# EXTRACTION, PHARMACOLOGICAL AND PHARMACOINFORMATIC EVALUATION OF ACTIVE CONSTITUENTS OF *DALBERGIA LATIFOLIA* AS POTENTIAL ANTIDIABETIC AGENTS

Thesis Submitted for the Award of the Degree of

**DOCTOR OF PHILOSOPHY**

**in**

**Pharmacology**

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## DECLARATION

I hereby declare that the presented work in the thesis entitled "**Extraction, Pharmacological, And Pharmacoinformatic Evaluation Of Active Constituents Of *Dalbergia latifolia* As Potential Antidiabetic Agents**" in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is the outcome of research work carried out by me under the supervision of **Dr. Rashmi Saxena Pal**, working as Professor (Pharmacognosy), in the School of Pharmaceutical Sciences of Lovely Professional University, Punjab, India. In keeping with the general practice of reporting scientific observations, due acknowledgements have been made whenever the work described here has been based on the findings of other investigators. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

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Name of the scholar: Srinivas Sutrapu

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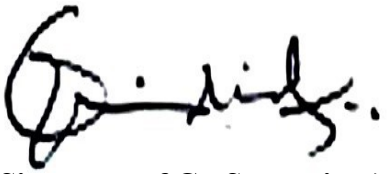
## CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled "**Extraction, Pharmacological, And Pharmacoinformatic Evaluation Of Active Constituents Of *Dalbergia latifolia* As Potential Antidiabetic Agents**" submitted in fulfilment of the requirement for the award of the degree of **Doctor of Philosophy (Ph.D.)** in the School of Pharmaceutical Sciences of Lovely Professional University, is a research work carried out by Srinivas Sutrapu, 41800404, is a bonafide record of his/her original work carried out under my supervision and that no part of the thesis has been submitted for any other degree, diploma or equivalent course.



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## ABSTRACT

Diabetes mellitus (DM) is a serious and complex metabolic disorder characterized by elevated blood glucose levels. In recent years, researchers have gained much more interest in natural products as alternative sources for diabetes treatment. Though many potential agents have been identified, their clinical utility is limited because of their adverse effects. Therefore, there is a keen interest in discovering natural compounds to treat diabetes efficiently with fewer side effects. *Dalbergia latifolia* (DL) has been explored extensively because of its diverse pharmacological activities, including diabetes. Therefore, the present research work aimed to identify and isolate the potential antidiabetic agents from the heartwood of DL.

Initially, the thirteen active constituents of DL were identified based on the literature, and the identified compounds were pharmacoinformatically evaluated using SwissADME, Molinspiration, Protox-ii, and PyRx web tools. Based on the results obtained from the pharmacoinformatic evaluation, compounds with good potential for diabetes were selected and further screened by *In silico* docking. In the Lipinski rule of 5, except compound 7 (molecular weight ≥500), all the compounds obeyed this rule. All compounds except compound 1 exhibited better aqueous solubility. In the pharmacokinetic study, all the compounds except compounds 7 and 12 showed improved gastric absorption if taken orally, and compounds 7, 8, 9, and 12 are less likely to cross the BBB. In the lead likeness, compounds 1, 3, 4, 5, 9, 10, and 13 are likely lead-like compounds. In the bioactivity score, among the GPCR ligands, compounds 7, 8, 10, and 12 are highly active in ion channel modulator activity, and compounds 3 and 12 are highly active in kinase inhibitor activity. All the compounds are moderately active in nuclear receptor activity. Compounds 3, 8, 9, 11, and 12 are highly active in protease inhibitor activity, and only compound 12 is highly active in enzyme inhibitory activity, except for compounds 1, 2, 4, 5, and 6, which are highly active. Among the predicted toxicology classes, compounds 1, 4, and 13 belong to class 5, indicating that the $LD_{50}$ values range from 2500 to 4000 mg/kg.

Auto dock vina was used to check the binding affinity of the compounds with the target (α-amylase). Among all compounds 1,2,7,8,9, and 11 showed lower binding affinity in the range of -9.0 k/cal and -7.4 k/cal for α-amylase enzyme (1HNY). They

also formed bonds with the crucial amino acids of 1HNY. Among all the compounds that showed better therapeutic potential, we successfully extracted Dalbergin (DGN) and Isoliquiritigenin (ISG) from the heartwood. We evaluated their antidiabetic potential both *in vivo* and *in vitro*. α-amylase activity inhibition of ISG and DGN was found to be 99.05 ± 8.54% (IC50 = 0.6025 µg/mL) and 84.68 ± 5.2% (IC50 = 0.0216 µg/mL) respectively. Glucose uptake assay revealed DGN (158%) promoted maximum uptake compared to ISG (77%), which was over control. *In vivo*, anti-diabetic activity was evaluated by inducing diabetes in SD rats with the help of low-dose (35 mg/kg ) Streptozotocin (STZ) and a High-fat diet (HFD). After the continuous administration of DGN (5 mg/kg, 10 mg/kg) and ISG (5 mg/kg, 10 mg/kg) for 14 days, we observed a reduction in the blood glucose levels, body weight, blood urea, serum creatinine, serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, alkaline phosphatase levels, low-density lipoprotein, total cholesterol, very low-density lipoprotein than diabetic control group indicates the potency of ISG and DGN against diabetes.

