have elevated blood pressure (hypertension), chronic high levels of insulin (hyperinsulinemia) and unhealthy levels of cholesterol and other blood fats (hyperlipidemia).[3,4] All of these factors contribute to the long-term complications of diabetes, which include:

Vascular disease (diabetic angiopathy), atherosclerosis, heart conditions and stroke: These cardiovascular disorders are the leading cause of death in people with diabetes.

Kidney disease (diabetic nephropathy): Diabetes is the chief cause of end-stage renal disease, which requires treatment with dialysis or a kidney transplant.

Eye diseases: These include diabetic retinopathy, glaucoma and cataracts. Diabetes is a leading cause of visual impairment and blindness.

Nerve damage (diabetic neuropathy): This includes peripheral neuropathy, which often causes pain or numbness in the limbs, and autonomic neuropathy, which can impede digestion (gastroparesis) and contribute to sexual dysfunction and incontinence. Neuropathy may also impair hearing and other senses.

Infections and wounds: Foot conditions and skin disorders, such as ulcers, make diabetes the leading cause of nontraumatic foot and leg amputations. People with diabetes are also prone to infections including periodontal disease, thrush, urinary tract infections and yeast infections.

Cancer: Diabetes increases the risk of malignant tumors in the colon, pancreas, liver and several other organs.

Musculoskeletal disorders: Conditions ranging from gout to osteoporosis to restless legs syndrome to myofascial pain syndrome are more common in diabetic patients than nondiabetics.

Pregnancy complications: Diabetes increases the risk of preeclampsia, miscarriage, stillbirth and birth defects.

Emotional difficulties: Many but not all of the studies exploring connections between diabetes and mental illness have found increased rates of depression, anxiety and other psychological disorders in diabetic patients. In addition to chronic hyperglycemia, diabetic patients can experience acute episodes of hyperglycemia as

well as hypoglycemia (low glucose). Severe cases can cause seizures, brain damage and a potentially fatal diabetic coma. Acute glucose emergencies include:

Diabetic ketoacidosis: A lack of insulin can force the body to burn fats instead of glucose for energy. The result is a toxic byproduct called ketones, along with severe hyperglycemia.

Hyperosmolar hyperglycemic nonketotic state: This involves severe hyperglycemia and dehydration.

These dangerous glucose complications are most common in patients with unstable diabetes, but they can develop even in individuals who do not realize they have diabetes. About one-third of the estimated 20.8 million Americans with diabetes have not yet been diagnosed, according to the U.S. Centers for Disease Control and Prevention.

INCIDENCE OF DIABETES MELLITUS

The incidence of diabetes has soared worldwide in recent years and is expected to keep growing, with the greatest increase seen in metabolic forms of diabetes, notably type 2. This is blamed largely on the rise of obesity and the global spread of Western-style habits: physical inactivity along with a diet that is high in calories, processed carbohydrates and saturated fats and insufficient in fiber- rich whole foods. The aging of the population is also a factor. However, other factors, such as environment may also be contributing, because cases of autoimmune diabetes (type 1) are also becoming more common.

The estimated number of people with diabetes has jumped from 30 million in 1985 to 150 million in 2000 and then to 246 million in 2007, according to the International Diabetes Federation. It expects this number to hit 380 million by 2025. Seven percent of Americans have diabetes, according to the CDC, which predicts that one in three Americans born in 2000 will eventually become diabetic. Health agencies are warning that diabetes is a becoming an unprecedented epidemic even as other major diseases including cancer and nondiabetic heart disease are being controlled [5].

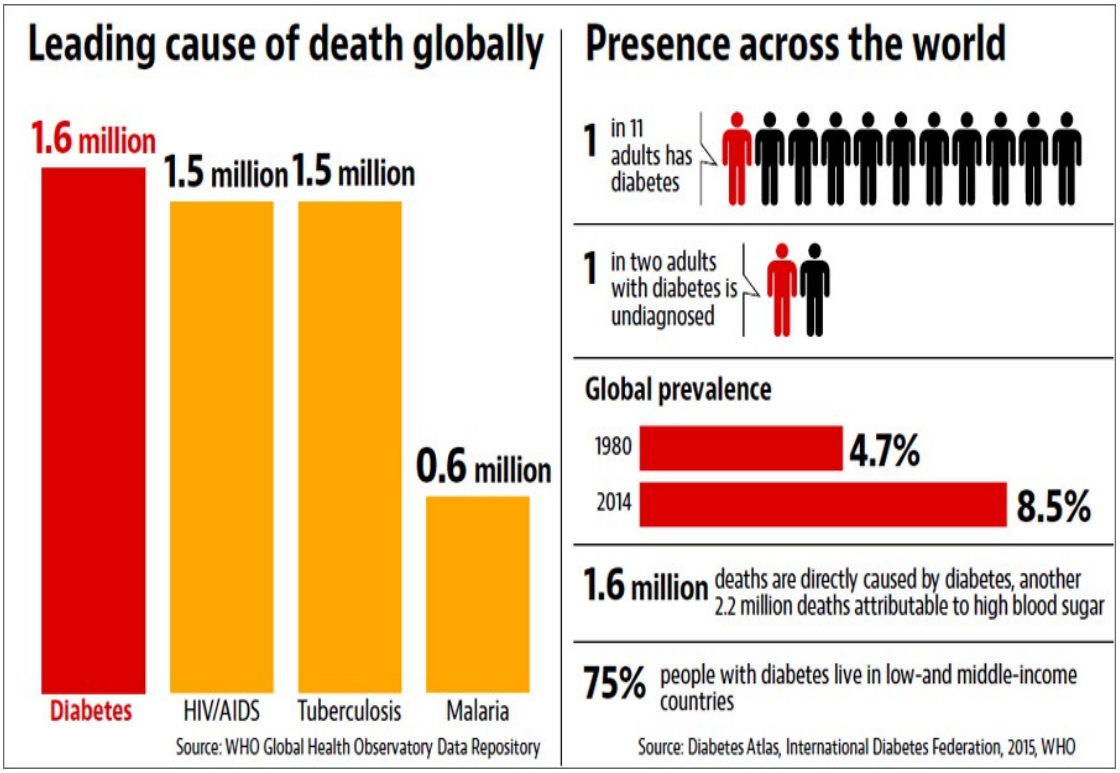


Fig. no: 2 Global cause of death across the world.

TYPES AND DIFFERENCES OF DIABETES

There are several forms of diabetes. Scientists are still defining and categorizing some of these variations and establishing their prevalence in the population. Types of diabetes include:

Type 1 diabetes: An autoimmune disease in which the immune system mistakenly destroys the insulin-making beta cells of the pancreas. It typically develops more quickly than other forms of diabetes. It is usually diagnosed in children and adolescents, and sometimes in young adults. To survive, patients must administer insulin medication regularly.

Type 1diabetes used to be called juvenile diabetes and insulin-dependent diabetes mellitus (IDDM). However, those terms are not accurate because children can develop other forms of diabetes, adults sometimes develop type 1, and other forms of diabetes can require insulin therapy.

A variation of type 1 that develops later in life, usually after age 30, is called latent autoimmune diabetes of adulthood (LADA).

Sometimes patients with autoimmune diabetes develop insulin resistance because of weight gain or genetic factors. This condition is known as double diabetes.

Type 2 diabetes: A disorder of metabolism, usually involving excess weight and insulin resistance. In these patients, the pancreas makes insulin initially, but the body has trouble using this glucose-controlling hormone. Eventually the pancreas cannot produce enough insulin to respond to the body's need for it. Type 2 diabetes is by far the most common form of diabetes, accounting for 85 to 95% of cases in developed nations and an even higher percentage in developing nations, according to the International Diabetes Federation.

This disease may take years or decades to develop. It is usually preceded by prediabetes, in which levels of glucose (blood sugar) are above normal but not high enough yet for a diagnosis of diabetes. People with pre- diabetes can often delay or prevent the escalation to type 2 diabetes by losing weight through improvements in exercise and diet, as the Diabetes Prevention Program and other research projects have demonstrated.

Type 2 diabetes used to be called adult-onset diabetes and non-insulin-dependent diabetes mellitus (NIDDM). Those terms are not accurate because children can also develop this disease, and some patients require insulin therapy.

Gestational diabetes: A temporary metabolic disorder that any previously nondiabetic woman can develop during pregnancy, usually the third trimester. Hormonal changes contribute to this disease, along with excess weight and family history of diabetes. About 4% of pregnant women develop gestational diabetes, according to the American Diabetes Association.

Gestational diabetes can cause problems for the mother and baby, including preeclampsia, premature delivery, macrosomia (oversized infant), and jaundice and breathing difficulties in the infant. This disease typically ends when the pregnancy does, but it increases the risk of type 2 diabetes later in life for the mother and the child.

Secondary diabetes: Diabetes caused by another condition. The many potential sources of secondary diabetes range from diseases such as pancreatitis, cystic fibrosis, Down syndrome and hemochromatosis to medical treatments including corticosteroids, other immune suppre-ssives, diuretics and pancreatectomy.

Maturity onset diabetes of the young (MODY). An uncommon disease caused by a genetic defect inherited from a parent. It is usually diagnosed before age 25 in people of normal weight. MODY is sometimes classified as a form of type 2 or secondary diabetes but is often considered a separate condition [6].

RISK FACTORS AND CAUSES OF DIABETES

The causes of diabetes are complex and only partly understood. This disease is generally considered multi- factorial, involving several predisposing conditions and risk factors. In many cases genetics, habits and environment may all contribute to a person's diabetes.

To complicate matters, there can be contrary risk factors for the various forms of the disease. For example, autoimmune diabetes (type 1 and latent autoimmune diabetes of adulthood, LADA) is more common in white people, but metabolic diabetes (type 2 and gestational diabetes) is more common in people of other races and ethnicities. Type 1 is usually diagnosed in children, but advancing age is a risk factor for type 2 and gestational diabetes. Insulin resistance, prediabetes and metabolic syndrome are strong risk factors for type 2 diabetes. Other diabetic risk factors and causes include:

Genetics and family history: Certain genes are known to cause maturity-onset diabetes of the young (MODY) and Wolfram syndrome. Genes also contribute to other forms of diabetes, including types 1 and 2.

Family medical history is also influential to varying degrees:
For example, a person whose parents both have type 1 diabetes has a 10 to 25% chance of developing that disease, according to the American Diabetes Association, and someone whose parents both have type 2 diabetes has a 50% chance of developing that disease.

Weight and body type: Overweight and obesity are leading factors in type 2 diabetes and gestational diabetes. Excess fat, especially around the abdomen (central obesity), promotes insulin resistance and metabolic syndrome [7].

SIGNS AND SYMPTOMS OF DIABETES

Diabetes often goes undetected because symptoms can be attributed to many other causes and some patients experience no symptoms or fail to heed warning signs. Possible indicators of diabetes include [8]:

- Excessive thirst (polydipsia)
- Excessive urination (polyuria) and dehydration
- Excessive hunger or appetite (polyphagia)
- Unexplained weight loss
- Blurred vision, nearsightedness or other vision problems
- Frequent infections, including skin infections, thrush, gingivitis, urinary tract infections and yeast infections, Slow healing of sores, skin problems, such as itchiness or acanthosis nigricans
- Fatigue, lethargy or drowsiness
- Shakiness or trembling
- Mood swings or irritability
- Dizziness or fainting , Numbness, tingling or pain in the feet, legs or hands

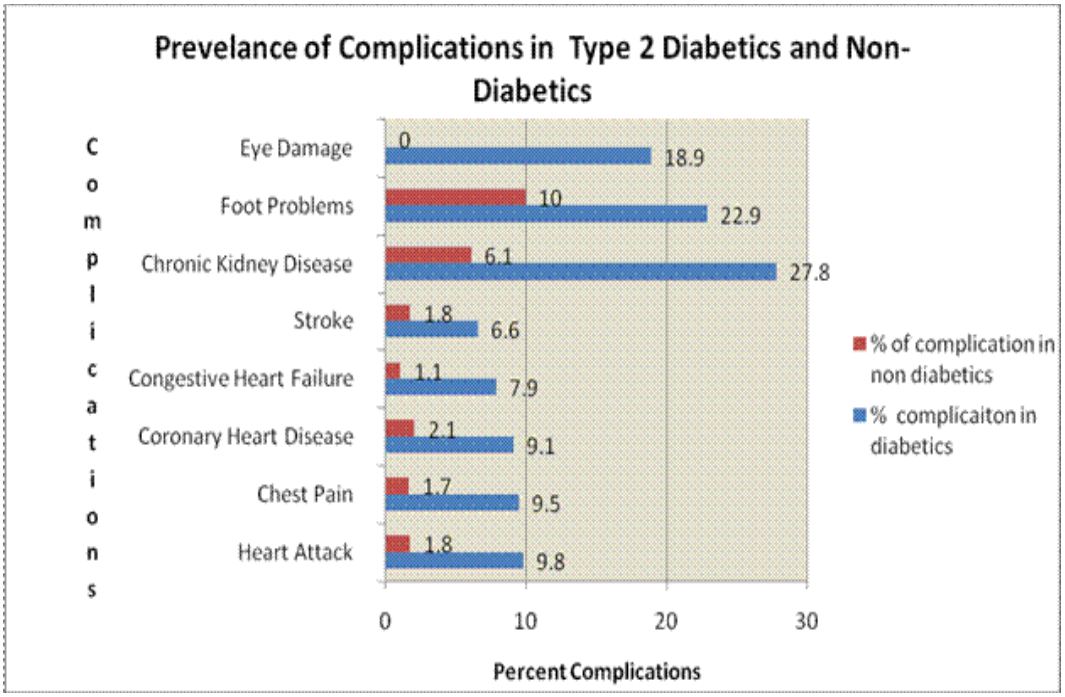


Fig. no: 3 Complications of Type 2 diabetes

CURRENT MANAGEMENT OF TYPE 2 DIABETES

The pharmacological management of type 2 diabetes is based on altering the effects of the ominous octet that lead to hyperglycaemia. The eight pathophysiological mechanisms include reduced insulin secretion from pancreatic β cells, increased glucagon secretion from pancreatic α cells, increased hepatic glucose production, neurotransmitter dysfunction and insulin resistance in the brain, increased lipolysis, increased renal glucose reabsorption, reduced incretin effect in the small intestine and reduced glucose uptake in peripheral tissues such as skeletal muscle, liver and adipose tissue. Currently available glucose-lowering therapies target one or more of these key components [9].

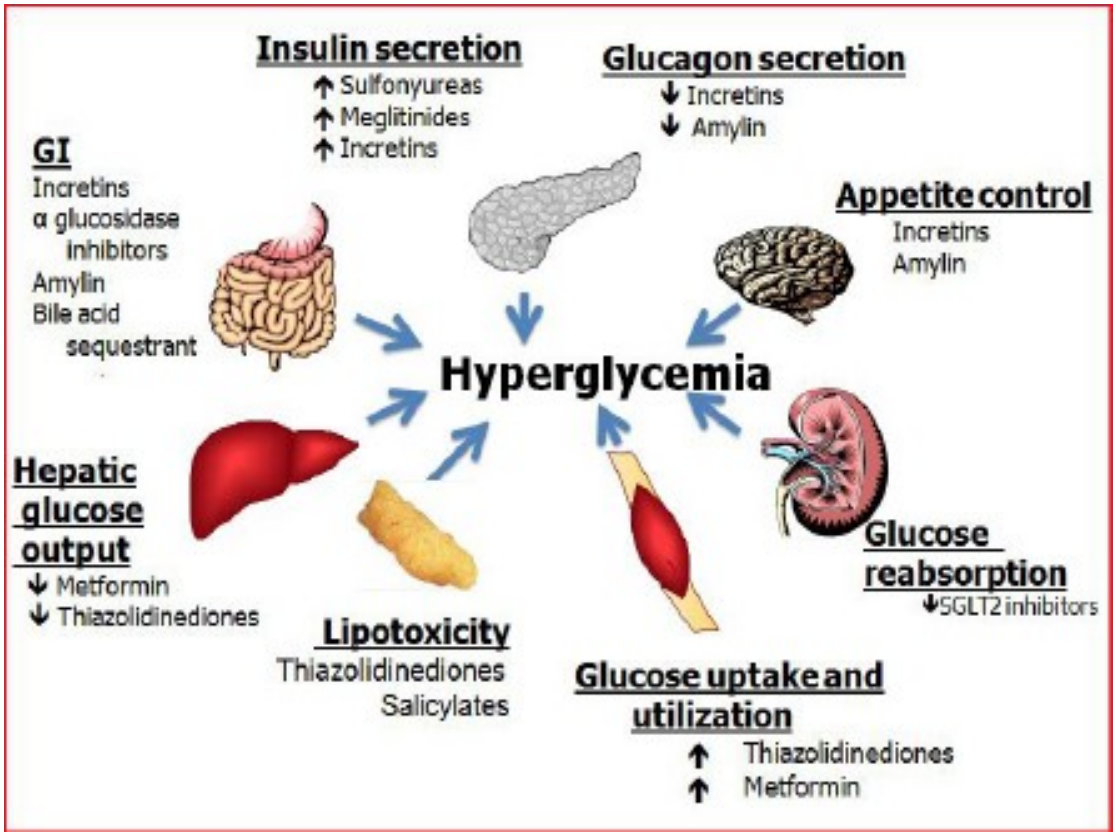


Fig. no: 4 Mechanism and site of action of synthetic drugs

ROLE OF MEDICINAL PLANTS IN DIABETES MELLITUS

In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects.. In Indian systems of medicine most practitioners formulate and dispense their own recipes. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world.

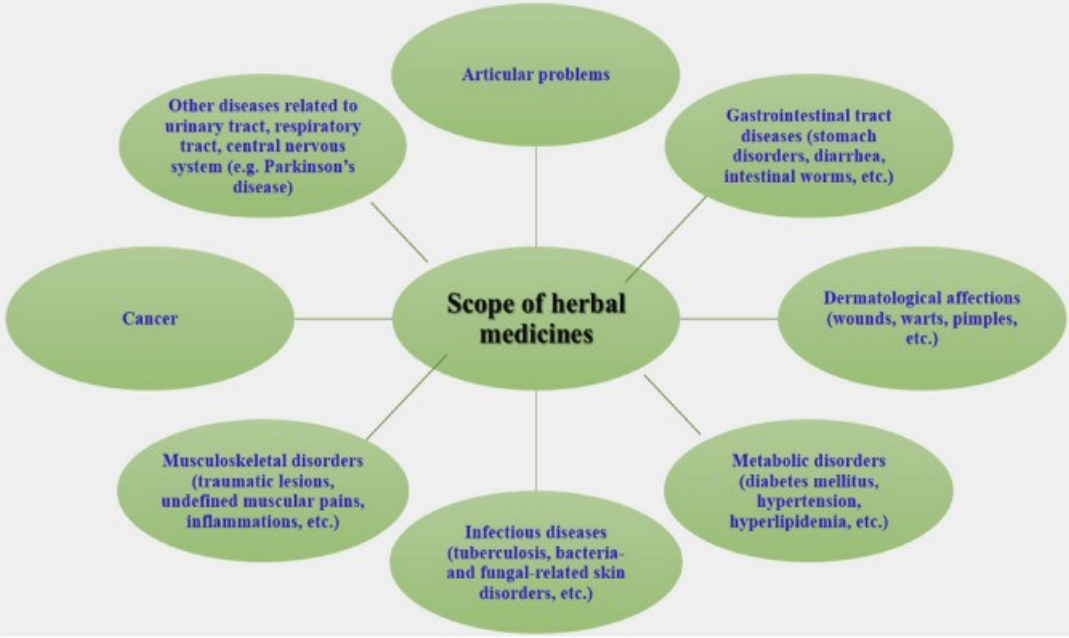


Fig. no: 5 Scope of Herbal Medicines against various diseases.

Medicinal plants are being looked up once again for the treatment of diabetes. Many conventional drugs have been derived from prototypic molecules in medicinal plants. Metformin exemplifies an efficacious oral glucose-lowering agent. Its development was based on the use of *Galega officinalis* to treat diabetes. *Galega officinalis* is rich in guanidine, the hypoglycemic component. Because guanidine is too toxic for clinical use, the alkyl biguanides synthalin A and synthalin B were introduced as oral anti-diabetic agents in Europe in the 1920s but were discontinued after insulin became more widely available. However, experience with guanidine and biguanides prompted the development of metformin. To date, over 400 traditional plant treatments for diabetes have been reported, although only a small number of these have received

scientific and medical evaluation to assess their efficacy. The hypoglycemic effect of some herbal extracts has been confirmed in human and animal models of type 2 diabetes. The World Health Organization Expert Committee on diabetes has recommended that traditional medicinal herbs be further investigated [10].