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Mr. Srinivas Sutrapu

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## LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| **ADA** | : | American Diabetes Association |
| **ADMET** | : | Absorption, Distribution, Metabolism, Elimination, Toxicity |
| **ALP** | : | Alkaline phosphatase |
| **ANOVA** | : | Analysis of variance |
| **ATP** | : | Adenosine triphosphate |
| **DGN** | : | Dalbergin |
| **DL** | : | *Dalbergia Latifolia* |
| **DM** | : | Diabetes mellitus |
| **DMEM** | : | Dulbecco's modified eagle's medium |
| **DPP-4** | : | Dipeptidyl peptidase 4 |
| **DPPH** | : | 2,2-diphenyl-1-picrylhydrazyl |
| **E.Coli** | : | *Escherichia coli* |
| **EMA** | : | European Medical Agency |
| **FBS** | : | Fetal bovine serum |
| **GAD** | : | Glutamic Acid Decarboxylase |
| **GIP** | : | Gastric inhibitory polypeptide |
| **GLP-1** | : | Glucose-like peptide 1 |
| **GLUT-4** | : | Glucose transporter 4 |
| **GOD-POD** | : | Glucose oxidase and peroxidase |
| **HbA1C** | : | Hemoglobin A1C |
| **HDL** | : | High density lipoprotein |
| **HFD** | : | High fat diet |
| **IDDM** | : | Insulin-dependent diabetes mellitus |
| **IDF** | : | Indian Diabetic Federation |
| **IR** | : | Insulin resistance |
| **ISG** | : | Isoliquiritigenin |
| **IUCN** | : | International Union of Conservation of Nature |
| **LDL** | : | Low density lipoprotein |
| **MODY** | : | Maturity onset diabetes of the young |
| **NCCS** | : | National center for cell science |

| | | |
|---|---|---|
| **NIDDM** | : | Non-insulin-dependent diabetes mellitus |
| **NMR** | : | Nuclear magnetic resonance |
| **NRU** | : | Neutral red uptake |
| **PBS** | : | Phosphate buffer solution |
| **PDB** | : | Protein Data Bank |
| **PPARs** | : | Peroxisome proliferator-activated receptors |
| **SC** | : | Serum creatinine |
| **SD** | : | Sprague Dawley |
| **SGOT** | : | Serum glutamic oxaloacetic transaminase |
| **SGPT** | : | Serum glutamate pyruvate transaminase |
| **SOD** | : | Superoxide dismutase |
| **STZ** | : | Streptozotocin |
| **T1DM** | : | Type 1 diabetes mellitus |
| **T2DM** | : | Type 2 diabetes mellitus |
| **TC** | : | Total cholesterol |
| **TG** | : | Triglycerides |
| **TZD** | : | Thiazolidinediones |
| **UTI** | : | Urinary tract infections |
| **VLDL** | : | Very low-density lipoprotein |
| **WFS** | : | Wolframin ER Transmembrane Glycoprotein |
| **WHO** | : | World health organization |

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| S.No | : | Name |
|---|---|---|
| 1 | : | Candidacy letter of Ph.D |
| 2 | : | Approval letter BY IAEC |
| 3 | : | List of publications, patents, awards, and certificates |

# CHAPTER-1

## 1. INTRODUCTION

DM is a complicated metabolic disorder that causes high blood sugar levels, excessive thirst, increased hunger, and frequent urination (1). Elevated blood glucose levels are mainly caused by insufficient insulin from the pancreas, problems with insulin secretion, unresponsive pancreatic β cells, and an excess of glucose produced from the liver (2). The prevalence of high blood sugar levels significantly affects the occurrence of both nervous and vascular complications (3).

The International diabetes federation (IDF) released its latest data Nov, 2021, revealing that 536.6 million people worldwide have diabetes, with projections indicating up to 783.7 billion cases worldwide by year 2045 (4) is shown in Fig 1. Among all types of DM, type 2 diabetes was the most prevalent in all age groups (96%) (5). Recent research conducted by multiple investigators has revealed that closely monitoring and controlling blood glucose levels can significantly reduce the occurrence of DM and its associated long-term complications (6).

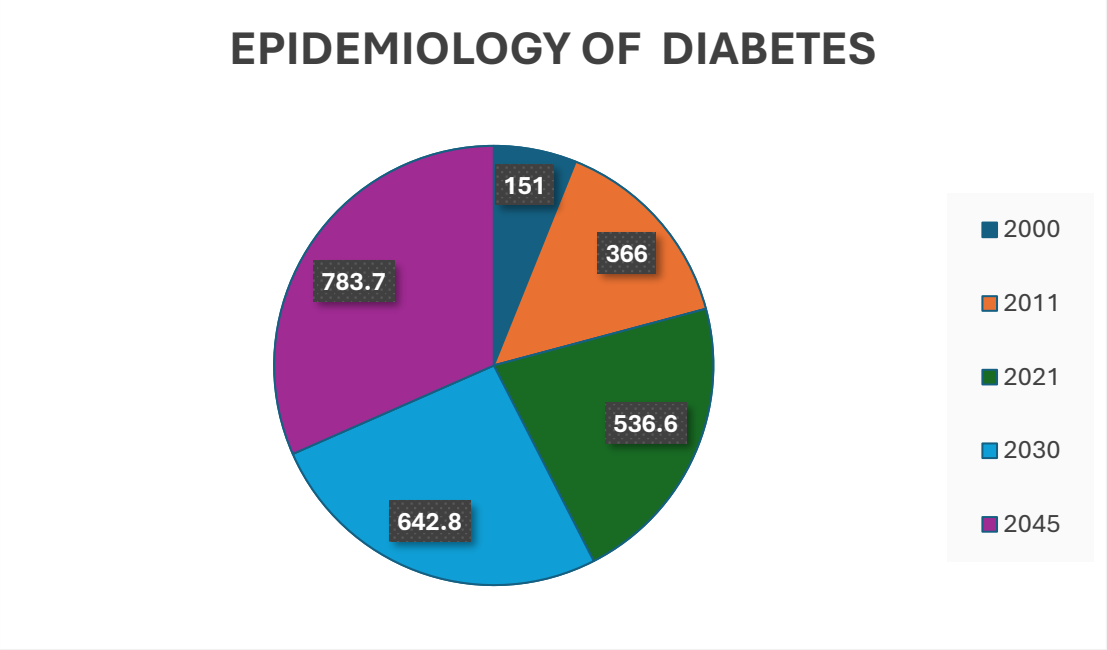



Fig.1. Prevalence of diabetes and its projection by 2045 . (Data was shown in millions)

A glucose rich environment over time causes several physiological and pathological alterations. The harmful effects of hyperglycemia on cells , tissues, and organ systems are intensified through various mechanisms. In addition to causing oxidative stress,

hyperglycemia can also increase the polyol pathway, activate protein kinase C, improve the hexosamine biosynthetic pathway, encourage the production of advanced glycation end products and gene expression. Further, chronic hyperglycemia damages the pancreatic β cell and causes insulin resistance, which results in severe complications (3). In T2DM patients, strict blood glucose management has successfully decreased microvascular consequences, according to the United Kingdom Prospective Diabetes Study (UKPDS)(7).

Controlling blood sugar levels after meals helps minimize the risk of side effects and improves the overall health of individuals with diabetes (8). An effective way to do this is to promote glucose uptake into skeletal muscle by restricting or delaying the release of glucose into the bloodstream by inhibition of alpha amylase (9,10). Inhibiting this enzyme can significantly lower the blood glucose level after a meal, assisting in controlling blood sugar. The researchers aim to use α-amylase inhibitors to regulate carbohydrate digestion and increase GLUT4 activity for targeted sugar control and better metabolic health.

Increasing GLUT4 translocation may greatly enhance insulin sensitivity and overall glucose uptake by muscle and adipose tissues, which are essential factors in managing conditions like T2DM and obesity (11). In addition, initial findings from clinical trials indicate encouraging benefits, with participants demonstrating improved glycaemic management and decreased insulin resistance after incorporating α-amylase inhibitors (12,13).

Our body's organs have specific functions that help to regulate blood sugar levels. These functions open exciting possibilities for developing medications that can assist in managing diabetes and enhancing overall health. Synthetic antidiabetic drugs are readily accessible and target different body areas to lower blood glucose levels effectively. These targets include the placenta (α-amylase and glucosidase inhibitors), brain and stomach (DPP4 inhibitors, GLP-1 analogues), pancreas (sulfonylureas, meglitinides, DPP4-inhibitors, GLP-1 analogues), liver (biguanides, thiazolidinediones), muscle, and adipose tissue (glitazones-muscles and adipose tissue) (10). Empagliflozin, canagliflozin, dapagliflozin, and ertugliflozin are new medications approved by the European Medical Agency (EMA) (14), and sotagliflozin (LX4211), LX2761, semaglutide, HMS5552, and advanced smart insulin formulations are in

clinical trials (15). These synthetic drugs are showing inadequate efficacy and producing many undesirable effects like hypoglycemia, weight gain, metabolic acidosis, reduced vitamin $B_{12}$ absorption, edema, cardiac failure, flatulence, and inflammation of the pancreas (16).