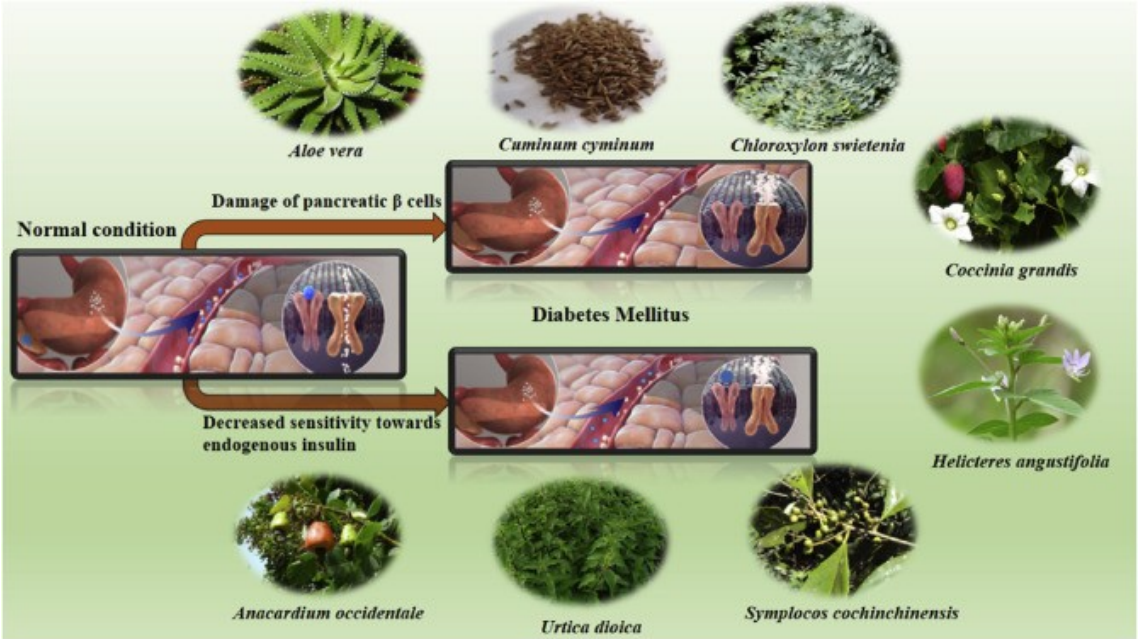


Fig. no: 6 Medicinal plants with Antidiabetic activity

Table no. 1. Indian medicinal plants with antidiabetic and related beneficial properties

Medicinal Plants	Ayurvedic/common name	Antidiabetic and other beneficial effects in traditional medicine
Annona squamosa	Sugar apple	Hypoglycemic and antihyperglycemic activities of ethanolic leaf-extract, Increased plasma insulin level[11, 12.]
Artemisia pallens	Davana	Hypoglycemic, increases peripheral glucose utilization or inhibits glucose reabsorption [13].
Areca catechu	Supari	Hypoglycemic [14]
Beta vulgaris	Chukkander	Increases glucose tolerance in OGTT [15]

Boerhavia diffusa	Punarnava	Increase in hexokinase activity,decrease in glucose-6-phosphatase and fructose bis-phosphatase activity, increase plasma insulin level, antioxidant
Bombax ceiba	Semul	Hypoglycemic [16]
Butea monosperma	Palasa	Antihyperglycemic [17]
Camellia sinensis	Tea	Anti-hyperglycemic activity, antioxidant
Capparis decidua	Karir or Pinju	Hypoglycemic, antioxidant, hypolipidaemic
Caesalpinia bonducella	Sagarghota, Fevernut	Hypoglycemic, insulin secretagogue, hypolipidemic [18,19]
Coccinia indica	Bimb or Kanturi	Hypoglycemic
Emblica officinalis	Amla, Dhatriphala	"Triphala" Decreases lipid peroxidation, antioxidant, hypoglycemic
Enicostema littorale	Krimihrita	Increase hexokinase activity, Decrease glucose 6-phosphatase and fructose 1,6 bisphosphatase activity. Dose dependent hypoglycemic activity
Ficus bengalenesis	Bur	Hypoglycemic, antioxidant
Gymnema sylvestre	Gudmar or Merasingi	Anti-hyperglycemic effect, hypolipidemic
Hemidesmus indicus	Anantamul	Anti snake venom activity, anti-inflammatory
Hibiscus rosasinesis	Gudhal or Jasson	Initiates insulin release from pancreatic beta cells Ipomoea batatas Sakkargand Reduces insulin resistance
Momordica cymbalaria	Kadavanchi	Hypoglycemic, hypolipidemic [20]
Musa sapientum	Banana	Antihyperglycemic, antioxidant [21,22]

Phaseolus vulgaris	Hulga, white kidney bean	Hypoglycemic, hypolipidemic, inhibit alpha amylase activity, antioxidant. Altered level of insulin receptor and GLUT-4 mRNA in skeletal muscle [23]
Salacia reticulata	Vairi	Inhibitory activity against sucrase, α-glucosidase inhibitor [24]
Scoparia dulcis	Sweet broomweed	Insulin- secretagogue activity, antihyperlipidemic, hypoglycemic, antioxidant [25,26,27]
Swertia chirayita	Chirata	Stimulates insulin release from islets [28,29]
Syzygium alternifolium	Shahajire	Hypoglycemic and antihyperglycemic
Terminalia belerica	Behada	constituent of "Triphala" Antibacterial, hypoglycemic
Vinca rosea	Sadabahar	Anti-hyperglycemic [30]
Withania somnifera	Ashvagandhawinter cherry	Hypoglycemic, diuretic and hypocholesterolemic [31]

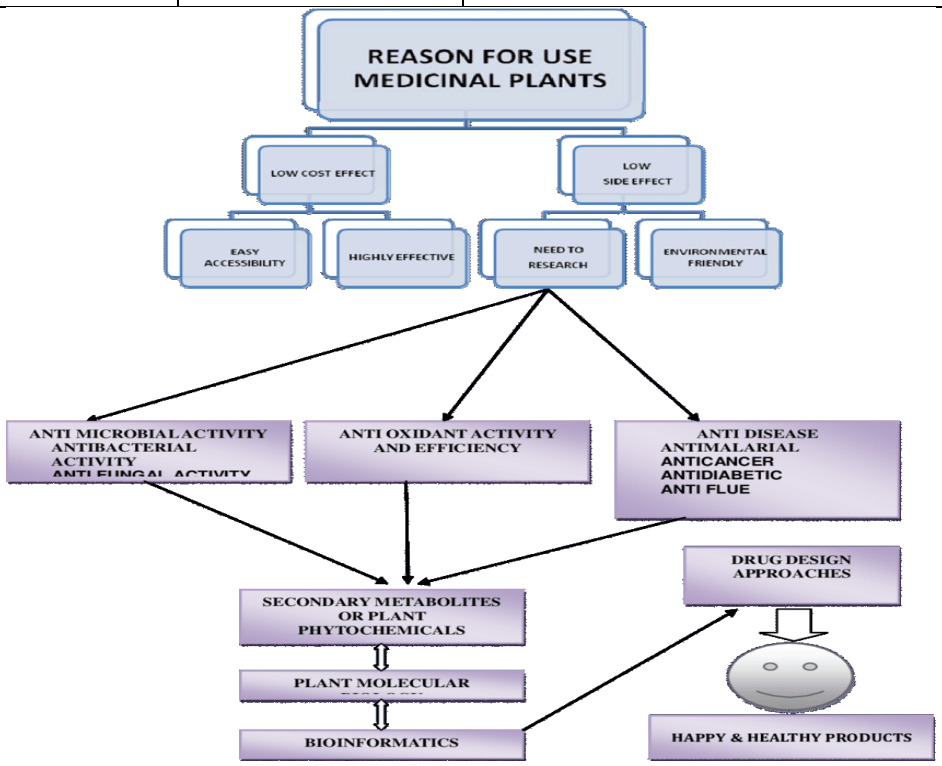


Fig. no: 7 An approach of Medicinal Plants in Bio informatics and Drug Design

NATURAL PRODUCT DRUG DISCOVERY

Current challenges to the use of natural products and difficulty in accepting their therapeutic efficacy include:

(1) Lack of standardization procedures (2) Lack of isolation of pure chemical products or compounds (3) Lack of elucidation of biological mechanisms and rarely undergoing so-called controlled documented clinical trials according to "standards". Historically, there is scientific evidence on the therapeutic efficacy of natural products and as previously mentioned this led to development of some blockbuster conventional medicines. Searching for new drug candidates from natural products is often made difficult by the complexity of the molecular mixtures. The therapeutic activity of plant extracts is usually because of the synergistic and simultaneous action of several chemicals. Given the complex nature of many diseases including cancer and degenerative diseases, it is not surprising that the reliance on single compound-based drug discovery has failed to provide effective cures.

Plant-based drug discovery therefore must start with a combinatorial approach when evaluating candidate compounds. The advent of novel technologies including quantum computing, profiling techniques, computational biology techniques, big data, microfluidics and artificial intelligence will enable scientists to use a combinatorial approach to harness the therapeutic properties of plant-based natural products and simultaneously study their molecular effects in physiological conditions.

It is however possible that not all components of plant extracts have measurable effects. It has been suggested that one way to improve screening and simplify extracts is through the removal of possible interfering components such as polyphenolic tannins. There are several reported innovative strategies which can be used to achieve this and these include pre-fractionation and extraction methods such as semi-bionic extraction, supercritical fluid extraction, microwave-assisted, ultrasonic-assisted and enzyme-assisted extraction, molecular distillation methods and membrane separation technology can be used to extract natural compounds efficiently from plants. These extractions strategies have been shown to have similar simulation to traditional methods allowing the extraction process to get most compounds from the natural product [32].

Despite the presence of anti-diabetic drugs in the pharmaceutical market, the treatment of diabetes with medicinal plants is often successful. Herbal medicines and plant components with insignificant toxicity and no side effects are notable therapeutic options for the treatment of this disease around the world [33]. The most common herbal active ingredients used in treating diabetes are flavonoids, tannins, phenolic, and alkaloids [34]. Tannins improves the function of pancreatic Beta-cells and increases insulin secretion. Quercetin is an antioxidant that acts in several mechanisms related with the removal of oxygen radicals, so prevents lipid peroxidation and metal ion chelation. These factors are mostly responsible for the reduction or elimination of diabetes complications. Streptazotocin rats are the most common animal model used to investigate anti-diabetic activity of plant extracts.

In this present research, A potent anti diabetic plant Merremia hederacea has been selected. The literatures pertaining to phytochemical and the antidiabetic activity of this plant are very scarce. Hence an attempt has been made to evaluate the in vitro and in vivo anti diabetic activity of Merremia hederacea and to isolate the active constituent responsible for anti diabetic activity. The isolated pure compound has been attempted for molecular docking analysis.

REVIEW OF LITERATURE

Phytochemical Review of *Merremia hederecea*

1. Wang, Wen-qiong, Song, Wei-bin, Lan, Xiao-jing et al; (2014) Isolated five new pentasaccharide resin glycosides, named merremins A-E (1-5), two new pentasaccharide resin glycoside methyl esters, named merremins F and G (6, 7), and four known resin glycosides, murucoidin IV, murucoidin V, stoloniferin IV, and murucoidin XVII, from the aerial parts of ***Merremia hederacea.*** This is the first report of resin glycosides obtained from *M. hederacea.* In addition, the new compounds can be divided into three types: those possessing an 18-membered ring (1-4), compound 5 with a 20-membered ring, and those with an acyclic core (6, 7). Furthermore, the different types of resin glycosides were evaluated for their multidrug resistance reversal activities. Compounds 1, 5, 6, and murucoidin V were noncytotoxic and enhanced the cytotoxicity of vinblastine by 2.3-142.5-fold at 25 μM. Compound 5 and murucoidin V, with 20-membered rings, were more active than compound 1, with an 18-membered ring [35].

2. Charles A, Joseph M and Alex Ramani V; (2012) investigated the ethanolic extract of the medicinal plant ***Merremia hederacea***, which contains alkaloids, steroids, carbohydrates, terpenoids, tannin, phenolic compounds and flavonoids and all of them were confirmed through phytochemical screening and GC-MS analysis. The phytoconstituents present in this medicinal plant have been found to be studied by standard methods [36].

Patent

1. Shang Y ; (2016) Traditional Chinese medicine comprises 1-5 borneol, 1-5 Chinese angelica, 7-13 pts. wt. pangolins, 3-7 pts. wt. salicylic acid, 1-5 pts. wt. bezoar, 13-17 pts. wt. sulfur, 10-20 pts. wt. butter, 5-10 pts. wt. ethanol, 1-3 pts. wt. trichlorfon, 5-8 pts. wt. ***Merremia hederacea***, 1-3 pts. wt. *Hyssopus officinalis*, 10-12 pts. wt. Hydrangea, 13-17 pts. wt. *Thalictrum foliolosum* and 6-9 pts. wt. cortex acanthopanacis radicism The composition useful for treating scabies on head (claimed), clearing away heat, detoxifying,

eliminating dampness, treating paralysis, promoting blood circulation, removing blood stasis and providing bactericidal effect.

2. Chen K ; (2016) A Chinese medicine contains 25 pts. wt. *Sida alnifolia*, 15 pts. wt. *Ixeris sonchifolia*, 10 pts. wt. *Prinsepia uniflora* kernels, 25 pts. wt. ***Merremia hederacea***, 10 pts. wt. *Chenopodium glacum*, 6 pts. wt. walking fern, 9 pts. wt. toothed clubmoss, 9 pts. wt. Chrysanthemum flower heads, and 15 pts. wt. Murdannia loriformis grass. USE - Chinese medicine used for treating chronic lacrimal gland inflammation. ADVANTAGE - The Chinese medicine provides safe and effective treatment for chronic lacrimal gland inflammation

3. Yang C; (2014) Prepared merremoside B by taking ***Merremia hederacea*** stem, crushing, adding 60-80% methanol solution in which added amount of methanol solution is 8-10 times amount of crushed *M. hederacea*, performing ultrasonication to extract for 2-3 times, filtering to obtain extracted liquid, adding activated carbon, bleaching, filtering to remove activated carbon, concentrating, putting in macroporous resin column, and adding 60-70 vol.% concentrated methanol solution in which added amount of methanol solution is 4-8 times amount of liquid. ADVANTAGE - Preparation process is simple and generates less pollution.

4. Zhang X; (2014) Investigated the chinese medicinal composition comprises (in pts.wt.): Eupatorium (20-30), ***Merremia hederacea*** (15-20), Astragalus root (10-15), *Salvia miltiorrhiza* root (12-18), *Atractylodes rhizome* (5-10), green tangerine orange peel (8-12), *Stephania tetrandra* root (15-20), Verbena (14-18), Polyporus (10-15), tangerine pith (5-10), Polygonatum rhizome (11-17), *Achyranthes bidentata* root (18-24), Lepidium seed (10-15), Areca nut (5-10), Luffa (16-22) and watermelon peel used for treating ascites due to cirrhosis (claimed), and increasing blood circulation, removing blood stasis, nourishing spleen and qi, accelerating liver cell regeneration, promoting urination and preventing liver fibrosis. The Chinese medicinal composition is safe and has quick and long lasting action with high curative rate and without toxic and side effects and adverse effects, and treats disease in short course.

Pharmacological review of other Medicinal Plants having antidiabetic activity

1. Nasreddine El Omari , Karima Sayah et al; (2019) evaluated the *in vitro* antioxidant activity and inhibitory potential of organic extracts from Aristolochia longa roots against key enzymes linked to hyperglycemia. Antioxidant activity was performed using 2,2 -diphenyl-1- picrylhydrazyl (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radicals and ferric reducing/antioxidant power (FRAP) methods. Te-Glucosidase and -Galactosidase inhibitory activities were investigated using an in vitro model [37].

2. Guy Sedar Singor Njateng et al; (2018) To determine inhibitory activity of methanolic leaf extract of *Piper umbellatum* and *Persea americana* (traditionally used in Cameroon against diabetes) on -glucosidase, -glucosidase, maltase-glucoamylase, aldose reductase and aldehyde reductase activities, enzymes involved in starch digestion or diabetic complications. To assess relative efficacy of these extracts, the determination of concentrations that were needed to inhibit 50% of enzyme activity was done, whereas, gas chromatography-mass spectrum was used to identify components from extracts that may be responsible for the activities [38].

AIM AND OBJECTIVE

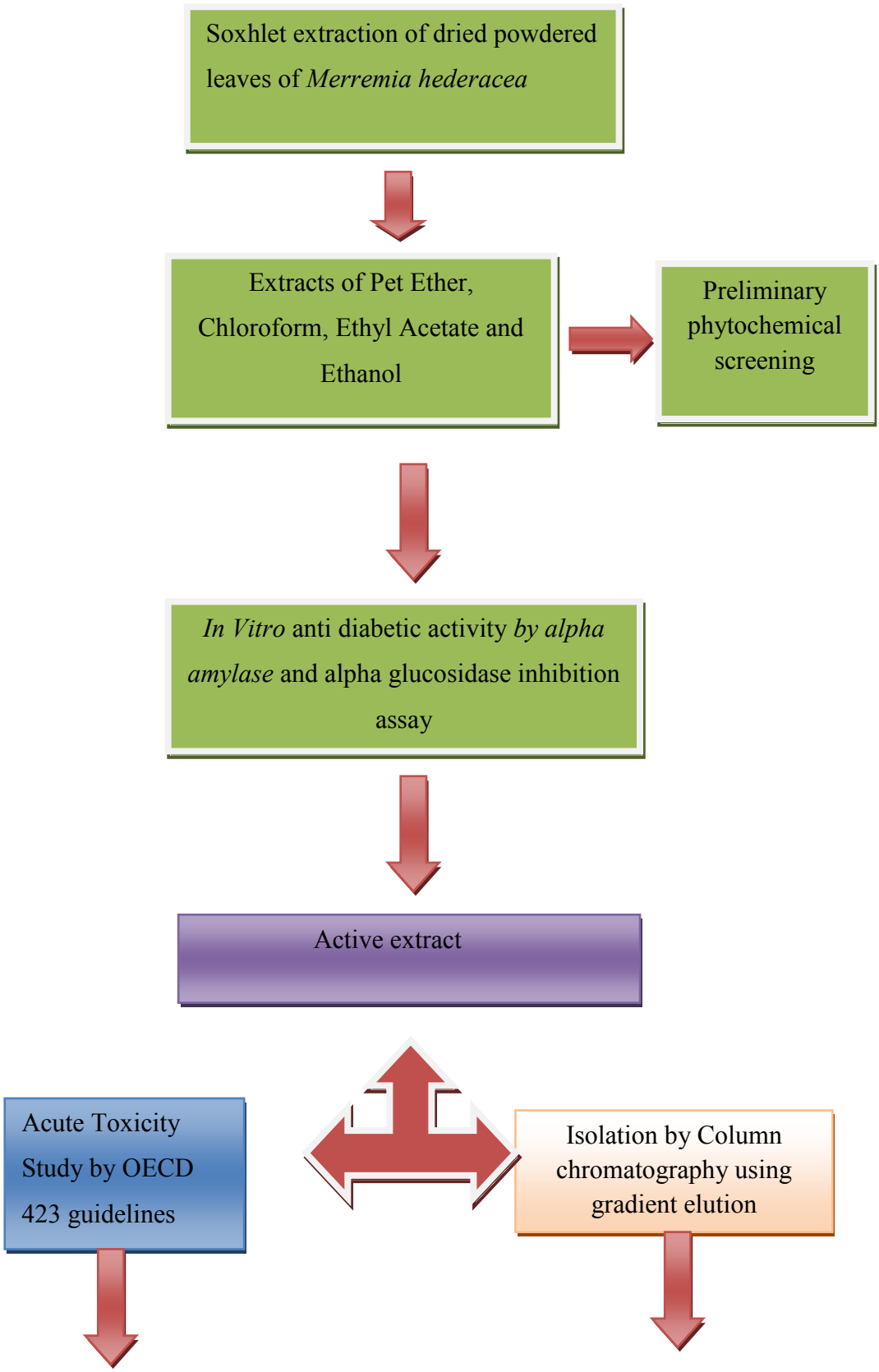
AIM:

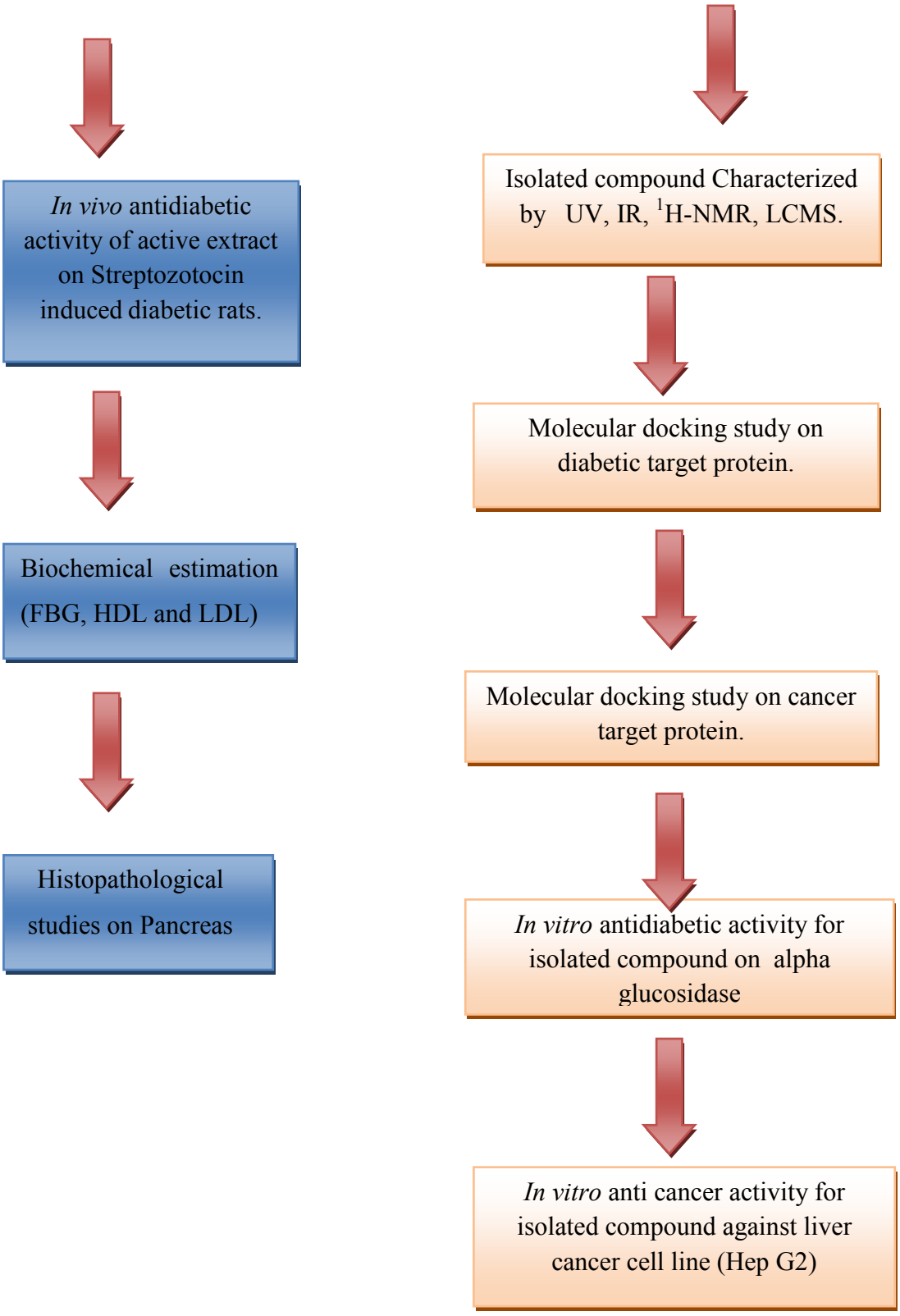
- To isolate and characterize an antidiabetic compound from the leaves of *Merremia hederacea (Burm. f.) Hallier f.*

OBJECTIVE:

- To perform soxhlet extraction on dried powdered of leaves using solvents such as petroleum ether, chloroform, ethyl acetate and ethanol.
- To carry out preliminary phytochemical screening for the extracts using standard procedures.
- To carry out *in vitro* antidiabetic activity for the extracts by alpha amylase and alpha glucosidase inhibition assay.
- The carry out column chromatographic isolation for an active extract by using gradient elution technique.
- The isolated compound will be characterized by using UV, IR, ^{1}H-NMR and LC-MS.
- Molecular docking study of the isolated compound will be performed on diabetic target protein using Auto dock software version 1.5.6.
- Molecular docking study of the isolated compound will be performed on cancer target protein using Auto dock software version 1.5.6.
- *In vitro* antidiabetic activity on alpha glucosidase inhibition assay will be carried out for the isolated compound.
- *In vitro* anticancer activity will be carried out for isolated compound against liver cancer cell line (Hep G2).
- Acute toxicity study will be carried out for an active extract by OECD 423 guidelines.
- *In vivo* antidiabetic *activity of an active extract will be investigated in* streptozotocin induced diabetic rats.

RESEARCH FLOW




Isolated compound Characterized by UV, IR, ^{1}H-NMR, LCMS.
Molecular docking study on diabetic target protein.
Molecular docking study on cancer target protein.
In vitro antidiabetic activity for isolated compound on alpha glucosidase
In vitro anti cancer activity for isolated compound against liver cancer cell line (Hep G2)
In vivo antidiabetic activity of active extract on Streptozotocin induced diabetic rats.
Biochemical estimation (FBG, HDL and LDL)
Histopathological studies on Pancreas

PLANT PROFILE

Fig. No: 8 ***Merremia hederacea (burm. f.) hallier f.***

***MERREMIA HEDERACEA* (BURM. F.) HALLIER F.**

Synonym	: *Evolvulus hederaceus*
Family	**:** Convulvulaceae
Tamil Name	: Elikkatutalai,
Manipuri Name	: Komalata.
Malayalam	: Kudicivalli.

Distribution

The genus Merremia (Convolvulaceae) comprises 70 species, which are primarily distributed throughout the warm and tropical regions of Asia and Africa.

Morphology

Merremia hederacea (Burm.f.) Hallier f., belongs to Convolvulaceae family, twinning or prostrate herb. The plant has slender stems; glabrous or pubescent, sometimes rooting at the nodes. The leaf-blades are ovate in outline, 1.5- 5cm long and 1.25-4cm wide. Petioles are 0.5-6cm long and sparsely tuberculate. Besides, the pedicels are 2-4mm long. The sepals are obovate to spathulate, notched at the apex, and are sparsely pilose. The corolla is in white or yellow, 6-12mm long, glabrous outside, pilose at the base.

Ethno Medical uses

Merremia hederacea can be used to treat colds, febrile disease, sunstroke, oliguria, tonsil inflammation, laryngitis as well as leucorrhoea. The seeds can be used to treat fevers, colds, sore throats, haematuria, conjunctivitis and boils. Leaves of M. hederacea can be used in the treatments of chapped hands and feet. No culinary and other uses have been reported for this herb. The genus Merremia reports to contains phenolics, flavonoids,, aliphatic pyrolidine amides, tropane containing alkaloid, the species possess to antioxidant, anti inflammatory, alpha amylase inhibitory activity. Traditionally, *M. hederacea* is widely used for the treatment of pharyngitis and furuncles. [39]

MATERIALS AND METHODS

Collection of Plant:

The leaves of *Merremia hederacea* were collected from Tamil Nadu (forest of kalakatu) Tirunelveli District, India. Taxonomic identification was made from botanical survey of medicinal plants, Siddha Unit, Government of India, Palayamkottai authenticated by Chelladurai Botonist. Fresh plant leaves were shade dried at room temperature, ground into coarse powder and stored in airtight containers.

Preparation of extracts:

The dried powder of leaves was extracted sequentially by hot continuous percolation method by soxhlet apparatus using petroleum ether, chloroform, ethyl acetate and ethanol as solvent. The extracts were concentrated by using a rotary vacuum evaporator.

Table no: 2 Percentage yield of leaf extracts of *Merremia hederacea*

Extracts	Nature of the extract	Percentage yield
Pet ether	Yellowish brown	9.5%
Chloroform	Dark Green	12.6%
Ethyl acetate	Greenish Yellow	14.5%
Ethanol	Reddish yellow	18.65%

Phytochemical Screening of active extracts from *Merremia hederacea*:

All the extracts were subjected to preliminary phytochemical screening for the detection of various plant constituents present. The pharmacological actions of crude drugs were determined by the nature of their constituents and the phytoconstituents are responsible for the desired therapeutic properties.

To obtain these pharmacological effects, the plant materials itself or extracts in a suitable solvent or isolated active constituent may be used. The pet ether, chloroform, ethyl acetate and ethanolic extracts of *Merremia hederacea* was subjected to the following chemical tests used for the identification of various active constituents.[40, 41.]

TESTS FOR ALKALOIDS

Dragendroff's Test

The extract was treated with Dragondroff's reagent and observed for the formation of yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test

The extract was treated with Wagner's reagent and observed for the formation of a reddish brown precipitate indicates the presence of the alkaloids.

Mayer's Test

The extract was treated with mayer's reagent formation of white precipitate or creamy coloured precipitate indicates the presence of alkaloids.

Hager's Test

The extract was treated with Hager's reagent and observed for the formation of yellow precipitate indicates the presence of alkaloids.

TEST FOR CARBOHYDRATES

Molisch's Test

To 2 ml of the extract 1 ml of alpha- naphthol solution was added and concentrated sulphuric acid was added through the sides of test tube. Purple or reddish violet colour at the junction of the two liquids revealed the presence of carbohydrates.

Fehling's Test

To 1ml of the extract equal quantities of Fehling's solution A and B were added while heating formation of a brick red precipitate that indicates the presence of carbohydrates.

Benedict's test

To 5ml of Benedict's reagent 1ml of the extract solution was added and boiled for 2 minutes and cooled. Formation of red precipitate shows the presence of carbohydrates.

TESTS OF GLYCOSIDES

Legal's Test

The extract was dissolved in pyridine and sodium nitroprusside solution was added to make it an alkaline. The formation of pink red to red colour shows the presence of glycosides.

Baljet Test

To 1 ml of the ethanolic extract was added with 1 ml sodium picrate solution and the yellow to orange colour reveals the presence of glycosides.

Borntrager's Test

A few ml of dilute Hydrochloric acid was added to 1 ml of the extract solution. It was then boiled, filtered and the filtrate was extracted with chloroform. The chloroform layer was then treated with 1ml of ammonia. The formation of red colour shows the presence of anthraquinone glycosides.

Keller Killiani Test

The extract was dissolved in acetic acid containing traces of ferric chloride and it was then transferred to a test tube containing sulphuric acid. At the junction, formation of a reddish brown colour, which gradually became blue confirms the presence of glycosides.

TESTS FOR PHYTOSTEROL

Libermann Burchard Test

3ml of extract was mixed with 3ml of acetic anhydride. It was heated and then cooled. Few drops of concentrated sulphuric acid was added. Appearance of blue colour shows the presence of phytosterol.

Salkowski's Test

Dissolve the extract in chloroform and equal volume of concentrate sulphuric acid was added. Formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer represents the steroid components present in the extract.

TEST FOR FLAVONOIDS

Shinoda test

The extract was treated with 5ml of 95% ethanol. Add few drops of concentrated hydrochloric acid and 0.5g of magnesium turnings. Pink colour was observed which shows the presence of flavonoids.

TEST FOR TANNINS AND PHENOLIC COMPOUNDS

Ferric chloride test

1 ml of the extract was added with ferric chloride and observed for the formation of a dark blue or greenish black colour indicates the presence of Tannins and Phenolic compounds.

Gelatin Test

The extract was treated with 1%gelatin containing 10%NaCl and observed for the precipitation which indicates the presence of Tannins and Phenolic compounds.

TESTS FOR PROTEINS AND AMINO ACIDS

Biuret Test

1ml of the extract was treated with 1ml of 40% sodium hydroxide solution followed by 2 drops of 1% copper sulphate solution. Formation of a violet colour shows the presence of proteins.

Xanthoproteic Test

1ml of the extract was treated with 1ml of concentrated nitric acid. A white precipitate was formed, it was boiled and cooled. Then 20% of sodium hydroxide or ammonia was added orange colour was formed indicates the presence of aromatic amino acids.

Lead Acetate Test

The extract was treated with 1ml of lead acetate. Formation of a white precipitate indicates the presence of proteins.

TEST FOR SAPONINS

About 1 ml of ethanol extract was diluted separately with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. A1% 1 cm layer of foam indicates the presence of saponins.

TEST FOR TRITERPENOIDS

Two or three granules of tin metal in 1ml thionyl chloride solution were dissolved and 1ml of the extract was added into a test tube. The formation of pink colour indicates the presence of Triterpenoids.

TEST FOR FIXED OILS

Spot Test

A small quantity of the extract was pressed between two filter papers. Oil stains on the filter paper indicates the presence of fixed oils.

Table no 3. Preliminary Phytochemical analysis on leaf extracts of *Merremia hederacea*

TEST	PET ETHER	CHLOROFORM	ETHYL ACETATE	ETHANOL
ALKALOIDS	-	+	-	+
CARBOHYDRATES	-	+	-	-
GLYCOSIDES	-	-	+	+
PHYTOSTEROLS	+	+	-	-
FLAVONOIDS	-	+	+	+
TANNINS & PHENOLIC COMPOUNDS	-	-	-	+
PREOTEINS & AMINO ACIDS	+	+	-	-
SAPONINS	-	-	-	+
TERPENOIDS	+	-	+	-
FIXED OILS	+	-	-	-

NOTE: + POSITIVE; - NEGATIVE

RESULT:

From the preliminary phytochemical screening, it was observed that Pet. ether extract shows the presence of phytosterol, triterpenoids, proteins, amino acids and fixed oils. Chloroform extract shows the presence of alkaloids, carbohydrates, phytosterol, flavonoids, proteins and amino acids. Ethyl acetate extract shows the presence of glycosides, flavonoids and terpenoids. Ethanolic extract shows the presence of glycosides, flavonoids, tannins and Phenolic compounds.

***In vitro* antidiabetic activity:**

All the four extracts were subjected to *in vitro* antidiabetic activity by alpha amylase inhibition assay and alpha glucosidase inhibition assay.

IN VITRO ANTIDIABETIC ACTIVITY

PROCEDURE:

Alpha - amylase inhibitory activity:

This study was performed by a modified starch iodine protocol. In short, plant extract and standard concentration (10, 20, 40, 80, 160, 320µg/mL.) was taken in pre labelled test tubes. A volume of 20 mL of α-amylase was added to each test tube and incubated for 10 min at 37 °C. After the incubation 200 µL of 1% starch solution was added to each test tube and the mixture was re-incubated for 1 h at 37 °C. Then 200 µL of 1% iodine solution was added to each test tube and after that, 5 mL distilled water was added. Absorbance of the mixture was taken at 565 nm. Blank were undertaken under the same conditions. IC_{50} value was calculated by using regression analysis [42, 43]

$$\%\ inhibition = \frac{As - Ac}{As} \times 100$$

Where, **Ac** is the absorbance of the control and; **As** is the absorbance of the sample.

Alpha - Glucosidase Inhibition assay:

The effect of the plant extracts on α-glucosidase activity was determined using α-glucosidase enzyme. The substrate solution p-nitro phenyl glucopyranoside (pNPG) was prepared in 20mM phosphate buffer, and pH 6.9. 100 µL of α-glucosidase was pre-incubated with 2.5ml of the different concentrations of the extracts for 10min. Then 50 µL of 3.0mM (pNPG) as a substrate dissolved in 20mM phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37 ˚C for 20min and stopped by adding 0.5mL of Na_2CO_3 (0.1M). The yellow-coloured reaction mixture, 4-nitrophenol, released from pNPG was measured at 405nm using UV - VIS spectrophotometer [44].Acarbose was used as a positive control and the inhibitory activity of α-glucosidase was calculated using the following formula,

% Inhibition = [(Abs Control - Abs Sample) / Abs Control] x 100.

Table no: 4 Effect of extracts of *Merremia hederacea* on Alpha-amylase

Concentration in µg level	Pet ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract	Acarbose (standard)
10	6.55	9.25	10.47	13.68	47.86
20	10.93	35.23	40.11	57.14	69.75
40	19.21	40.21	45.62	59.47	76.31
80	26.08	45.18	52.62	76.95	90.44
160	29.32	50.23	60.41	89.49	90.62
320	38.23	55.54	65.44	91.44	95.44

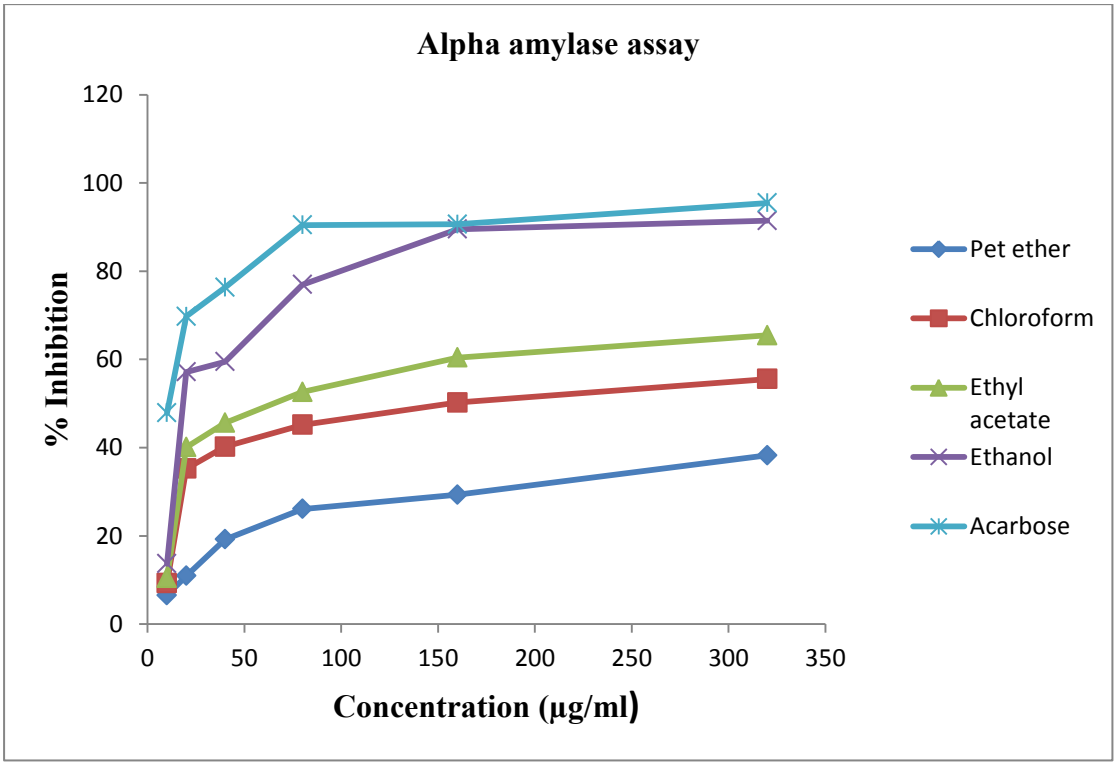


Fig. No: 9 α- amylase inhibition assay for the extracts of *Merremia hederacea*

Table no : 5 Effect of extracts of *Merremia hederacea* on alpha glucosidase

Concentration in µg level	Pet ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract	Acarbose (standard)
10	3.15	4.33	3.15	19.24	47.86
20	6.72	9.94	8.93	22.12	69.75
40	23.86	25.21	19.81	40.27	76.31
80	31.82	34.01	27.94	55.27	90.44
160	72.96	71.40	59.38	78.58	90.62
320	62.24	70.33	69.82	85.50	95.45

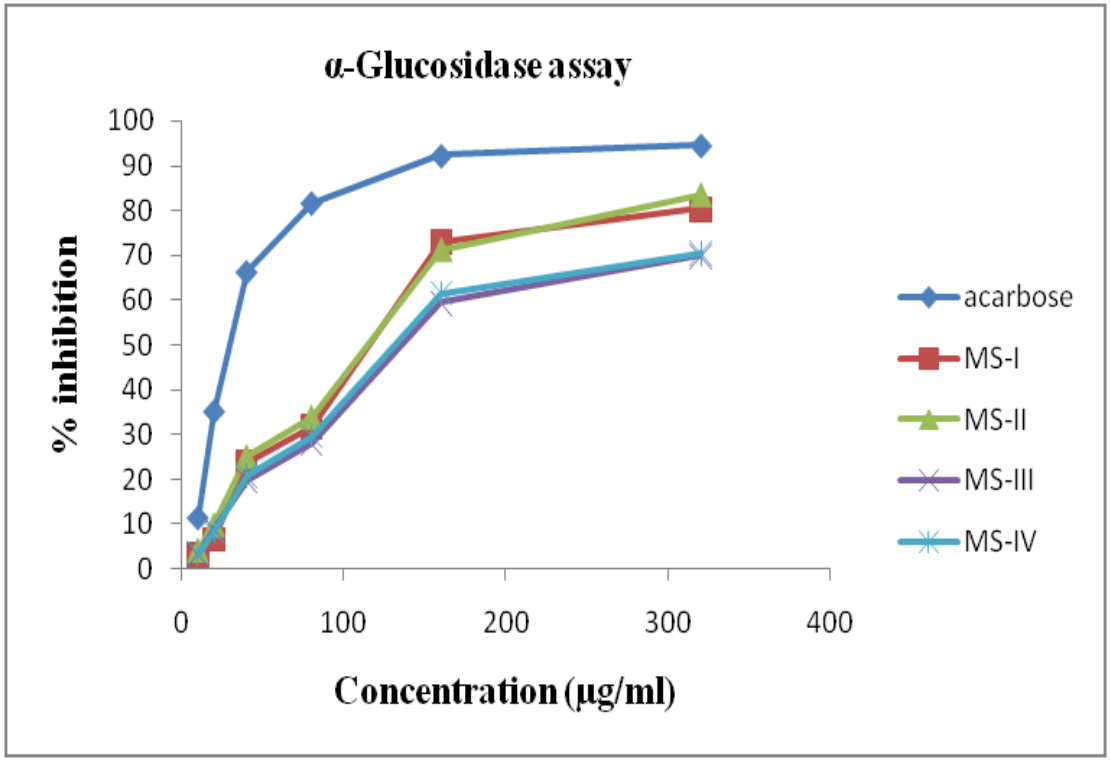


Fig. No: 10. α-glucosidase inhibition assay for the extracts of *Merremia hederacea*

RESULTS:

***IN VITRO* ANTIDIABETIC ACTIVITY**

Alpha amylase assay

As illustrated in table 4, alpha amylase assay was carried out for the extracts of *Merremia hederacea*. It was found that ethanol extract shows good antidiabetic activity compared to other extracts. At highest concentration 320µg, ethanol extract shows greater percentage inhibition of 91.44%. The standard acarbose also shows good alpha amylase inhibition with a percentage inhibition of 95.44%

Fig 9 shows the percentage inhibition of all the extracts and standard acarbose against alpha amylase.