Nevertheless, the exorbitant prices and side effects associated with these medications have intensified the exploration of alternative therapies. Historically, using plant parts or crude extracts has been common in treating various diseases, a traditional approach documented in the research literature. Incredible plant sources have demonstrated remarkable efficacy in managing diabetes and offer a potential approach to this problem (17). A surprising variety of natural phytoconstituents have been found to have hypoglycemic properties. These include steroids, chalcones, carotenoids, tannins, saponins, peptides, lipids, glycopeptides, flavonoids, alkaloids, terpenoids, phenolics, and even lesser-known compounds such as iridoids and imidazolines (18). Their proven antidiabetic properties make them an exciting avenue for seeking relief and improving overall wellness. More than 800 plants have been studied scientifically for their antidiabetic activity (19)(20). In conventional medical systems across the globe, numerous species of Dalbergia are utilized to treat a wide range of disorders (21).

*Dalbergia latifolia* (DL) (synonymous name *Dalbergia emarginata* Roxb, *Amerimnon latifolium* Roxb Kuntze.), widely recognised as Indian rosewood, is a member of the genus *Dalbergia* and family Fabaceae (Leguminosae). Traditionally, the decoction of bark was given in diarrhoea (22), bark and leaves were used for leprosy (23,24), and oil was used for skin diseases (25). In recent years, DL has been in the limelight due to its pharmacological actions. Recent studies have shown that DL has antitermitic action (26), anthelminthic activity (27), antibacterial activity (25), antifungal activity (26), cerebroprotective effects (28), antioxidant activity (29), hemolytic activity (30), immunomodulatory activity(31), nootropic, anxiolytic activity (32), *in vitro* anticancer activity (33) and antidiabetic activity (34).

*In silico* analysis using advanced computational techniques allows researchers to test and analyse plant-derived pharmaceutical compounds virtually. By mapping the interactions between these compounds and biological targets, scientists can identify promising candidates for further research, saving valuable time and resources. This

data-driven approach enables rapid screening of a large drug area for new effective and safe plant-based therapies and increases opportunities related to the discovery (35)

ADMET (absorption, distribution, metabolism, excretion, and toxicity) assays are important complements to *in silico* studies and provide important insights into the pharmacokinetics and toxicology of plant-derived compounds. These comprehensive assays help to understand the composition of these compounds and their derivatives in the human body, ensuring that only promising candidates move forward in the drug development process. ADMET assays effectively mitigate the risks associated with conventional drug-discovery methods by providing a pre-assessment priority that can control drug potential absorption, distribution, metabolism, and toxicity management. Combining *in silico* and ADMET assays could revolutionise the biopharmaceutical industry by accelerating the discovery and development of plant-based therapeutics (36).

These compounds bind to an enzyme called α-amylase, reducing its ability to break down carbohydrates efficiently. Plant-derived α-amylase inhibitors are increasingly important in promoting overall health and well-being, supported by a growing body of scientific evidence.

Recent studies have shown that various extracts of plant parts consist of alkaloids, glycosides, flavonoids, phenols, resins, tannins, steroids, and carbohydrates (32,37,38). The bark extract of DL exhibited antidiabetic activity (34), while the leaf extract showed *in-vitro* α-glucosidase activity (39). However, the specific constituents responsible for this activity and their underlying mechanism have yet to be evaluated thus far. In this study, we initially identified the active constituents of DL and performed *in-silico* molecular docking against the α-amylase enzyme. We also studied the ADMET properties of the molecules, which found more potential against α-amylase. Finally, two compounds were isolated, namely Dalbergin (DGN) and Isoliquiritigenin (ISG). Next, our study investigated *in vitro* α-amylase inhibitory activity and *in vivo* DGN and ISG antidiabetic activity using low-dose STZ and HFD.

DGN belongs to the neoflavones class of chemical compounds and is extracted from *Dalbergia* species. The structure of these neoflavonoids is derived from the skeleton of 4-phenylcoumarin. It possesses anti-inflammatory, anti-cancer (40), anti-ulcerogenic, anti-osteoporotic (41) and antioxidant properties (42).

One of the most prevalent chalcone type flavonoids ISG is extracted from the roots of the liquorice plant, *Glycyrrhiza* species. Because of its potential health benefits and pharmacological properties, including antioxidant, anti-inflammatory (43), anti-microbial(44), anti-diabetic(45), hepatoprotective(46), cardioprotective (47) and anticancer activities, ISG has attracted a lot of attention in the medical and nutritional fields in recent years (48).