Alpha glucosidase assay

As illustrated in the table 5, alpha glucosidase assay was carried out for the extracts of *Merremia hederacea*. It was found that, ethanol extract shows greater percentage inhibition of 85.50% at 320µg. The standard agarbose also shows greater alpha glucosidase inhibition with a percentage inhibition of 95.45. Fig 10, shows the percentage inhibition of all the extracts and standard against alpha glucosidase.

Discussion

The use of herbal drugs as complementary approaches in existing medications for the treatment of diabetes and its complications is growing worldwide and many plants in different countries are known to have antidiabetic effects.[45] The ancient Indian literature reports more than 800 plants with antidiabetic properties while ethnopharmacological surveys indicate that more than 1200 plants can be used for hypoglycemic activity [46]. Mainly two carbohydrate hydrolyzing enzymes (α-amylase and α-glucosidase) are responsible for postprandial hyperglycemia. α-amylase begins the process of carbohydrate digestion by hydrolysis of 1, 4-glycosidic linkages of polysaccharides (starch, glycogen) to disaccharides and α-glucosidase catalyzes the disaccharides to monosaccharides, which leads to postprandial hyperglycemia. Hence, inhibitors of α-amylase and α-glucosidase are useful in the control of hyperglycemia as they delay carbohydrate digestion, which consequently reduce the postprandial plasma glucose level [47, 48].

Many bioactive compounds from different plants have been reported to have hypoglycemic effect, in that mostly phenolics, resin glycosides and flavonoids have a positive correlation as antidiabetic agents [49]. The presence of flavonoids, phenolic compounds in extracts of *Merrimia hederaceae* may act against diabetes mellitus either through their capacity to avoid glucose absorption or to improve glucose tolerance by competitive inhibition of sodium-dependent glucose transporter-1 Another possible mechanism followed by flavonoid compounds (luteolin, kaempferol, chrysin, and galangin) to control blood glucose levels is the inhibition of α-amylase and α-glucosidase activity in the intestine [50,51].

ISOLATION BY COLUMN CHROMATOGRAPHY

The ethanol extract is active and shows good *in vitro* anti diabetic activity compared to other extracts. Hence the bioactive guided isolation was performed and the ethanol extract of *Merremia hederacea* (ETMH) was subjected for column chromatographic isolation by gradient elution technique.

Packing of column:

- Wet packing technique was used for the isolation. Initially cotton plug is kept at the bottom of the column and eluting solvent (Hexane) was added to $3/4^{th}$ of the column.
- Pre heated Silica gel (60-120 mesh size) is mixed with eluting solvent (Hexane) to make it as slurry and added to the column and the silica gel was allowed to settle down to from a uniform packing.
- Then the stop-cock of the column was opened and the excess of solvent over the column head was allowed to run.

Packing of active extract:

After the silica gel packing, the ethanol extract (5g) was mixed with the desired solvent then silica gel was added slowly with stirring and heated to get a free flowing powder. The powdered sample was then applied carefully on the top of the prepared column.

Elution Process:

- The active extract was eluted by gradient elution technique by solvent/solvent system using various solvent systems such as Hexane, Hexane: chloroform, chloroform, chloroform: ethyl acetate, ethyl acetate, Ethyl acetate: methanol to separate the eluate. When the mixture of solvent system used, the ratio of mixtures are prepared as 100%, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90.
- Elutes were collected and marked from fractions 1-29. Each 50ml fractions were collected.
- All the fractions were spotted successfully on TLC plate and the fractions having same R_f value are pooled together and evaporated to dryness.

Table no: 6 Number of fractions collected and solvent systems

S. NO.	FRACTIONS	SOLVENT SYSTEM
1	F-1	Hexane
2	F-2 to F-8	Hexane : chloroform
3	F-9	Chloroform
4	F-10 to F-18	Chloroform : ethyl acetate
5	F-19	Ethyl acetate
6	F-20 to F-24	Ethyl acetate : methanol
7	F-25 to F 29	Ethyl acetate : methanol : glacial acetic acid

The yellow colour band was eluted in fractions 20-24 in the solvent system ethyl acetate: methanol (80:20). The fractions show similar R_f value in thin layer chromatography and contains 2 spots and were mixed together and evaporated. The reddish brown band was eluted in fraction 25 -29 in the solvent system ethyl acetate: methanol: glacial acetic acid (60.30:10). The fractions 25 -29 were tested in TLC with various solvent systems. The fractions 25- 29 show similar R_f values and shows single spot and were mixed together and evaporated to yield a yellow amorphous compound.

Table no: 7 Thin layer chromatography of isolated compound

S.no	Fractions	Solvent systems	No of spot	Rf value
1	20 – 24	Chloroform: methanol (90: 10)	2	0.6, 0.8
		Chloroform: methanol (80: 20)	2	0.7,0.9
		Ethyl acetate: methanol (90: 10)	2	0.8,0.9
		Ethyl acetate: methanol (80: 20)	2	0.9
2	25 – 29	Chloroform: methanol (90: 10)	1	0.8
		Chloroform: methanol (80: 20)	1	0.8
		Ethyl acetate: methanol (90: 10)	1	0.9
		Ethyl acetate: methanol (80: 20)	1	0.9

The compound is a yellow amorphous powder. FeCl test was performed and shows positive (dark green) and it is characterized by UV, IR, ^{1}H-NMR & LCMS.

Characterization of an isolated compound (10mg)

Ultraviolet spectroscopy was performed in (MeOH) λ_{max} 253.0, 278.0 nm.

Infra red spectroscopy (KBr Pellet) 3425.33 cm^{-1} Aromatic O-H Stretching, 1643.23 cm^{-1} C =O (Chelated carbonyl group), 1396.36 cm^{-1} CH_3, 2877 cm^{-1} CH – Aliphatic, 1728 cm^{-1} Ketones (Carbonyl group)

HPLC + PDA+ MS: HPLC + PDA+ MS with a molecular ion peak, $[M]^+$ at 325.10 $[M+ H]^+$ and other fragments at 269.95, 369.05. From, the previous literature survey, the molecular ion peak and fragments matched with a related prenylated derivatives of chalcone.

^{1}H NMR (Bruker DMSO 500 MHz): δ 1.642 (3H, s), δ 1.753 (3H, s), δ 3.25 (2H, d), δ 5.201 (1H, t), δ 6.501 (1H, d), δ6.850 (2H, d), δ7.254 (1H, d), δ 7.710 (1H, d), δ 7.772 (2H,d), δ 8.131, δ 10.12(1H, s), (s, δ2.50 (DMSO solvent peak.).

Discussion

ULTRA VIOLET SPECTROSCOPY

Ultraviolet spectroscopy was performed in (MeOH) λ_{max} 253.0 nm,, 278.0 nm.

^{1}H NMR (Bruker DMSO 500 MHz)

The ^{1}H NMR exhibited a set of 1.642 and 1.753 (3H δ signals for a prenyl substituent at 3.250 (2H, d), δ 3.252 (2H, d), δ 5.201 (1H, t), δ 6.850 (2H,d) Signals observed at 7.254, 7.710 and 7.772 (1H each) are characteristic for trans-olefinic protons. A set of doublet at δ6.850 and a doublet at δ7.772 (1H each, d) was attributable to ortho coupled aromatic protons and H- α H- β in chalcone skeleton [52]

HPLC + PDA+ MS

From the observations in correspondent in HPLC + PDA+ MS the m/z value325.10 $[M+ H]^{+}$ with the retention time 4.297 and the other fragments at 269.95, 369.05. The isolated compound was found to be **2′, 4, 4′-Trihydroxy-3′-(3-methyl-2-butenyl)-chalcone** with the molecular formula $C_{20}H_{20}O_4$.[53]

INFRA RED SPECTROSCOPY (KBr Pellet)

3425.33 cm^{-1} shows the presence of aromatic O-H Stretching, 1643.23 cm^{-1} C =O shows the presence of chelated carbonyl group), 1398.36 cm^{-1} characteristic of CH_3 group , 2877 cm^{-1} CH – Aliphatic, 1728 cm^{-1} shows the presence of ketones (Carbonyl group).

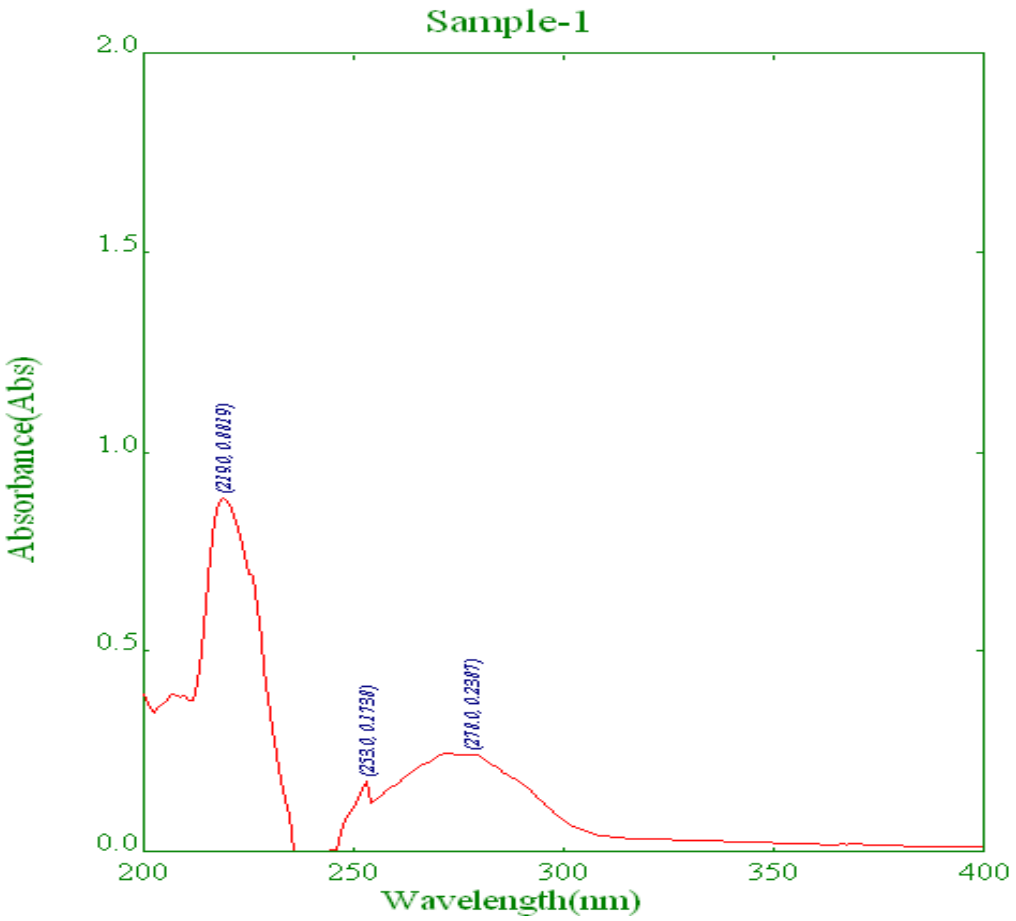


Fig.no 11: UV Spectrum of isolated compound from ethanol extract of *Merremia hederaceae* (ETMH)

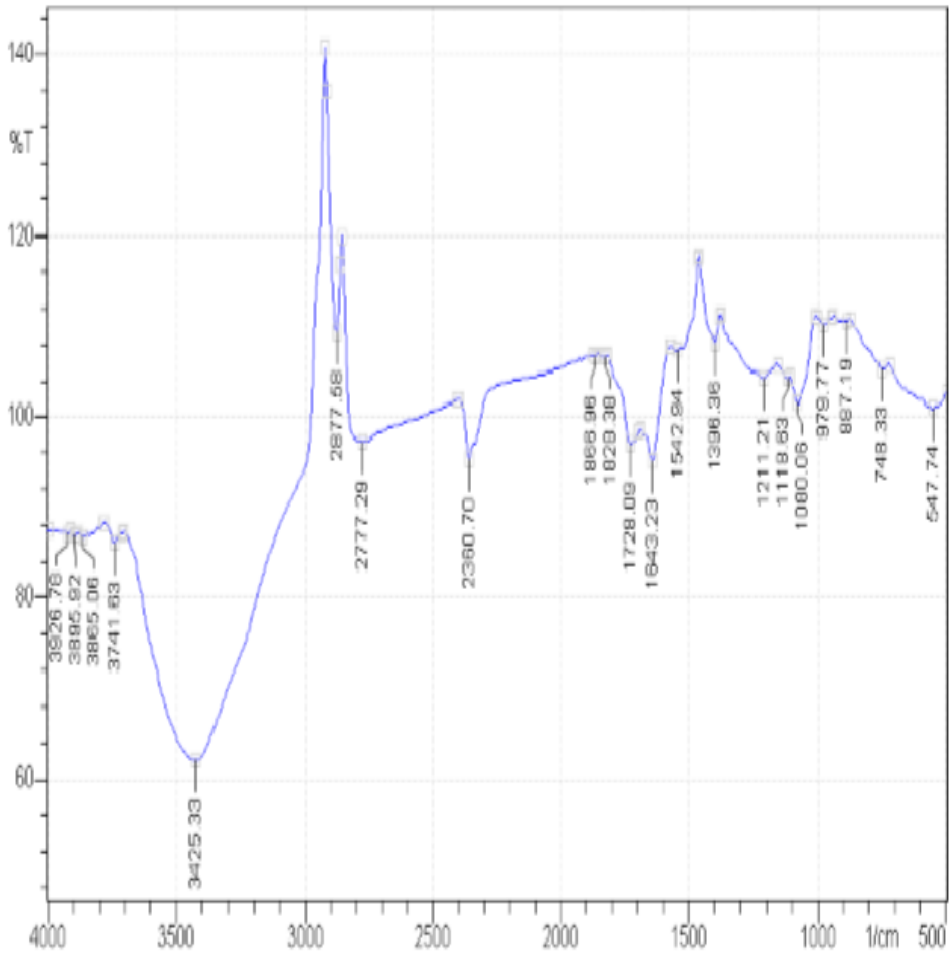


Fig.no 12: IR Spectrum of isolated compound from ethanol extract of *Merremia hederacea* (ETMH)

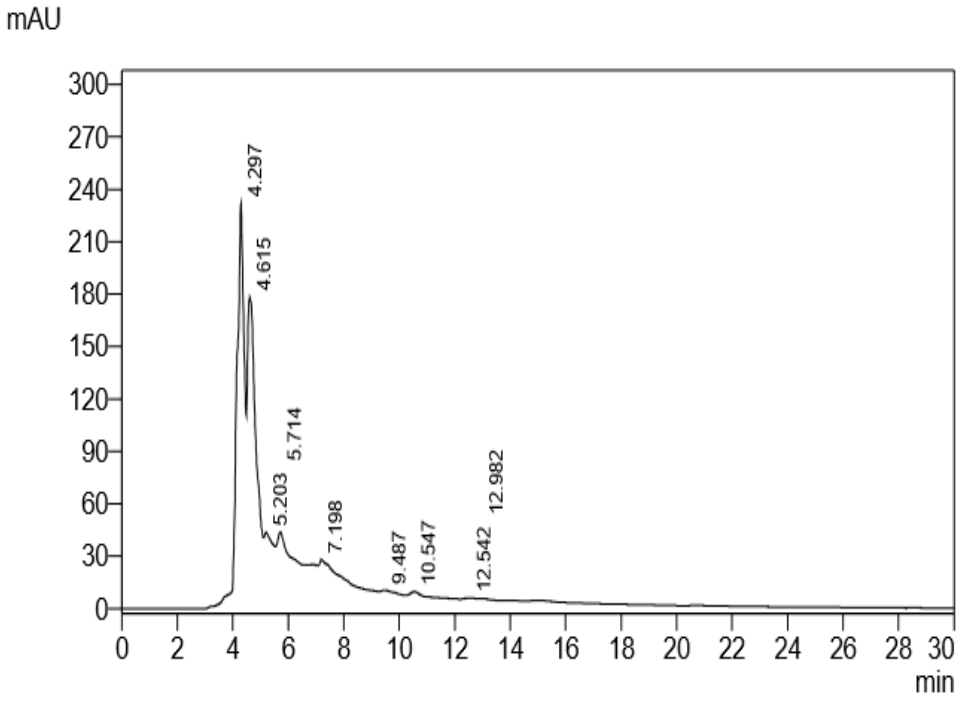


Fig.no: 13 LCMS/MS Chromatogram of isolated compound from ethanol extract of *Merremia hederaceae* (ETMH)

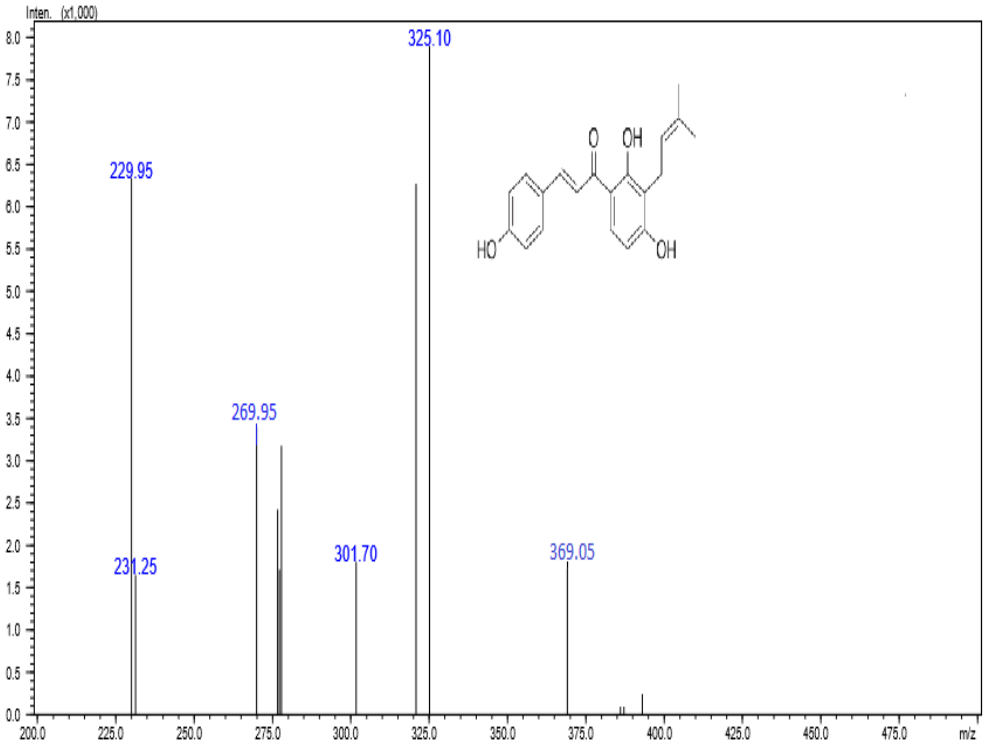


Fig.no: 14 LC-MS/MS of 2′, 4, 4′ -Trihydroxy-3′-(3-methyl-2-butenyl)chalcone.

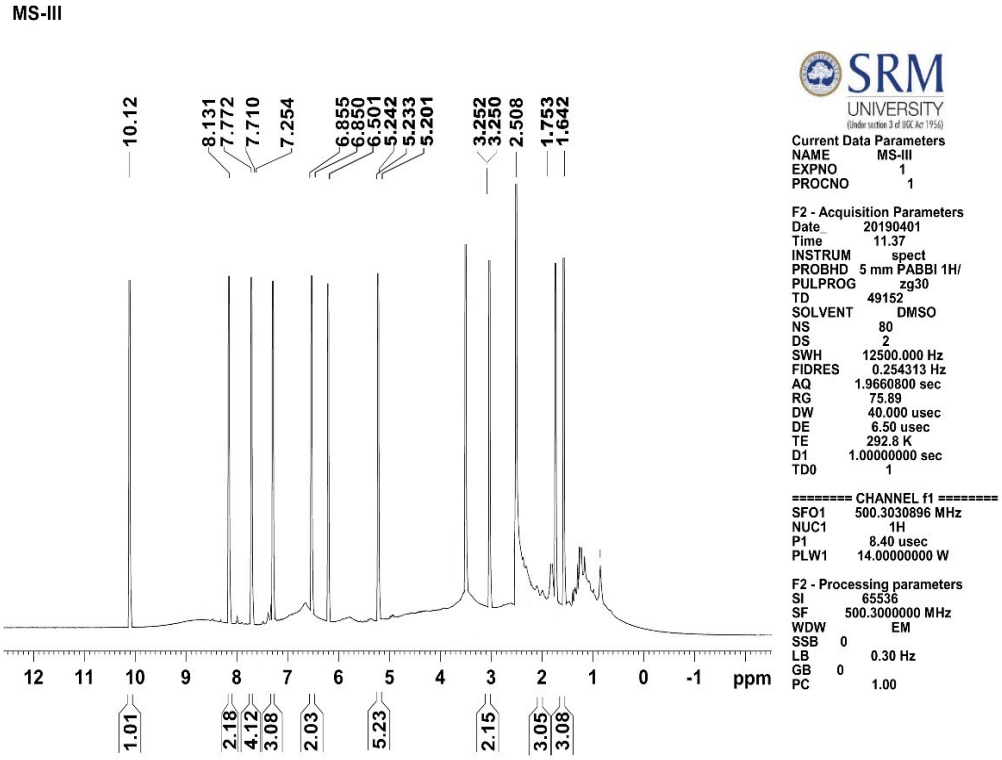


Fig.no: 15 ^{1}H-NMR of 2′, 4, 4′ -Trihydroxy-3′-(3-methyl-2-butenyl) chalcone.

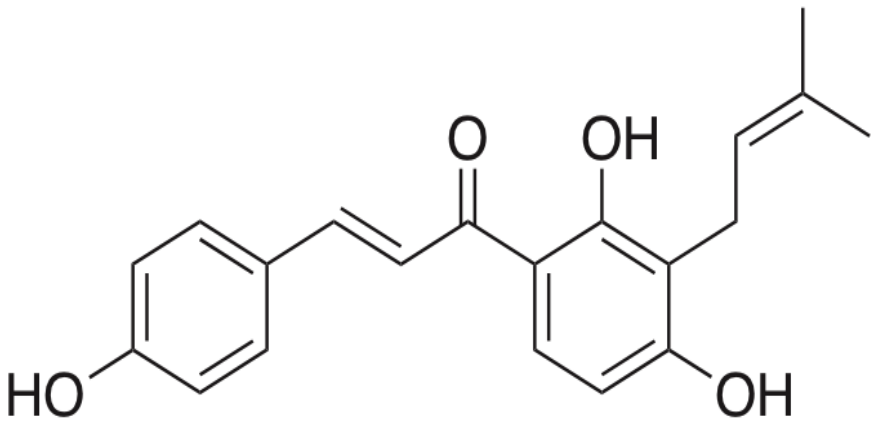


Fig no: 16. Structure of 2′, 4, 4′ -Trihydroxy-3′-(3-methyl-2-butenyl)chalcone

MOLECULAR DOCKING STUDY OF 2', 4, 4'-TRIHYDROXY-3'–(METHYL-2-BUTENYL) CHALCONE AGAINST DIABETIC TARGET PROTEIN

Molecular docking is generally used to detect the protein-ligand orientation and interaction. Auto Dock Tools package version 1.5.6 was utilized to create the docking input files. The grid region was surrounded by the active site for binding. So, grid region was selected on the basis of amino acid residues representing the binding site of Miglitol as the standard drug obtained from PDB with ID- 3L4W and considered as the best active region for the favourable interaction. The grid box was set at 126 × 126 × 126 A˚ for x, y and z axis and covered the active site of the target protein. The Lamarckian Genetic Algorithm (LGA), a local search algorithm was utilized for ligands conformers searching. During the docking process, a maximum of 10 conformers were considered for the compound. After completion of the conformer the lowest binding energy was chosen. The conformational similarity by visualizing the binding site and its energy (Kcal/mol) and the docked amino acid residues forming hydrogen bonds and other parameters like intermolecular energy (Kcal/mol) and inhibition constant (μM) were analysed by Auto Dock tool. Ten best poses were generated for the ligand and scored using Auto Dock -11.28 $kcal^{-1}$ scoring functions. Based on the docked energy the ligand was selected for anti diabetic activity. The ligand interacting residues with the target protein were analyzed using Auto Dock tools, Molegro Molecular viewer.[54]

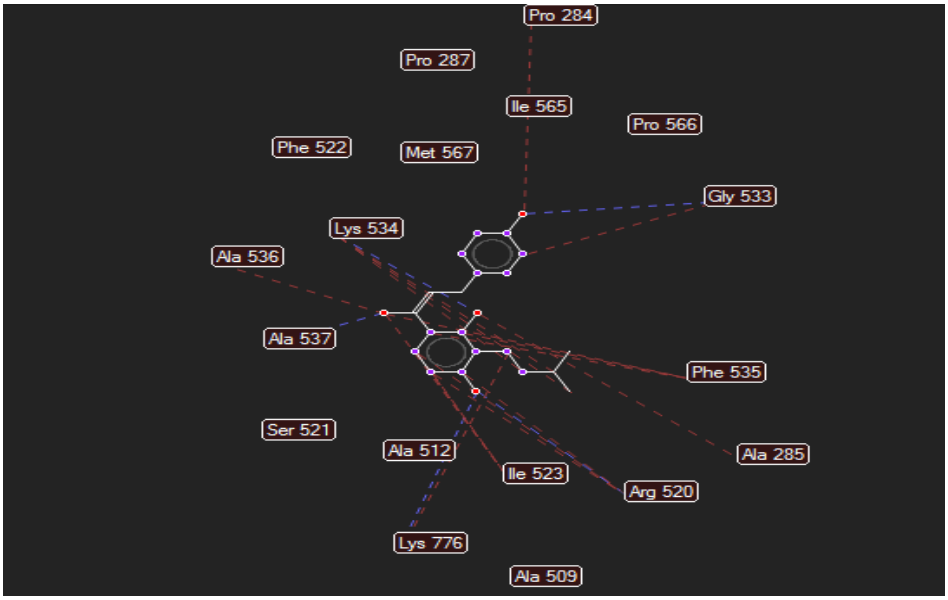


Fig.no 17. Number of amino acid interaction

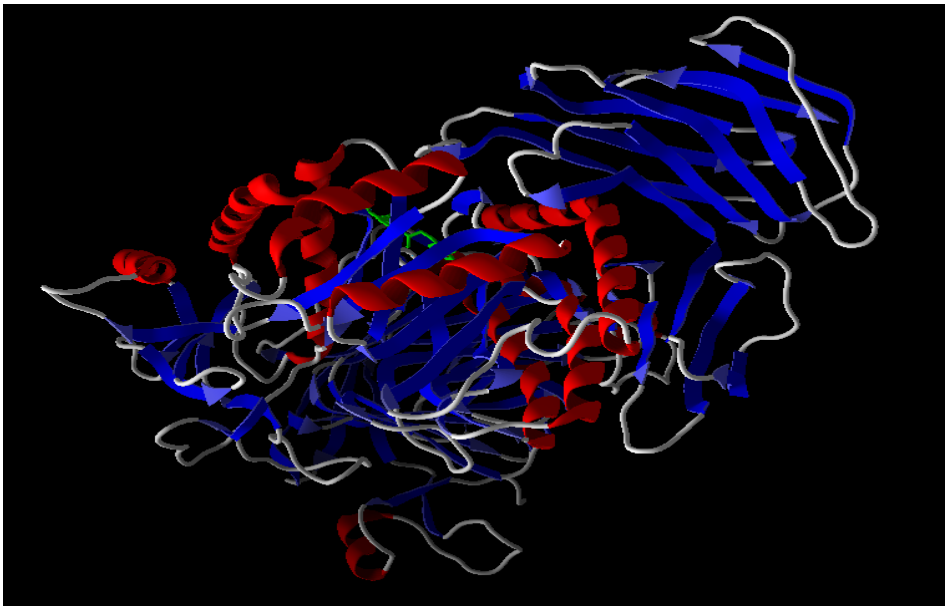


Fig.No:18 protein ligand interaction

AUTO DOCK and ADT

Short Tutorial:

Overview:

Working with AutoDock4 includes 3 steps:

1. Preparation of receptor & ligand files.

2. Calculation of affinity maps by using a 3D grid around the receptor & ligand.

3. Defining the docking parameters and running the docking simulation.

The preparation step starts with pdb files of receptor (protein.pdb) and ligand (ligand.pdb), which are added hydrogens and then saved as protein.pdb & ligand.pdb. The calculation of affinity maps in the "Grid" section requires the above pdb files to be assigned charges & atom types, and also that the nonpolar hydrogens are merged. This is done automatically by ADT, and the resulting files need to be saved as protein.pdbqt&ligand.pdbqt, which is the only format AutoGrid&AutoDock can work with. Calculation of affinity maps is done by AutoGrid and then docking can be done by AutoDock. The newest docking algorithm is LGA (Lamarckian Genetic Algorithm).

For running ADT, you should be following next steps:

1. Install MGL tools.

2. Download autogrid4.exe, autodock4.exe and AD4.1_bound.dat files.

3. Install Molegro Molecular Viewer.

4. Install ChemDraw including 3D Pro.

After having finished installation of above tools, you can run ADT. Here, I recommend you to follow my current protocol for running it:

1- Create a work folder for your job and tag it based on your favorite name. (e.g. project)

2- running ADT in start menu in windows:

after running you should set its directory: following these steps:

a) open ADT> File> Preferences>set>Startup Directory (in this section you should put on

yourworkfolder path and set it). for example: “C:\Users\project

3- prepare ligand and protein in ".pdb" format. Notice, you must have labeled them by "Ligand" and "Protein" keywords. Protein can be preprocessed by Molegrow Molecular

Viewer & Ligand can be initially prepared using in ChemBioDraw and can be saved as pdb.

After having finished these steps you can open ADT: Well! you should do the below ways:

1- open ADT:

File > Read Molecule> Select Protein File (“.pdb” file)

Then:

Edit >Hydrogens>Add>>>>>> Polar Only >OK

Molecular Docking

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Edit >Hydrogens> Merge Non-Polar > Continue (in this step if you see any warning please

click on "continue")

then:

Edit > Charges > Compute Gasteiger> Ok

Edit >Misc> Repair Missing Atoms> Ok/Select all > Dismiss

File > Save > Write PDB>>Sort Nodes (Check) > OK > (Overwrite) YES

Well! in the next step you should try prepare the ligand:

Ligand > Input > Open> Select Ligand File (".pdb" file) > OK

Now repeat above steps and then: (in this section you must add charge to your ligand. So,

click on

Edit menu and:

Edit> Charges> Add Kollman charges

Ligand > Output > Save as PDBQT

****

Now you should be executing AutoGrid4:

For AutoGrid following the below steps: (preparing gpf file)

1-Grid >Macromole> Choose> Click (Protein) > Select Molecule > OK > save Save as

PDBQT

2-Grid> Set Map types >Choose Ligand> Click (Ligand) > Select Ligand

3-Grid >GridBox> Set the BOX> File > Close Saving Current

4-Grid > Output > Save GPF>grid.gpf> save

****

After having finished above steps following the below ways:

For Autodock following the below steps: (preparing dpf file)

1)Docking> Macromolecule > Set Rigid filename> Select „Protein.pdbqt" > Open

2-Docking > Ligand > Choose> Click „Ligand" > Select Ligand> Accept

3-Docking > Search Parameters > Genetic Algorithm> Accept

4-Docking > Output > Lamarckian GA> Save file as „dock.dpf‟

****

Now copy the autogrid4.exe, autodock4.exe and AD4.1_bound.dat files into the destination

folder (working folder) and check whether 2 pdbqt files and grid.gpf and dock.dpf files are

there.

After having finished above steps following the below ways:

1-open cmd (command prompt). Windows +R and type cmd enter.

2-in command prompt go the destination folder by cd commands.

3-run the following commands

4- autogrid4.exe -p grid.gpf -l grid.glg (wait for the response)

5- autodock4.exe -p dock.dpf -l dock.dlg (wait for the response)

Now the dock.dlg file is your docking result.

****

1-Analyze > Docking > Open> Select „dock.dlg‟ > Open >Assign Ligand New Name > OK

2-Analyze > Macromolecule > Choose> Click „Protein‟ > Select Macromolecule

3- Analyze> Conformations> Play, Ranked By Energy or Play > Click on the „&‟ Button

4-Set Play Options >Check „Build H-Bonds‟ > View the hydrogen bonds formed > Check

„Show Info‟ > View the Interaction Energy > Build Current Write Complex> Save as „Result.pdb‟ save....

For better ligand interactions you can open the result.pdb in Molegro Molecular Viewer and

View it and save the ligand interaction image.

Results:

From the previous literature review of the docking results of a standard ligand (Miglitol) had a binding energy of -8.04 kcal/mol. The hydrogen bond forming amino acid residues of the target protein was found to be His 626 and Asp 232.Excluding hydrogen interaction, the ligand was also formed nine electrostatic interaction with the amino acids.

The ligand **(2',4,4'-trihydroxy-3'–(methyl-2-butenyl) chalcone**) was predicted to have better binding energy of –11.28 kcal/mol as compared to standard drug. It forms hydrogen bond interactions with amino acid residues of the target protein, Lys534, Ala 537, Lys 776, Arg 520, Gly 533.

Discussion

In this docking study, we present an approach to control blood glucose levels in individuals with type 2 diabetes by targeting maltase- glucoamylase and intestinal glucosidases using a ligand **(2',4,4'-trihydroxy-3'–(methyl-2-butenyl) chalcone)** isolated from *Merremia hederacea*. One of the intestinal glucosidases targeted the N-terminal catalytic domain of maltase-glucoamylase (ntMGAM) which is responsible for the hydrolysis of terminal starch products into glucose. Hence to slow down the glucose level and targeted the ntMGAM. Previously acarbose and miglitol were found to be potent inhibitors alpha glucosidase having poly hydroxy groups in their structure . Hence novel ligand (2',4,4'-trihydroxy-3'–(methyl-2-butenyl) chalcone) which has three hydroxyl groups and is chalcone based isolated from *Merremia hederacea* was docked for the binding sites of alpha glucosidase. The ligand (2',4,4'-trihydroxy-3'–(methyl-2-butenyl) chalcone) was predicted to have better binding energy of –11.28 kcal/mol as compared to standard drug. It forms hydrogen bond interactions with amino acid residues of the target protein, Lys534, Ala 537, Lys 776, Arg 520, Gly 533 (fig 17, 18). Hence the isolated compound has better binding energy into alpha glucosidase than the standard drug miglitol.[55]

MOLECULAR DOCKING STUDY OF 2',4,4'-TRIHYDROXY-3'–(METHYL-2-BUTENYL) CHALCONE AGAINST CANCER TARGETED PROTEIN

Molecular docking is generally used to detect the protein-ligand orientation and interaction. Auto Dock Tools package version 1.5.6 was utilized to create the docking input files. The grid region was surrounded by the active site for binding. So, grid region was selected on the basis of amino acid residues representing the binding site of 5-Fluoro uracil as the standard drug obtained from PDB with ID- 1FP2 and considered as the best active region for the favourable interaction. The grid box was set at 126 × 126 × 126 A˚ for x, y and z axis and covered the active site of the target protein. The Lamarckian Genetic Algorithm (LGA), a local search algorithm was utilized for ligands conformers searching. During the docking process, a maximum of 10 conformers were considered for the compound. After completion of the conformer the lowest binding energy was chosen. The conformational similarity by visualizing the binding site and its energy (Kcal/mol) and the docked amino acid residues forming hydrogen bonds and other parameters like intermolecular energy (Kcal/mol) and inhibition constant (μM) were analysed by Auto Dock tool. Ten best poses were generated for the ligand and scored using Auto Dock 1.5.6 scoring functions. Based on the docked energy the ligand was selected for anti cancer activity. The ligand interacting residues with the target protein were analyzed using Auto Dock tools, Molegro Molecular viewer.

Results & Discusion

From the previous literature review of the docking results of a standard ligand (5-Fluoro uracil) had a binding energy of -4.67 kcal/mol. The hydrogen bond forming amino acid residues of the target protein was found to be Leu 103 and Val 37. Excluding hydrogen interaction, the ligand was also formed nine electrostatic interaction with the amino acids.

The ligand **(2',4,4'-trihydroxy-3'–(methyl-2-butenyl) chalcone)** was predicted to have better binding energy of – 9.56 kcal/mol as compared to standard drug. It forms

hydrogen bond interactions with amino acid residues of the target protein Asn 165, Asp 239, Asp 219, Gly 196 (fig 19 & 20).

Chalcone O-methyltransferase (ChOMT) and isoflavone O-methyltransferase (IOMT) are potent targets for antitumour activity and are SAM dependent methyltransferases whose crystal structures with flavonoids have been determined. The crystal structure of ChOMT in complex with the product S-adenosyl-l-homocysteine and the substrate isoliquiritigenin (4,2',4'-trihydroxychalcone) as well as the crystal structure of IOMT in complex with the products S-adenosyl-l-homocysteine and isoformononetin (4'-hydroxy-7- methoxyisoflavone) have been determined.[56]

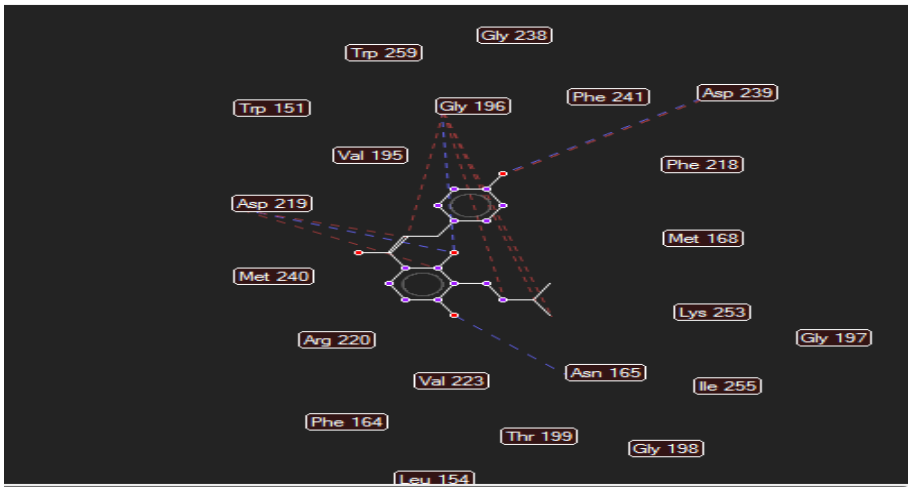


Fig.no 19.Number of amino acid interaction

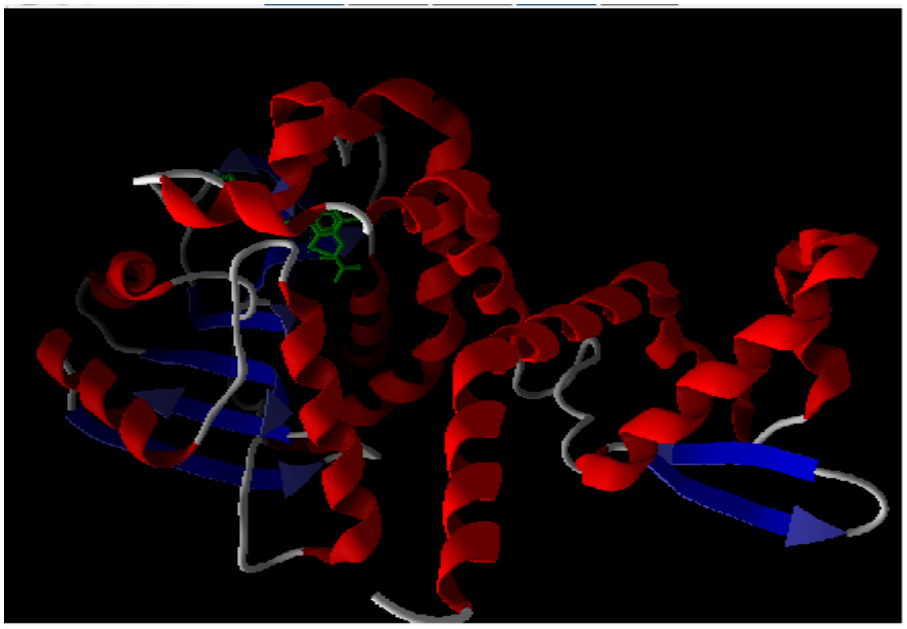


Fig.no 20.Protein ligand interaction

IN VITRO ANTIDIABETIC ACTIVITY OF 2′, 4, 4′ - TRIHYDROXY-3′-(3-METHYL-2-BUTENYL) CHALCONE AGAINST ALPHA GLUICOSIDASE

PROCEDURE:

Alpha - Glucosidase Inhibition assay:

The effect of the plant extracts on α-glucosidase activity was determined using α-glucosidase enzyme. The substrate solution p-nitro phenyl glucopyranoside (pNPG) was prepared in 20mM phosphate buffer, and pH 6.9. 100 µL of α-glucosidase was pre-incubated with 2.5ml of the different concentrations of the extracts for 10min. Then 50 µL of 3.0mM (pNPG) as a substrate dissolved in 20mM phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37 ˚C for 20min and stopped by adding 0.5mL of Na_2CO_3 (0.1M). The yellow-coloured reaction mixture, 4-nitrophenol, released from pNPG was measured at 405nm using UV - VIS spectrophotometer [57] voglibose was used as a positive control and the inhibitory activity of α-glucosidase was calculated using the following formula,

% Inhibition = [(Abs Control - Abs Sample) / Abs Control] x 100.