The search for antidiabetic drug activity in animal models is an essential step in developing novel therapies for diabetes. Intensive preclinical studies in animal models can yield important information about potential antidiabetic agents' efficacy, safety, and mode of action. Well-designed animal studies using appropriate mouse or mouse strains can study the glucose-lowering effects of candidate drugs and other key markers of diabetic health, pancreatic function, and diabetic complications; their impact can be seen more clearly (49). A high-fat diet (HFD) with streptozotocin (STZ) supplementation is a common animal model used to study type 2 diabetes and its complications in rats. This model mimics human type 2 diabetes, which, many times, begins with insulin resistance, followed by progressive pancreatic beta cell dysfunction. STZ by itself merely induces betacell dysfunction, whereas high fat diet induces insulin resistance (50).

# CHAPTER-2

## 2. REVIEW OF LITERATURE

One of the most significant public health challenges worldwide after cancer and heart disease is diabetes. Despite the advancement of medications and medical approaches, effective diabetes care remains a challenge, and these aspects are the focus of studies worldwide (51).

Elevated blood glucose levels and abnormalities in the metabolism of fat, protein, and carbohydrates are the hallmarks of diabetes mellitus. Insulin is the hormone secreted by the endocrine gland pancreas (52). Disturbances in insulin secretion or decreased insulin function in our body lead to increased blood glucose levels. Elevated blood glucose increases water intake, frequent urination, hunger, and weight loss. Full or Partial insufficiency of insulin secretion damages the eyes, kidneys, neurons, brain, heart, and extremities. Many predisposing parameters play a vital role in the development of diabetes. Age, sex, genetics, ethnicity, and family history also contribute to the development of diabetes (53).

### 2.1. Epidemiology

Diabetes is currently one of the most significant public health challenges worldwide, after cancer and heart disease. It is widely prevalent and poses a considerable health risk in people of all ages due to its involvement in multiple body systems and the occurrence of serious complications (51). The World Health Organization (WHO) estimates that diabetes affects more than 537 million individuals worldwide. According to projections, this number would rise to 700 million by 2045.

Almost ninety percent of the cases are suffering from Type 2 diabetes, which is mainly induced by overweight and decreased physical activity. According to the Indian Diabetic Federation (IDF) 2021 data, the prevalence of diabetes in the top three countries is China (10.6%), India (9.6%), and the USA (10.7%). Without strict guidelines, the trend is expected to reach 10.4 % in 2030 and 10.8 % in 2045 in India (54).

### 2.2. Etiology

The specific root cause of diabetes is unknown, but many factors contribute to its development, including overweight, sedentary lifestyle, unhealthy diet, and genetic predisposition (55). Insulin is the hormone secreted by the endocrine gland of the pancreas to control the elevated levels of blood glucose (52). β cell dysfunction and

insulin resistance (IR) are triggering factors of hyperglycemia, leading to the development of Type 2 diabetes (56). Elevated blood glucose increases water intake, frequent urination, hunger, and weight loss. Full or partial insufficiency of insulin secretion damages the eyes, kidneys, neurons, brain, heart, and extremities. Age, sex, genetics, ethnicity, and family history also contribute to the development of diabetes (57).

## 2.3. Types of Diabetes

According to the WHO, diabetes is classified into four major types.

### *2.3.1. Type 1 diabetes (Insulin-dependent diabetes mellitus -IDDM)*

Total deficiency of insulin secretion in the body occurs in type 1. 5- 10% of the diabetic population belongs to this kind of diabetes. People who have Type 1 diabetes require a regular dose of insulin. It often occurs in children (Juvenile diabetes) and adults. Autoantibodies are the primary reason and are initially found in 85–90% of patients with fasting hyperglycemia. The development of this specific type of diabetes can be attributed to the damage caused to pancreatic cells by cellular-mediated autoimmunity. This mechanism plays a significant role in triggering the condition. Markers of autoimmunity include autoantibodies against the protein tyrosine phosphatases IA-2 and IA-2b, islet cell autoantibodies, insulin autoantibodies, and Glutamic Acid Decarboxylase GAD (GAD65) autoantibodies. In certain people, such as newborns and young children, the rate of β-cell death is quick, whereas in adults, it is slow (58).

### *2.3.2. Type 2 diabetes (T2DM) (non-insulin-dependent diabetes mellitus-NIDDM)*

It arises from a decrease in the body's insulin production or insufficient insulin secreted to reduce elevated blood glucose levels caused by insulin resistance. T2DM accounts for 90-95% of the diabetic population, and these patients do not require insulin for a lifetime. It may be asymptomatic in some patients, but gradually, the sugar level will increase daily. In many patients, insulin resistance is the major root cause of obesity, diabetes, thyroid, autoimmune disorders, and cancer. When there are no symptoms, and this kind of diabetes goes misdiagnosed for a long time, hyperglycemia slowly rises (58).