Table no: 8. Effect of 2′, 4, 4′ -Trihydroxy-3′-(3-methyl-2- butenyl)chalcone against alpha glucosidase

Concentration in µg level	Standard (voglibose)	Percentage inhibition
10	11.28809	4.466759
20	35.07618	30.87262
40	66.23961	42.98427
80	81.6482	65.23876
160	92.20914	75.23854
320	94.49446	80.57829

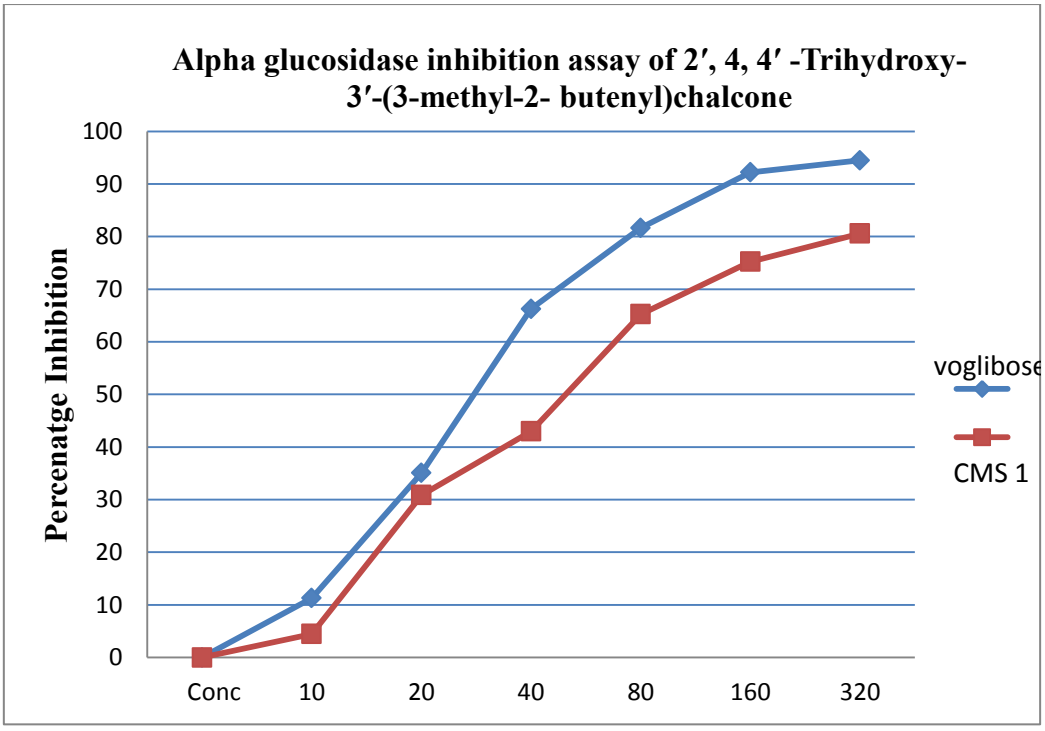


Fig: no. 21. Alpha glucosidase inhibition assay of 2′, 4, 4′ -Trihydroxy-3′-(3-methyl-2- butenyl) chalcone

Discussion:

Alpha glucosidase inhibition assay of ***2', 4, 4' -Trihydroxy-3'-(3-methyl-2-butenyl)chalcone***

As illustrated in table 8 & fig 21, the isolated compound, was evaluated against alpha glucosidase inhibition assay. It was found that ***2', 4, 4' -Trihydroxy-3'-(3-methyl-2-butenyl) chalcone*** showed good anti diabetic activity with a percentage inhibition of 80.57% at 320µg compared to the standard voglibose with a percentage inhibition of 94.49 at 320 µg.

Discussion

2', 4, 4' -Trihydroxy-3'-(3-methyl-2- butenyl) chalcone is a class of flavonoids, possess good antidiabetic activity. Chalcones are secondary metabolites of terrestrial plants and precursors of the flavonoids biosynthesis, have been used for a long time in traditional medicine due to their wide-range of biological activities, from which the anti-diabetic activity stands out. Chalcones are able to exert these properties by acting in different therapeutic targets:

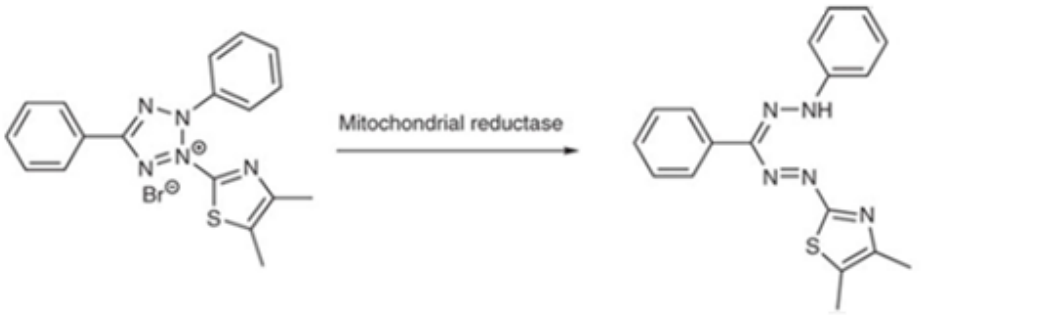
dipeptidyl peptidase 4 (DPP-4); glucose transporter type 4 (GLUT4), sodium glucose cotransporter 2 (SGLT2), α-amylase, α-glucosidase, aldose reductase (ALR), protein tyrosine phosphatase 1B (PTP1B), peroxisome proliferator-activated receptor-gamma (PPARγ) and adenosine monophosphate (AMP)-activated protein kinase (AMPK). Chalcones are, undoubtedly, promising anti-diabetic agents, and some crucial structural features have already been established. From the structure-activity relationships analysis, it can generally be stated that the presence of hydroxyl, prenyl and geranyl groups in their skeleton improves their activity for the evaluated antidiabetic targets [58].

IN VITRO ANTICANCER ACTIVITY OF 2′, 4, 4′ -TRIHYDROXY-3′-(3-METHYL-2- BUTENYL)CHALCONE AGAINST LIVER CANCER CELLS (Hep G2)

Anticancer Activity studies using MTT

Introduction

The *in vitro* determinations of toxic effects of unknown compounds have been performed by counting viable cells after staining with a vital dye. Alternative methods used are measurement of radioisotope incorporation as a measure of DNA synthesis, counting by automated counters and others which rely on dyes and cellular activity. The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases. The MTT method is simple, accurate and yields reproducible results. The key component is (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) or MTT, is a water soluble tetrazolium salt yielding a yellowish solution when prepared in media or salt solutions lacking phenol red. Dissolved MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of viable cells. This water insoluble formazan can be solubilized using DMSO, acidified isopropanol or other solvents (Pure propanol or ethanol). The resulting purple solution is spectrophotometrically measured. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the test material.



(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide))

MTT

(2E,4Z)-(4,5-Dimethylthiazol-2-yl)-3,5-diphenylformazan

(Formazan)

Materials & Methods

1. MTT reagent (the solution is filtered through a 0.2 μm filter and stored at 2–8 °C for
frequent use or frozen for extended periods)
2. DMSO
3. CO_2 incubator
4. Micro Plate reader
5. Inverted microscope
6. Refrigerated centrifuge

Preparation of test solutions

For Anticancer studies, serial two fold dilutions (200-1000μg/ml) were prepared from this for carrying out Anticancer studies.

Cell lines and culture medium

Liver cancer (HepG2) cell lines were procured from NCCS, stock cells was cultured in medium supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 μg/ml) in an humidified atmosphere of 5% CO_2 at 37^oC until confluent. The cell was dissociated with TPVG solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The viability of the cells are checked and centrifuged. Further 50,000 cells / well was seeded in a 96 well plate and incubated for 24 hrs at 37^oC, 5% CO_2 incubator. [59]

Source of reagents: DMEM, FBS, Pen strip, Trypsin procured from Himedia.

Procedure

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100μl of the diluted cell suspension (50,000cells/well) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100μl of different test concentrations of test drugs were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37^oC for 48hrs in 5% CO_2 atmosphere. After incubation the test solutions in the wells were discarded and 100μl of MTT (5 mg/10 ml of MTT in PBS) was added to each well. The plates were incubated for 4h at 37^oC in 5% CO_2 atmosphere. The supernatant was removed and 100μl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm. The

percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) values is generated from the dose-response curves for each cell line. [60]

IC_{50} Value:

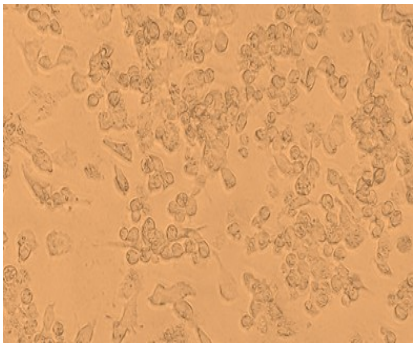
The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half.

The IC_{50} of a drug can be determined by constructing a dose-response curve and examining the effect of different concentrations of antagonist on reversing agonist activity. IC50 values can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist. IC_{50} values for cytotoxicity tests were derived from a nonlinear regression analysis (curve fit) based on sigmoid dose response curve.

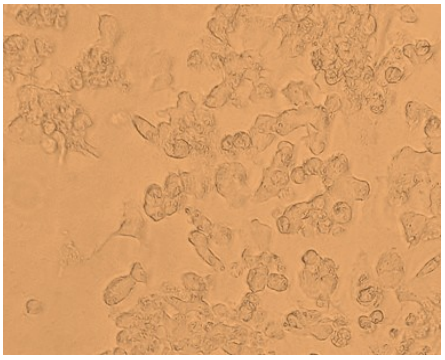
The direct Microscopic Observations of Drug Treated Images of Cell lines by Inverted Biological Microscope was enclosed with the report.

Table: no 9 Anticancer activity of 2′, 4, 4′ -Trihydroxy-3′-(3-methyl-2-butenyl) chalcone against liver cancer cell (Hep G2)

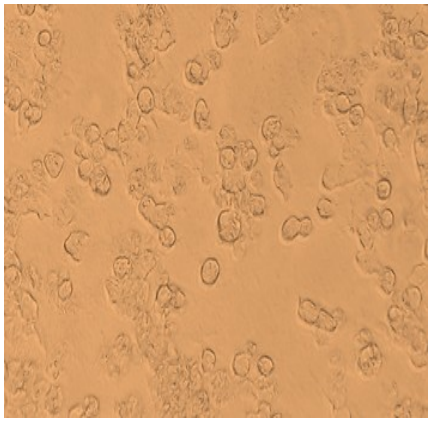
Concentration Unit: µG	**Isolated compound**	**Standard (5-Fluoro Uracil)**
3.12	63.52	48.73
6.25	54.81	40.04
12.5	52.78	30.58
25.00	40.96	20.81
50.00	29.88	14.52
100	18.75	5.29
IC_{50} value	**10.57**	**2.73**



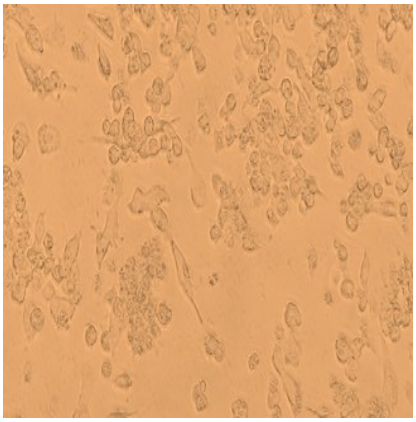
3.12µg



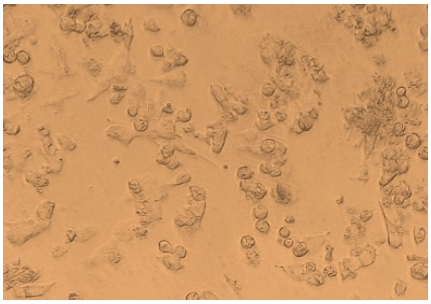
6.25µg



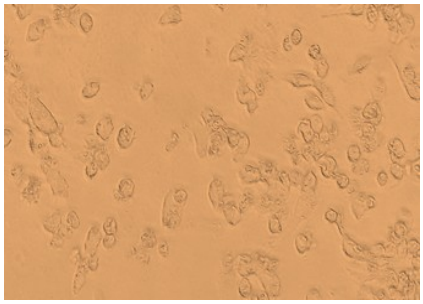
12.5 µg



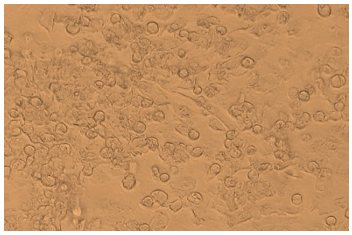
25µg



50 µg

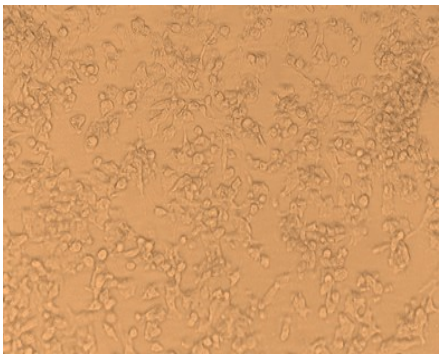


100 µg

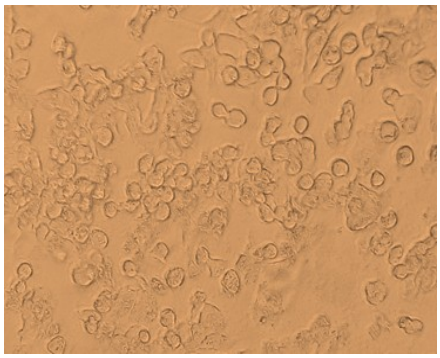


control

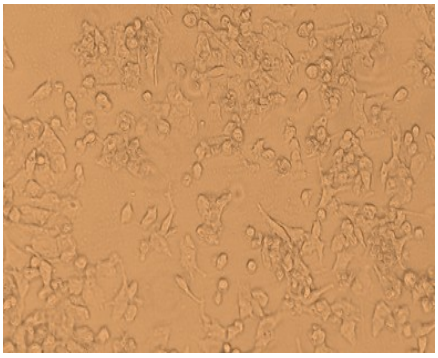
Fig: no 22. Percentage cell growth of Liver cancer cells on treatment with 2′, 4, 4′ -Trihydroxy-3′-(3-methyl-2-butenyl)chalcone at various concentrations.



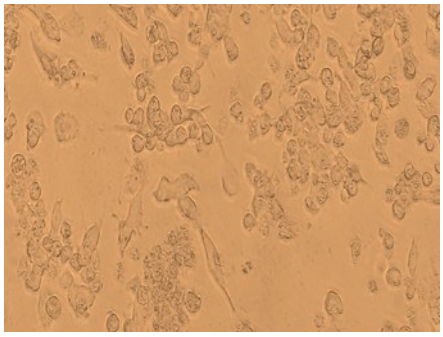
3.12µg



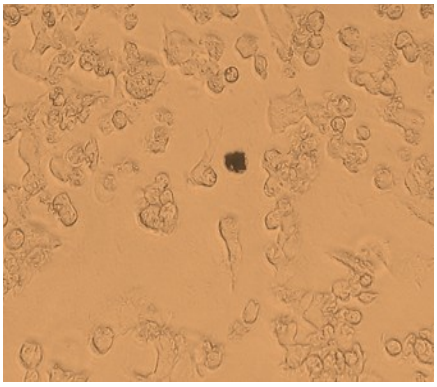
6.25 µg



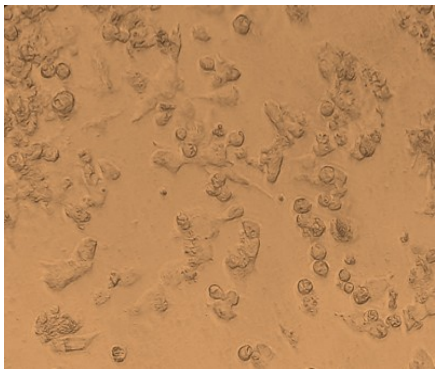
12.5µg



25 µg



50µg



100 µg

Fig: no 23. Percentage cell growth of Liver cancer cells on treatment with 5 - Furo Uracil at various concentrations.

In vitro Anticancer Activity of 2′, 4, 4′ -Trihydroxy-3′-(3-methyl-2- butenyl) chalcone

MTT Assay

As illustrated in table 9, *in vitro* anticancer activity was carried out for the isolated compound **2′, 4, 4′ -Trihydroxy-3′-(3-methyl-2- butenyl) chalcone** against liver cancer cell line (HepG2). The percentage viability at 100µg of the isolated compoundwas found to be 18.75 and an IC_{50} value of 10.57. The **2′, 4, 4′ -Trihydroxy-3′-(3-methyl-2- butenyl) chalcone** shows good anticancer activity against liver cancer cell line. The standard drug 5 flurouracil shows greater anticancer activity than the isolated compound with an IC_{50} value of 2.73 and percentage viability of 5.29 at 100 µg. The cell growth against various concentrations used for the compound and standard were depicted in fig 22 & 23.

Chalcones mainly belongs to flavonoids family and known as open chain flavonoid in which two aromatic rings A and B structurally joined by α,β-unsaturated carbonyl system. Plants containing chalcones have been used traditionally as anti-inflammatory, antioxidant, antimalarial, antimicrobial, antifungal, antitubercular, cytotoxic, antiviral, antitumor and chemopreventive agent.

The IC_{50} values for prenylated chalcone and having wide range of biological activities including anticancer, it ranged from 0.20 µM (towards CCRF-CEMcells) to 195.12 µM (towards leukemia CEM/ADR 5000 cells) for doxorubicin. It induces apoptosis in CCRF-CEM leukemia cells, mediated by caspase activation and the disruption of MMP[61].

ACUTE TOXICITY ACTIVE ETHANOLIC EXTRACT OF *MERREMIA HEDERACEA* IN RATS

Determination of acute oral toxicity is usually the initial screening step in the assessment and evaluation of the toxic characteristics of all compounds. The types of toxicity tests which are routinely performed by pharmaceutical manufacturers in the investigation of a new drug involve acute, sub-acute and chronic toxicity. Acute toxicity is involved in estimation of LD_{50} (the dose which has proved to be lethal (causing death) to 50% of the tested group of animals).

The acute toxicity study of active ethanolic extract of *Merremia hederacea* was carried out as per (OECD) draft guidelines 423 adopted on 17th December 2001 received from Committee for the purpose of Control and Supervision of Experimental Animals (CPCSEA). The acute toxic class method is based on biometric evaluation with fixed doses, adequately separated to enable a substance to be ranked for classification purpose and hazard assessment [62].

Acclimatization of Animals:

Female Wistar rats (130-150g) were maintained under standard laboratory condition at the centre for experimental animals in Madras Medical College. Animal ethical Committee's clearance 1917/ReBi/S/16/CPCSEA/25.10.2016 was obtained for the study. After seven days of Acclimatization, the 3 animals were housed in labelled cages with solid plastic sides and floor with stainless steel grid tops. Animals were allowed free access to standard pellet diet and water *ad libitum*. They were maintained in controlled laboratory conditions of 12hours dark/light cycle, 22±2ºC temperatures and 45-60% humidity.

Administration of ethanol extract:

Three animals were used for the study. Animals made to fast prior to dosing (food was withdrawn overnight and water was withdrawn 3hrs before drug administration) following the period of fasting. The animals were weighed and the extract was administered in a single dose, as 1% suspension in carboxy methyl cellulose by oral intubation. Food was withheld for further one hour after the

administration of drug. The starting dose level was selected for the study with a dose of 2000mg/kg body weight.

Observations:

Animals were observed individually after dosing periodically during the first 30 minutes to first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days.

The time at which signs of toxicity appear and disappear was observed systematically and recorded for each animal. Additional signs of toxicity such as changes in bodyweight, skin and fur, eyes and mucus membranes, respiratory system, circulatory system, autonomous system and central nervous system, somatomotor activity and behaviour pattern were also recorded. Attention was given to observe the tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

The rats were observed regularly for 14 days to note the compound related mortality/morbidity or other toxic symptoms. Any mortality during the experiment for 14 days was observed and recorded. If no deaths were reported, the study was repeated with same dose to confirm the results.

Table: no 10. Results of acute toxicity in rats on administration of ethanol extract of *Merremia hederacea* (ETMH) at the dose of 2000mg/kg.

OBSERVATION	30 MINS	4 HOURS	24 HOURS	14th DAY
BODY WEIGHT	-	-	-	-
PRE TERMINAL DEATH	-	-	-	-
CAGE SIDE OBSERVATION	+	+	+	+
MOTOR ACTIVITY	+	+	+	+
CONVULSIONS	-	-	-	-
PILORECTION	-	-	-	-
RIGHTING REFLEX	+	+	+	+
LACRIMATION	-	-	-	-
SALIVATION	-	-	-	-
RESPIRATION	+	+	+	+
SKIN COLOUR	-	-	-	-
DIARRHOEA	-	-	-	-
GROOMING	-	-	-	-
SEDATION	-	-	-	-
EXCITATION	+	+	+	+
AGGRESSION	+	+	+	+

NOTE: + indicates presence and - indicates absence.

Discussion:

The ethanolic extract of *Merremia hederacea* (ETMH) was found to be non-toxic upto the dose of 2000mg/kg and did not cause any death of the tested animals and the LD_{50} value is expected to exceed 2000mg/kg/body weight.

Since there was no death and behavioural changes observed. The extract was considered as safe to administer. From this $1/10^{th}$ and $1/5^{th}$ of the dose (200mg and 400mg) were selected for *in vivo* studies.

IN VIVO ANTIDIABETIC ACTIVITY OF ETHANOL EXTRACT OF *MERREMIA HEDERACEA* IN RATS

ACCLIMATIZATION OF ANIMALS:

Animals of either sex of wistar rats (7 to 8 week; 150–190 g), maintained in sanitized polypropylene cages (6 per cage) in air conditioned rooms (23 ± 2∘ C, 35–60% humidity with 12 h light-dark cycle), were obtained from Animal house, Madras Medical College. The rats were fed with pellet diet and water ad libitum. Prior approval was obtained from the Institutional Animal Ethical Committee (1917/ReBi/S/16/CPCSEA/25.10.2016). They were maintained in controlled laboratory conditions of 12hours dark/light cycle, 22±2ºC temperatures and 45-60% humidity.

GROUPING OF ANIMALS AND TREATMENT

30 Wistar rats of either sex (weighing about 150-190g) were selected for this experiment.

Table: no 11. Grouping of animals and Treatment

GROUP	DRUG	TREATMENT	NO. OF ANIMALS
1	Control	Normal saline	**6**
2	Disease control	Streptozotocin 60mg/kg	**6**
3	Standard	Glibenclamide 5mg/kg	**6**
4	Test Dose 1	Extract – Low dose (200mg/kg)	**6**
5	Test Dose 2	Extract – High dose(400mg/kg)	**6**
		Total	**30**

Preparation of 0.1 M Citrate Buffer.

Accurately weighed quantity of trisodium citrate (1.49 g) was dissolved in sufficient milli Q water to produce 100 mL and was adjusted to pH 4.5 with HCl.

Preparation of STZ and Nicotinamide Solution.

STZ is freely soluble in water and saline, but it is unstable in both. It is stable in 0.1 M citrate buffer. A solution of STZ of appropriate strength (depending on the required total dose per animal) was prepared by dissolving weighed quantity of STZ in freshly prepared ice cold citrate buffer and was administered i.p. in volumes of ~2 mL/kg (dose: 60 mg/kg). STZ was freshly prepared because it is unstable. Nicotinamide was dissolved in normal saline to yield a strength that is appropriate for administration .

Experimental induction of diabetes:

Diabetes was induced by STZ (60 mg/kg), 15 min after the intraperitoneal administration of nicotinamide (120 mg/kg). Diabetic rats were permitted to intake of 10% glucose solution overnight to overcome the initial drug induced hypoglycaemic death. After 7 days rats showing FBG between 180 and 220 mg/dL were considered diabetic and included in the study. Active extract and standard drug were administered orally by gastric intubation using a force feeding needle to the respective groups of rats for 21days.

Biochemical estimation:

After overnight fasting the blood was withdrawn from the tail vein about 0.1 mL and fasting blood glucose level was checked by using accu-check glucometer on 1, 7, 14 and 21day period. Animal body weight is also recorded before and after the treatment. At the end of the experiment 21st day, rats were fasted overnight and blood samples were withdrawn through the retro-orbital plexus (2 to 5 mL) under light isoflurane anaesthesia using a glass capillary tube and collected in a Vacutainer blood collection tubes. Blood was allowed to clot and serum separated by centrifugation at 4000 rpm for 10 minutes. LDL-C and HDL-C were measured by enzymatic colorimetric methods using commercial kits.

After the collection of blood samples animal was sacrificed and pancreas was isolated for histopathological studies.[63]

Parameters:

- Fasting Blood glucose level
- Body weight
- Serum HDL-C and LDL-C

Statistical analysis

Data is reported as Mean ± SEM. Statistical comparisons were determined by analysis of variance (ANOVA) and means were separated using Dunnett's t- test.

Table: no: 10 Effect of ethanol extract of *Merremia hederaceae* (ETMH) on fasting blood glucose level in STZ induced diabetic rats

Treatment	Day 0	Day 1	Day 7	Day 14	Day 21
Normal control (Saline)	98±2.42	99±2.12	100±2.03	103±1.76	99±2.21
Disease control (STZ Induced)	100±2.82	275±4.33 **	286±3.46	295±4.27	303±3.99 **
Positive control STZ + Glibenclamide	102±1.41	212±3.86 ***	141±2.84 **	119±2.95 ***	99±2.33 ***
STZ + extract Low dose (200mg/kg)	103±2.45	231±2.55 ***	160±2.18 ***	123±2.97 ***	106±2.85 ***
STZ + extract High dose (400mg/kg)	105±1.64	209±3.70 ***	157±2.73 ***	123±1.65 ***	102±1.84 ***

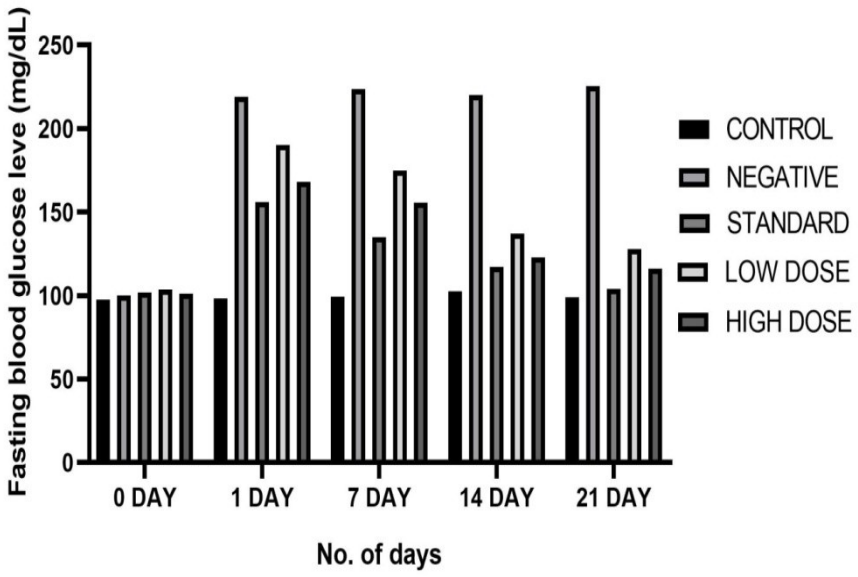
All values are expressed as mean ± SEM

*** P<0.001 vs Group II, significant by ANOVA followed by Dunnett's t – test (At 200 & 400mg/kg (ETMH), Negative control.

** P<0.01 vs Group III, significant by ANOVA followed by Dunnett's t – test. (Negative control).

*** P<0.001 There was a significant increase of FBG in STZ induced group of rats when compared with normal control group.

P<0.001, P<0.01, P<0.001 Treatment with positive control, low dose and high dose of ETM,H groups showed significant decrease in FBG level when compared with negative control group(STZ induced)



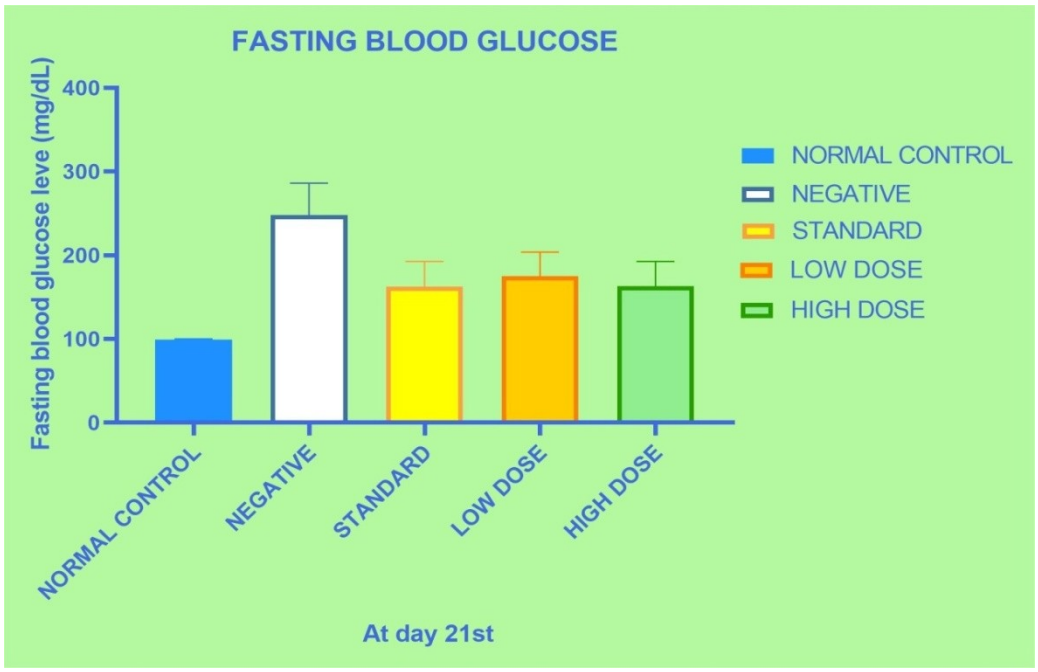


Fig no: 17 Effect of ETMH on fasting blood sugar level in STZ induced diabetic rats

Table no 11. Effect of ETMH on body weight in STZ induced diabetic rats

Treatment	Day 0	Day 1	Day 7	Day 14	Day 21
Normal control (Saline)	165±2.47	168±2.77	171±2.46	175±2.84	180±2.33
Disease control (STZ Induced)	163±2.17	157±1.78	146±1.02	129±1.11	113±1.51 **
Positive control STZ + Glibenclamide	166±2.67	172±2.57	179±3.28	187±2.86	201±3.83 ***
STZ + extract Low dose (200mg/kg)	161±1.55	163±2.11	174±2.12	185±1.98	189±1.23 **
STZ + extract High dose (400mg/kg)	165±3.59	169±.978	176±4.45	188±4.43	193±4.49 ***

All values are expressed as mean ± SEM

At 400mg/kg (ETMH), *** P<0.001 vs Group III, Significant by ANOVA followed by Dunnett's t – test.

The result showed that,

There was significant ** ($p< 0.01$) reduction in body weight in negative control group compared with normal control group

*** ($p<0.001$), ** ($p<0.01$), ***($p<0.001$) for group III, IV and V respectively. Here showed significant increase in body weight compared with disease control. When treatment with positive control low dose and high dose of ETMH

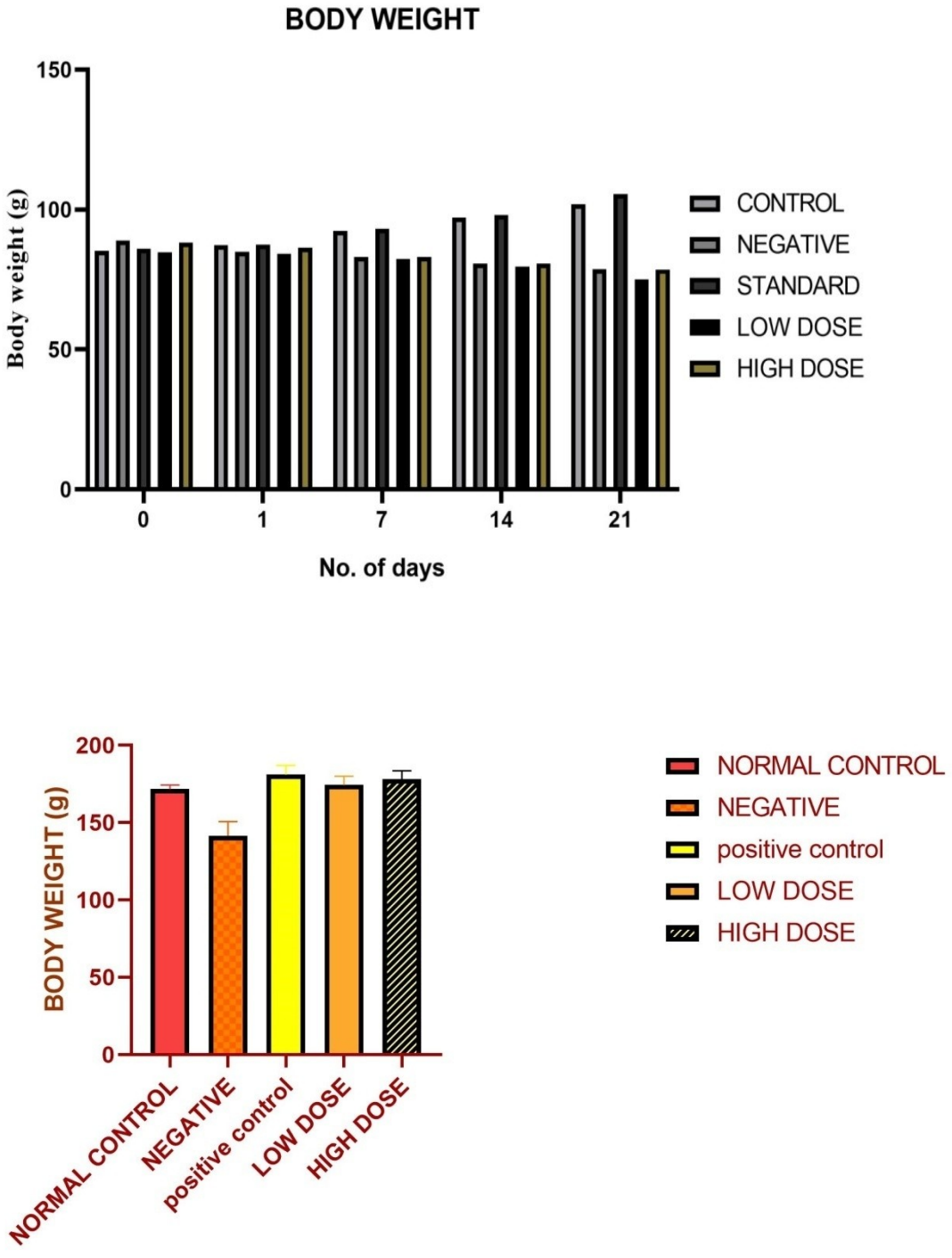


Fig no: 18 Effect of ETMH on body weight in STZ induced diabetic rats

Table no 12. Effect of ETMH on LDL-C and HDL-C in STZ induced diabetic rats on 21st Day

Test	LDL-C	HDL-C
Normal control	88±1.68	60±1.40
Diabetic control STZ induced	178±2.39 ***	27±1.08 **
Standard STZ + Glibenclamide	83±4.1.73***	58±1.67 ***
STZ + ETMH (200mg/kg)	103±1.52 **	34±1.20 **
STZ + ETMH (400mg/kg)	94±1.42 ***	54±1.65***

All values are expressed as mean ± SEM

LDL Cholesterol

At 400mg/kg of ETMH *** P<0.001 vs group III (positive control), significant by ANOVA followed by Dunnett's t – test

At 200mg/kg of ETMH ** P<0.01 vs group III (positive control), significant by ANOVA followed by Dunnett's t – test

HDL Cholesterol

At 400mg/kg ***P<0.001, vs Group III, significant by ANOVA followed by Dunnett's t – test.

At 200mg/kg ETMH ** P<0.01 vs Group III, significant by ANOVA followed by Dunnett's t – test

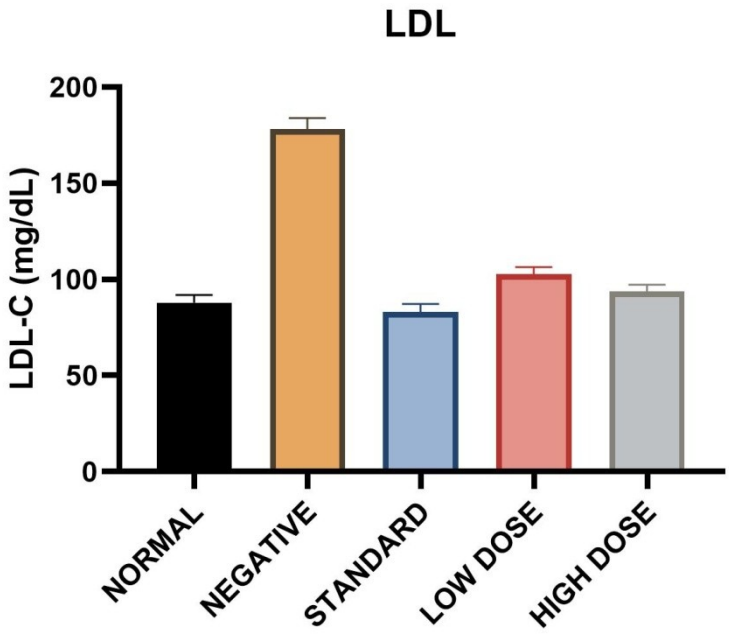


Fig no. 19: Effect of ETMH on LDL-C in STZ induced diabetic rats on 21st Day

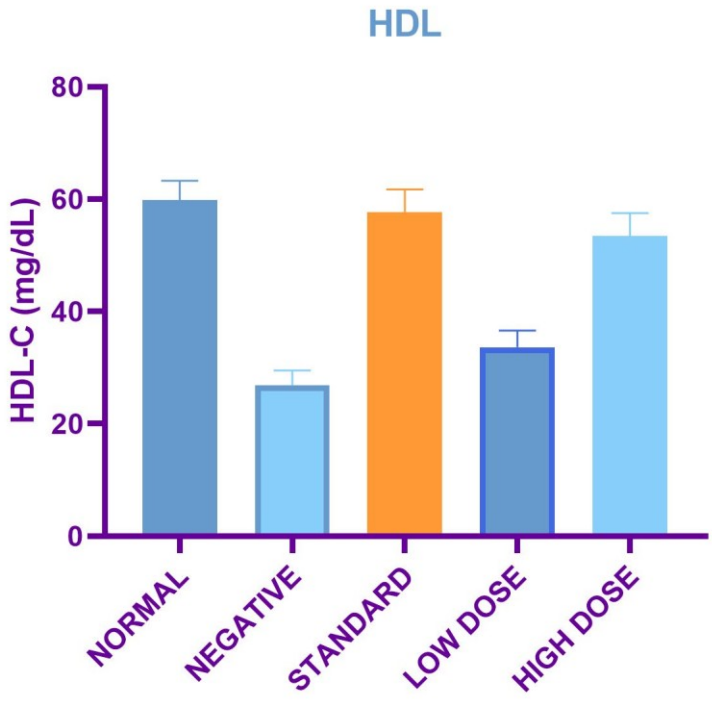


Fig no. 20: Effect of ETMH on HDL-C in STZ induced diabetic rats on 21st Day

Results and Discussion

Effect of ETMH in fasting blood sugar level in STZ induced diabetic rats.

Administration of STZ resulted in significant ($P < 0.001$) increase in mean blood glucose level on day 1, 7, day 14 & 21. Post treatment with an ETMH in STZ induced rats significantly reduce ($P < 0.001$) the increase of blood glucose level as compared to group II. The Administration of ETMH produces dose dependent effect as the high dose (400mg/kg) shows more significant anti diabetic activity than 200mg/kg. Treatment with glibenclamide after induction of diabetes significantly ($P < 0.001$) reduced the increased blood glucose level as compared to group II (Table no.12, Fig: no. 24)

Discussion

The objective of treatment in diabetic patient is to lower blood glucose to normal level. In the present study, ETMH showed significant lowering of blood glucose level, an index of diabetic control. STZ-induced diabetes is one of the widely used animal models that mimic the human diabetes mellitus. In addition, STZ generates potential free radicals such as nitric oxide (NO) by intracellular metabolism of STZ and precipitate further β-cells DNA damage by strand break.

The concentration of blood glucose was significantly increased in STZ induced diabetic as compared to normal control. Administration of ETMH (200 mg/kg and 400 mg/kg) significantly reduced the raised blood glucose level in STZ induced diabetic rats and the lowering was almost comparable to glibenclamide 5mg/kg.) (Table no.12). Further, this antidiabetic activity of ETMH was associated with an increase in the serum insulin level revealed that ETMH may stimulate insulin secretion from regenerated β cells and remaining β-cells. The blood glucose lowering effect of the ETMH could be due to the presence of flavonoid and phenolic compounds as reported in the preliminary phytochemical screening. These findings were also supported by the previous experimental findings wherein they reported the blood glucose lowering effect of flavonoids.[64,65]

Effect of ETMH in Body weight in STZ induced diabetic rats.

Table 13, fig 25, describes the changes in the body weight of the control and experimental rats treated with ETMH and glibenclamide. The body weight of the STZ-induced diabetic rats was lowered than that of the normal control groups at 7, 14 & 21st day. But the administration of ETMH of 400mg/kg, significantly increase of the body weight on 7,14, 21st day compared to standard glibenclamide.

Low Density Lipoprotein (LDL)

As illustrated in table 14 & fig 26, there was a significant rise (P< 0.001) in LDL level in STZ induced diabetic rats when compared to normal group. The administration of glibenclamide has significant (P< 0.001) lowering of LDL level at 21st day. The administration of ETMH, have showed a significant lowering (P < 0.001) of LDL level when compared to positive control.