### *2.3.3.* ***Gestational diabetes***

It occurs in pregnant women (gestational diabetes) due to elevated blood glucose levels. Pregnant women are strongly advised by the American Diabetes Association (ADA) to undergo diabetes testing at their initial prenatal appointment. In most cases, gestational diabetes disappears after delivery. The criteria were applicable whether the problem remained after pregnancy, and it did not rule out the potential that undetected hyperglycemia may have emerged concurrently during or before the pregnancy. However, in most cases, gestational diabetes is resolved with timely delivery (58). Gestational diabetes mellitus complicates around 7% of pregnancies, resulting in more than 200,000 cases per year (59).

### *2.3.4.* ***Secondary diabetes***

The term "secondary diabetes" refers to a type of diabetes that occurs when the beta cells in the pancreatic islets are damaged and when an acquired condition, such as endocrinopathies, leads to insulin resistance. In some cases, insulin secretion and β cell functioning are both normal. Other factors are contributing to increased blood glucose levels. Ex: Pancreatitis, steroid-induced diabetes, viral infections destroying β cells of the pancreas, etc (58).

### *2.3.5.* ***Other forms of diabetes***

#### ***2.3.5.1. Maturity onset diabetes of the young (MODY)***

Genetic mutation causes MODY. If a parent carries this gene mutation, there is a 50% chance that any of their children will inherit it. Irrespective of an individual's lifestyle, weight, or ethnicity, the manifestation of MODY in a child is typically observed before the age of 25 if they inherit the mutation (58) .

#### ***2.3.5.2. Neonatal diabetes***

A gene alteration that affects insulin synthesis contributes to the development of neonatal diabetes and increases glucose levels significantly. Neonatal diabetes is of two types: 1) Transient and 2) Permanent. Transient neonatal diabetes is a condition that typically resolves on its own before the child reaches one year of age. However, it's important to note that permanent neonatal diabetes, which accounts for 40% to 50% of cases, does not go away and lasts indefinitely (58).

#### *2.3.5.3. Wolfram syndrome*

It is a rare genetic syndrome consisting of four conditions: diabetes mellitus, Diabetes Insipidus, Deafness, and Optic Atrophy. It is caused by mutations in the WFS1 or WFS genes and affects one in 500,000 people (60).

#### *2.3.5.4. Alstrom syndrome*

It is an autosomal recessive genetic disorder that originates from mutations in the ALMS1 gene. Its clinical presentation encompasses a spectrum of manifestations, comprising obesity, type-II diabetes, cardiomyopathy, renal failure, retinal degeneration, and orthopedic and rheumatologic complications (60).

#### *2.3.5.5. Latent autoimmune disease in adults (LADA)*

It shares genetic features with type-I (HLA-DQB1, INS VNTR, PTPN22) and type-II (TCF7L2) diabetes. It appears to be a combination of both type 1 and type 2 diabetes. Because of this, some people refer to it as type 1.5 or type 1 1/2 diabetes (61).

#### *2.3.5.6. Type 3 and Type 3c diabetes*

Type 3 diabetes is associated with insulin resistance and insulin growth like factor dysfunction, which exclusively occurs in the brain, are the causes of Alzheimer's disease, one of the main causes of dementia. STZ induced experimental brain diabetes shares many characteristics with AD, such as disruptions in acetylcholine homeostasis and cognitive impairment (62).

Type 3c diabetes develops due to direct damage to the pancreas by external agents. This type of diabetes is associated with pancreatitis, pancreatic cancer, cystic fibrosis, and hemochromatosis (58).

#### *2.3.5.7. Steroid-induced diabetes*

It occurs in the patients when they are using steroids for prolonged periods. This form of diabetes is prevalent among individuals with a higher susceptibility to developing type 2 diabetes (58).

#### *2.3.5.8. Cystic fibrosis diabetes*

Cystic fibrosis is a hereditary disorder that affects certain people from birth. It is usually diagnosed before the child's first birthday and is caused by inheriting a damaged CFTR gene from both parents. Individuals diagnosed with cystic fibrosis produce dense and adhesive mucus, which has the potential to accumulate in the lungs, pancreas, and

various organs within the body. Symptoms include high blood sugar levels, breathing difficulties, lung infections, and digestion problems (58).

## 2.4. Signs and symptoms of diabetes

### *2.4.1.* ***Type 1 diabetes***

The primary symptoms are Nausea and vomiting. The later stage is diabetic ketoacidosis, a metabolic disturbance characterized by the smell of acetone; the breakdown of muscle and fat for energy leads to weight loss. In the advanced stage, it leads to a coma and sudden death (63).

### *2.4.2.* ***Type 2 diabetes***

Early symptoms are increased frequency of urination, water intake, excessive eating, and weight loss. Late symptoms are poor wound healing, blurred vision, infections, agitation, lethargy, and ketoacidosis (64).

## 2.5. Diagnosis of diabetes (65).

Physicians recommended general tests to detect diabetes and monitor blood sugar levels, as shown in **Table** 1.

**Table 1. Categories of diabetes based on Blood sugar levels**

| Categories | Based on | Values mg/dL |
|---|---|---|
| **Normal** | Pre-prandial blood sugar | 70-99 |
| **Prediabetic** | Pre-prandial blood sugar | 80-130 |
| **Borderline diabetes** | Pre-prandial blood sugar | 130-180 |
| **Diabetes** | Pre-prandial blood sugar | >200 |
| **Normal** | Postprandial blood sugar | > 140-160 |
| **Prediabetic** | Postprandial blood sugar | > 160-180 |
| **Diabetic** | Postprandial blood sugar | > 200 |
| **Normal** | Glycated hemoglobin (HbA1c) | < 5.7% |
| **Diabetes** | Glycated hemoglobin (HbA1c) | >6.0 % |
| **Diabetic** | Random blood sugar | ≥200 |

### *2.5.1.* ***Significance of Hba1c***

The hemoglobin A1c (HbA1c) blood test is a medical exam to check blood glucose levels. It measures the percentage of glycated hemoglobin in the blood and provides an average blood glucose reading for the previous two or three months.

Hemoglobin that has glucose bound to it is called glycated hemoglobin. HbA1c is a vital tool in diabetes management as it provides a long-term view of blood glucose control. It is crucial in preventing complications associated with high blood glucose levels. According to the American Diabetes Association, individuals with diabetes should get their HbA1c levels measured at least twice a year or more frequently if their blood glucose levels are not well controlled. Maintaining HbA1c levels within the recommended range can help reduce the risk of complications associated with diabetes. The target HbA1c level may vary depending on individual circumstances such as age, duration of diabetes, and other health conditions (53).

## 2.6. Clinically available antidiabetic medications and their mechanism of action

Subcutaneous administration of insulin is generally recommended in type I diabetes. However, insulin is also recommended in type 2 diabetes due to the progressive loss of pancreatic β cells. **Table 2**. gives the different classes of drugs used in type 2 diabetes, their mechanism of action, and side effects.

**Table 2. List of antidiabetic drugs and mechanisms of action in treating type 2 diabetes.**

| Oral hypoglycemic agent | Mechanism of action | Drugs | Side effects |
|---|---|---|---|
| Sulfonylureas | 1. Drugs bind to ATP-sensitive potassium channels in the pancreatic β-cell membrane, blocking the efflux of $K^+$ and leading to depolarization. This depolarization opens the $Ca^{2+}$ channel. Calcium in tissues releases insulin.<br>2. it enhances β cell sensitivity to insulin. | Tolbutamide,<br>Glimepiride<br>Glipizide<br>Glyburide | Risk of hypoglycemia,<br>Weight gain |
| Biguanides (metformin) | It reduces liver production of glucose, increases insulin sensitivity.<br>Enhances the sensitivity to insulin | Metformin | Metabolic acidosis, Weight loss, Reduced vitamin B12 absorption, weight loss |
| Thiazolidinediones (TZD) | It stimulates gamma isoform of PPARs (peroxisome proliferator-activated receptors) and reduces insulin resistance. | Rosiglitazone<br>Pioglitazone | Weight gain,<br>Edema,<br>Cardiac failure. |

| | | | |
|---|---|---|---|
| α-glucosidase inhibitors | It inhibits α-glucosidase, an enzyme that breaks down starch and disaccharides into monosaccharides. | Acarbose<br>Voglibose<br>Miglitol. | Flatulence, diarrhoea, the feeling of satiety |
| Meglitinides | Drugs bind to ATP-sensitive potassium channels in the pancreatic β-cell membrane, blocking the efflux of $K^+$ and leading to depolarization. This depolarization opens the $Ca^{2+}$ channel, and calcium in tissues releases insulin. | Nateglinide<br>Repaglinide | Risk of hypoglycemia<br>Weight gain |
| Amylin analogues | It mimics the effects of the pancreatic hormone amylin. It Reduces glucagon release and gastric emptying. | Pramlintide | Risk of hypoglycemia |
| DPP-IV inhibitors | Increase the levels of incretin, GLP, and GIP | Saxagliptin, Sitagliptin | Gastrointestinal complaints, inflammation of the pancreas |
| GLP-1 agonists (incretin mimetic drugs | Reduces glucagon secretion and reduces hepatic glucose output. | Exenatide,<br>Liraglutide,<br>Albiglutide | Vomiting, inflammation of the pancreas, and pancreatic cancer |
| GLT-2 inhibitors | Prevention of reabsorption of blood glucose from the proximal tubule of the kidney | (Canagliflozin,<br>Dapagliflozin, | Genital yeast infections, UTI, frequent urination. |

Overall, drug therapy in T2DM improves insulin sensitivity, increases glucose utilization by muscles, decreases hepatic glucose output, and reduces the gastrointestinal absorption of carbohydrates.