Patients with type 2 diabetes show reduced turn-over of their LDL particles with a reduction of catabolism, leading automatically to increased LDL plasma residence time. A^|^|6\6\6\ ugmented LDL residence time in plasma is likely to promote cholesterol deposition in the arterial wall

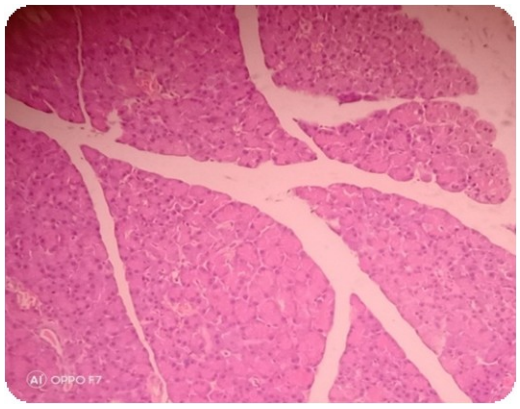
High Density Lipoprotein (HDL)

As illustrated in table 14 & fig 27, there was a significant decline (P< 0.001) in HDL level in STZ induced diabetic rats when compared to normal group. The administration of glibenclamide has significant (P< 0.001) increase of HDL level compared to group II. The administration of ETMH at 400mg/kg has showed a significant increase (P < 0.001) of HDL level on 21st day when compared to positive control.

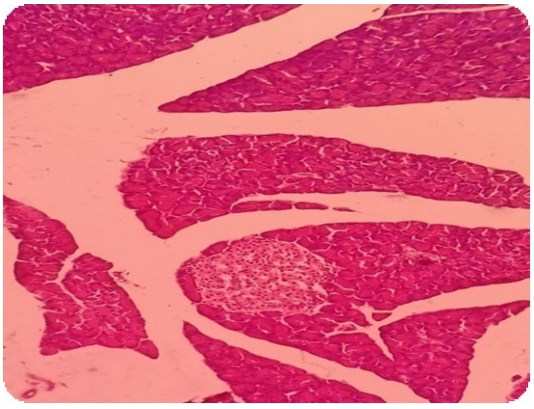
Type 2 diabetes is associated with decreased plasma HDL cholesterol levels related to reduction of the HDL 2 subfraction. Reduced HDL 2 level, in type 2 diabetes, has been shown to be correlated with both hypertriglyceridaemia and obesity. The decrease in HDL cholesterol, noted in patients with type 2 diabetes, is due to increased catabolism of HDL particles.[66]

HISTOPATHOLOGICAL STUDIES

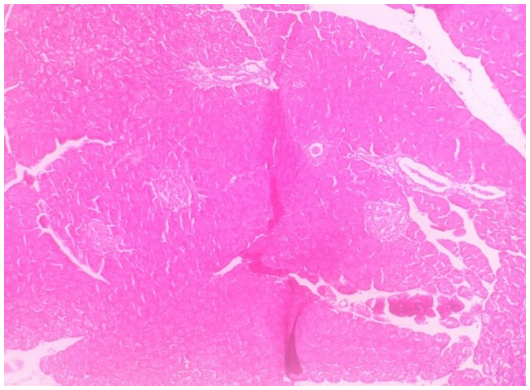
At the end of 21st day experiment, the selected rats were anesthetized by high dose of isoflurane and the animals were scarified and abdominally dissected. A portion of pancreatic tissue was dissected out and fixed in 10% buffered neutral formalin for 24 hours. After fixation the tissues were embedded in paraffin wax. About 5 microns thickness sections were prepared with microtome and mounted on clean glass slides and then they were de paraffinized in xylene twice for 5 minutes and then rehydrated with graded alcohol and stained with haematoxylin and eosin (H&E) dye. The stained sections were examined and photographed by using microscope.[67]



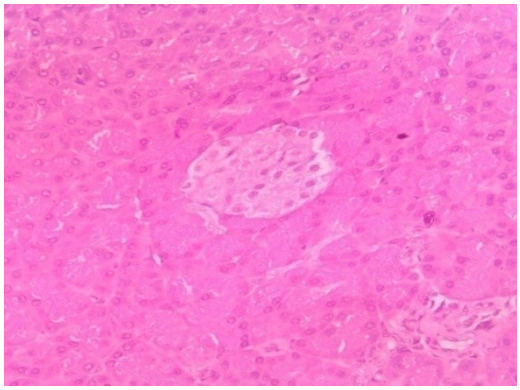
STZ Induced Diabetic rat



Standard (Glibenclamide



Low Dose (200mg/kg)



High Dose (400mg/kg)

Fig: no 28. Histopathology of STZ Induced Diabetic Rats Pancreas

Results:

STZ induced diabetic rat Pancrease:

Streptozotocin treated groups showing reduce islet of Langerhans. Low dose (200mg/kg), High dose (400mg/kg) and Glibenclamide (standard) treated groups showing recovery of islet of Langerhans. The number of islets cells was comparatively higher in 400mg/kg than low dose 200mg/kg. Hence the number of islets cells increases in dose dependent manner.

SUMMARY

In this present research, A potent anti diabetic plant Merremia hederacea has been selected. The literatures pertaining to phytochemical and the antidiabetic activity of this plant are very scarce. Hence an attempt has been made to evaluate the in vitro and in vivo anti diabetic activity of Merremia hederacea and to isolate the active constituent responsible for anti diabetic activity. The isolated pure compound has been attempted for molecular docking analysis.

Soxhlet Extraction & Preliminary Phytochemical Screening

The dried powder of leaves was extracted sequentially by hot continuous percolation method by soxhlet apparatus using Petroleum ether, chloroform, ethyl acetate and ethanol as solvent. Among all extracts, ethanol extract has the highest percentage yield of 18.65%. Preliminary phytochemical screening were carried out and found that ethanol extract shows the presence of glycosides, flavonoids, tannins and Phenolic compounds.

***In vitro* Antidiabetic activity**

All the extracts were subjected for *in vitro* anti diabetic activity against alpha amylase and alpha glucosidase. It was found in table 4 & 5, the ethanol extract shows greater percentage inhibition than other extracts in both enzyme assay with 91.44% and 85.50% respectively. The presence of flavonoids, phenolic compounds in ethanolic extract of *Merremia hederaceae* may act against diabetes mellitus. Mainly two carbohydrate hydrolyzing enzymes (α-amylase and α-glucosidase) are responsible for postprandial hyperglycemia. α-amylase begins the process of carbohydrate digestion by hydrolysis of 1, 4-glycosidic linkages of polysaccharides (starch, glycogen) to disaccharides and α-glucosidase catalyzes the disaccharides to monosaccharides, which leads to postprandial hyperglycaemia. Hence ethanol extract of *Merremia hederaceae* inhibits the α-amylase and α-glucosidase and control the hyperglycaemia as they delay carbohydrate digestion, which consequently reduce the postprandial plasma glucose level.

Isolation by Column Chromatography

Hence ethanol extract is bioactive than our extracts and considered for column chromatographic isolation, to isolate the anti diabetic component present in the extract. Wet packing method was followed and gradient elution technique was

adopted using various solvent systems such as Hexane, Hexane: chloroform, chloroform, chloroform: ethyl acetate, ethyl acetate, Ethyl acetate: methanol to separate the eluate. When the mixture of solvent system used, the ratio of mixtures are prepared as 100%, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90. The reddish brown band was eluted in fraction 25 -29 in the solvent system ethyl acetate: methanol: glacial acetic acid (60.30:10). The fractions 25 -29 were tested in TLC with various solvent systems. The fractions 25- 29 show similar R_f values and shows single spot and were mixed together and evaporated to yield an amorphous compound. The compound is a yellow amorphous powder. FeCl test was performed and shows positive (dark green) and it is characterized by UV, IR 1H NMR & LCMS. Ultraviolet spectroscopy (MeOH) λ_{max} 253.0, 278.0 nm. Infra red spectroscopy (KBr Pellet) Aromatic O-H Stretching 3440.78 cm^{-1}, C =O (Chelated carbonyl group) 1643.23 cm^{-1}, CH_3 1342.36 cm^{-1}, CH – Aliphatic 2877 cm^{-1}, Ketones (Carbonyl group) 1728 cm^{-1}

The molecular formula was determined to be $C_{20}H_{20}O_4$ from its HPLC + PDA+ MS with a molecular ion peak, $[M]^+$ at 325.10 $[M+ H]^+$ and other fragments at 269.95, 369.05. The molecular ion peak and fragments matched with a compound, **2′,4,4′-Trihydroxy-3′-(3-methyl-2-butenyl)-chalcone.**

^{1}H NMR (Bruker DMSO 500 MHz): δ 1.642 (3H, s), δ 1.753 (3H, s), δ 3.25 (2H, d), δ 5.201 (1H, t), δ 6.501 (1H, d), δ6.850 (2H, d), δ7.254 (1H, d), δ 7.710 (1H, d), δ 7.772 (2H, d), δ 8.131, δ 10.12 (1H,s), δ2.50 (DMSO solvent peak.). Hence the isolated compound from the ethanol extract of *Merremia hederacea* is elucidated as **2′,4,4′- Trihydroxy-3′-(3-methyl-2-butenyl)-chalcone.**

Molecular docking study of 2',4,4'-trihydroxy-3'–(methyl-2-butenyl) chalcone against diabetic target protein (alpha glucosidase)

Molecular docking study of 2',4,4'-trihydroxy-3'–(methyl-2-butenyl) chalcone was determined against the target with the PDB ID 3L4W. In this docking study, we present an approach to control blood glucose levels in individuals with type 2 diabetes by targeting maltase- glucoamylase and intestinal glucosidases using a ligand (2',4,4'-trihydroxy-3'–(methyl-2-butenyl) chalcone) isolated from *Merremia hederacea*. One of the intestinal glucosidases targeted the N-terminal catalytic domain of maltase-glucoamylase (ntMGAM) which is responsible for the hydrolysis of terminal starch

products into glucose. Hence to slow down the glucose level and targeted the ntMGAM. Previously acarbose and miglitol were found to be potent inhibitors alpha glucosidase having poly hydroxy groups in their structure . Hence novel ligand (2',4,4'-trihydroxy-3'–(methyl-2-butenyl) chalcone) which has three hydroxyl groups and is chalcone based isolated from *Merremia hederacea* was docked for the binding sites of alpha glucosidase.

From the previous literature review of the docking results of a standard ligand (Miglitol) had a binding energy of -8.04 kcal/mol. The hydrogen bond forming amino acid residues of the target protein was found to be His 626 and Asp 232.Excluding hydrogen interaction, the ligand was also formed nine electrostatic interaction with the amino acids.

The ligand (**2',4,4'-trihydroxy-3'–(methyl-2-butenyl) chalcone**) was predicted to have better binding energy of –11.28 kcal/mol as compared to standard drug. It forms hydrogen bond interactions with amino acid residues of the target protein, Lys534, Ala 537, Lys 776, Arg 520, Gly 533(fig 17 & 18)

Molecular docking study of 2',4,4'-trihydroxy-3'–(methyl-2-butenyl) chalcone against cancer target protein.

From the previous literature review of the docking results of a standard ligand (5-Fluoro uracil) had a binding energy of -4.67 kcal/mol. The hydrogen bond forming amino acid residues of the target protein was found to be Leu 103 and Val 37.

The ligand (**2',4,4'-trihydroxy-3'–(methyl-2-butenyl) chalcone**) was predicted to have better binding energy of – 9.56 kcal/mol as compared to standard drug. It forms hydrogen bond interactions with amino acid residues of the target protein Asn 165, Asp 239, Asp 219, Gly 196. (fig 19 & 20)

Chalcone O-methyltransferase (ChOMT) and isoflavone O-methyltransferase (IOMT) are potent targets for antitumour activity and are SAM dependent methyltransferases whose crystal structures with flavonoids have been determined.

Alpha glucosidase inhibition of 2′,4,4′- Trihydroxy-3′-(3-methyl-2-butenyl)-chalcone

The isolated compound, ***2', 4, 4' -Trihydroxy-3'-(3-methyl-2- butenyl)chalcone,*** was evaluated against alpha glucosidase inhibition assay as shown in table 8 & fig 21. It was found that the compound showed good anti diabetic activity with a percentage

inhibition of 80.57% at 320µg compared to the standard voglibose with a percentage inhibition of 94.49 at 320 µg.

2', 4, 4' -Trihydroxy-3'-(3-methyl-2- butenyl) chalcone is a class of flavonoids, possess good antidiabetic activity. Chalcones are secondary metabolites of terrestrial plants and precursors of the flavonoids biosynthesis, have been used for a long time in traditional medicine due to their wide-range of biological activities, from which the anti-diabetic activity stands out. Chalcones are, undoubtedly, promising anti-diabetic agents, and some crucial structural features have already been established. From the structure-activity relationships analysis, it can generally be stated that the presence of hydroxyl, prenyl and geranyl groups in their skeleton improves their activity for the evaluated antidiabetic targets.

In vitro **anticancer activity of 2′,4,4′- Trihydroxy-3′-(3-methyl-2-butenyl)-chalcone**

In vitro anticancer activity was carried out for the isolated compound **2′, 4, 4′ - Trihydroxy-3′-(3-methyl-2- butenyl) chalcone** against liver cancer cell line (HepG2) as shown in table 9. The percentage viability at 100µg of the isolated compound was found to be 18.75 and an IC_{50} value of 10.57. The **2′, 4, 4′ - Trihydroxy-3′-(3-methyl-2- butenyl) chalcone** shows good anticancer activity against liver cancer cell line. The standard drug 5 flurouracil shows greater anticancer activity than the isolated compound with an IC_{50} value of 2.73 and percentage viability of 5.29 at 100 µg. The cell growth against various concentrations used for the compound and standard were depicted in fig 22 & 23.

Chalcones mainly belongs to flavonoids family and known as open chain flavonoid in which two aromatic rings A and B structurally joined by α,β-unsaturated carbonyl system. Plants containing chalcones have been used traditionally as anti-inflammatory, antioxidant, antimalarial, antimicrobial, antifungal, antitubercular, cytotoxic, antiviral, antitumor and chemopreventive agent.

Acute toxicity study of ethanolic extract of *Merremia hederacea* (ETMH)

The active ethanolic extract of *Merremia hederacea* was found to be non-toxic upto the dose of 2000mg/kg and did not cause any death of the tested animals and the LD50 value is expected to exceed 2000mg/kg/body weight. Since there were no death and behavioural changes observed. The extract was considered as safe to administer.

From this $1/10^{th}$ and $1/5^{th}$ of the dose (200mg and 400mg) were selected for further studies.

In Vivo* anti diabetic activity of ethanolic extract of *Merremia hederacea

Determination of fasting blood sugar level in STZ induced diabetic rats.

Administration of STZ resulted in significant ($P < 0.001$) increase in mean blood glucose level on day 1, 7, day 14 & 21. Post treatment with an ETMH in STZ induced rats significantly reduce ($P <0.001$) the increase of blood glucose level as compared to group II. The Administration of ETMH produces dose dependent effect as the high dose (400mg/kg) shows more significant anti diabetic activity than 200mg/kg. Treatment with glibenclamide after induction of diabetes significantly ($P< 0.001$) reduced the increased blood glucose level as compared to group III (Table no.12, Fig: no. 24)

The concentration of blood glucose was significantly increased in STZ induced diabetic as compared to normal control. Administration of ETMH (200 mg/kg and 400 mg/kg) significantly reduced the raised blood glucose level in STZ induced diabetic rats and the lowering was almost comparable to glibenclamide 5mg/kg.) (Table no.10). Further, this antidiabetic activity of ETMH was associated with an increase in the serum insulin level revealed that ETMH may stimulate insulin secretion from regenerated β cells and remaining β-cells. The blood glucose lowering effect of the ETMH could be due to the presence of flavonoid and phenolic compounds as reported in the preliminary phytochemical screening.

Determination of Body weight in STZ induced diabetic rats.

Table 13, fig 25, represents the changes in the body weight of the control and experimental rats treated with ETMH and glibenclamide. The body weight of the STZ-induced diabetic rats was lowered than that of the normal control groups at 7, 14 & 21^{st} day. But the administration of ETMH of 400mg/kg, significantly increase of the body weight on 7,14, 21^{st} day compared to standard glibenclamide.

Determination of LDL & HDL in STZ induced diabetic rats.

There was a significant rise ($P< 0.001$) in LDL level in STZ induced diabetic rats when compared to normal group. The administration of glibenclamide has significant ($P< 0.001$) lowering of LDL level at 21st day as shown in table 14 & fig 26. The administration of ETMH, have showed a significant lowering ($P < 0.001$) of LDL level when compared to positive control.

Patients with type 2 diabetes show reduced turn-over of their LDL particles with a reduction of catabolism, leading automatically to increased LDL plasma residence time. Augmented LDL residence time in plasma is likely to promote cholesterol deposition in the arterial wall

High Density Lipoprotein (HDL)

There was a significant decline ($P< 0.001$) in HDL level in STZ induced diabetic rats when compared to normal group. The administration of glibenclamide has significant ($P< 0.001$) increase of HDL level compared to group II as shown in table 14 & fig 27, The administration of ETMH at 400mg/kg has showed a significant increase ($P < 0.001$) of HDL level on 21st day when compared to positive control.

Type 2 diabetes is associated with decreased plasma HDL cholesterol levels related to reduction of the HDL 2 subfraction. Reduced HDL 2 level, in type 2 diabetes, has been shown to be correlated with both hypertriglyceridaemia and obesity. The decrease in HDL cholesterol, noted in patients with type 2 diabetes, is due to increased catabolism of HDL particles.

Histopathological studies

Streptozotocin treated groups showing reduce islet of Langerhans. Low dose (200mg/kg), High dose (400mg/kg) and Glibenclamide (standard) treated groups showing recovery of islet of Langerhans. The number of islets cells was comparatively higher in 400mg/kg than low dose 200mg/kg. Hence the number of islets cells increases in dose dependent manner. (fig 28)

CONCLUSION

According to current educated guess, the global human population emerges to be in the midst of an epidemic of diabetes. In spite of the enormous steps that have been made in the perceptive and executive of diabetes, the disease and disease related problems are increasing spontaneously. Equivalent to this, current investigations and studies in understanding the pathophysiology of the disease process have opened up numerous novel avenues to recognize and expand new therapies to fight the diabetic condition. Simultaneously, phytochemicals bioactive compounds identified from traditional medicinal plants are presenting an exhilarating prospect for the improvement of novel therapeutics. This has step up the world wide effort to exploit and collect those medicinal plants that bear considerable amount of potential phytochemicals presenting multiple beneficial effects in combating with diabetes and diabetes-related difficulties. Therefore, as the disease is progressing alarming rates, there is an urgent need of identifying indigenous natural resources in order to procure them, and study in detail, their potential on different newly identified targets in order to develop them as new therapeutics. In these regard we have made an attempt to investigate the *in vitro* and *in vivo* anti diabetic activity of *Merremia hederacea*. The isolated pure compound has been attempted for molecular docking studies.

From all these observations it is concluded that ethanol extract of *Merremia hederacea* possess significant antidiabetic activity. The concentration of blood glucose was significantly increased in STZ induced diabetic as compared to normal control. Administration of ETMH (200 mg/kg and 400 mg/kg) significantly reduced the raised blood glucose level in STZ induced diabetic rats and the lowering was almost comparable to glibenclamide 5mg/kg.). The blood glucose lowering effect of the ETMH could be due to the presence of novel chalcone (**2',4,4'-trihydroxy-3'–(methyl-2-butenyl) chalcone)** which is isolated from the leaf of this plant by column chromatography in this study. The isolated compound is characterized by HPLC/PDA/MS, ^{1}HNMR & IR.

From the observations of molecular docking studies and *in vitro* anti diabetic and anti cancer activity of the isolated compound (**2',4,4'-trihydroxy-3'–(methyl-2-butenyl) chalcone)** it has been concluded that chalcones are, undoubtedly, promising anti-diabetic agents, and some crucial structural features have already been

established. From the structure-activity relationships analysis, it can generally be stated that the presence of hydroxyl, prenyl and geranyl groups in their skeleton improves their activity for the evaluated antidiabetic targets. The compound shows greater percentage inhibition for alpha glucosidase at 320µg compared to standard and has a good docking score of -11.28 $Kcal^{-1}$ against diabetic target protein, alpha glucosidase.

The **2′, 4, 4′ -Trihydroxy-3′-(3-methyl-2- butenyl) chalcone** shows good anticancer activity against liver cancer cell line (HepG2) compared to the standard 5 fluro Uracil. The compound has a docking score of – 9.56 kcal/mol against cancer target protein.

FUTURE PROSPECTS

- The isolated 2, 4, 4′ -Trihydroxy-3′-(3-methyl-2-butenyl) chalcone will be focused for lipid encapsulation nanotechnology and will be investigated in future for effective drug delivery system.

- The isolated 2, 4, 4′ -Trihydroxy-3′-(3-methyl-2-butenyl)chalcone will be evaluated in future for enzyme inhibition assay of Protein tyrosine Phosphatase 1b (the diabetic target protein).

- The isolated 2, 4, 4′ -Trihydroxy-3′-(3-methyl-2-butenyl)chalcone will be evaluated in future for in vitro anti tubercular activity.

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