## 2.7. Diabetic complications

Extremely high blood sugar levels in individuals with diabetes can pose significant threats to the health and quality of life for individuals living with this condition. There are two types (66).

### *2.7.1.* ***Microvascular***

Long term elevated blood sugar levels cause damage to small blood vessels all over the body, which results in complications like Diabetic retinopathy, diabetic nephropathy, and diabetic neuropathy. If diabetic retinopathy is not treated , it can cause blindness, similarly, diabetic nephropathy can cause renal failure that necessitates dialysis or a kidney transplant. Diabetic neuropathy can also result in severe pain and loss of sensation in the extremities, which raises the possibility of infections or injuries that might not be discovered until they are severe.

### *2.7.2.* ***Macrovascular***

Macrovascular complications of diabetes are a critical concern that demands immediate attention. Long term high blood sugar levels can cause blood vessels to harden and narrow, increasing the risk of macrovascular problems, such as cardiovascular disorders like heart attacks and strokes, in people with diabetes. This vascular damage not only increases the risk of life threatening events but also contributes to poor health outcomes that can diminish quality of life.

### *2.7.3.* ***Mechanisms of diabetic complications***

Increased intracellular hyperglycemia and insulin resistance in the target cells of diabetic complications, causes increased mitochondrial super oxide free radicals, which also directly deactivates two anti-atherosclerotic enzymes, prostacyclin synthase and eNOS, and activates five pathways involved in the pathophysiology of complications. These include increased flux of glucose and other sugars through polyol pathway expression of the receptor and production of advanced glycation end products (AGEs), hyper glycemia induced activation of protein kinase c (PKC) isoforms, and over activity of the hexosamine pathway . But T2DM lacks a definitive inheritance pattern, however

numerous affected individuals have atleast one close relative, such as a parent or sibling with the disease.

***2.7.3.1 Polyol pathway:*** It was first described in 966. The enzyme aldose reductase is the focus of polyol pathway. Normally, Aldose reductase converts harmful aldehydes into the cell to inert alcohol. However, when glucose content is too high, this enzyme converts glucose to sorbitol, which is subsequently oxidized to fructose by the enzyme sorbitol dehydrogenase (SDH), with $NAD^+$ as a cofactor. NADPH is the cofactor required to produce intracellular glutathione. Reduced glutathione levels are decreased by polyol pathway, making cells more vulnerable to intracellular oxidative stress (67).

***2.7.3.2 Advanced glycation end products (AGEs)***

When blood sugar and protein or fat mix together dangerous substances known as AGEs are created. There are 3 main pathways that intracellular AGE precursor production can harm cells (68).

1. Modification of the intracellular protein which is involved in the control of gene transcription.
2. AGE precursors are diffusing out of the cell and altering the nearby extracellular matrix molecules, which alters matrix cell signaling and results in cellular dysfunction.
3. The diffused AGE precursors diffuse out of the cell and alter blood proteins like albumin.

A variety of cells and tissues may be impacted by AGE-modified proteins in the blood stream. It has been demonstrated that a particular AGE receptor (RAGE) mediates signal transduction by producing ROS, activating NFkB. And p21 ras (69).

***2.7.3.3. Hyperglycemia induced activation of protein kinase c (PKC) isoforms***

A molecule known as Diacylglycerol, an essential activating cofactor for the classic isoforms of protein kinase-C, -β, -δ, and -α, is synthesized in greater amounts when cells experience internal hyperglycemia. When PKC is activated, it has range of effects include increase in vasoconstrictor endothelin-1, transforming growth factor-β, plasminogen activator inhibitor-1 and decrease in vasodilator producing endothelial nitric oxide synthase (eNOS)(70). Overall, activation of PKC leads to blood flow abnormalities, vascular occlusion, increased pro inflammatory gene expression and production ROS. Numerous animal studies, including Ishii H *et al.,* demonstrated that

inhibition of oral PKC inhibitor stopped early alterations in the diabetic kidney and retina (71).

#### *2.7.3.4. Hexosamine pathway and over modification of proteins by N-acetyl glucosamine.*

When cell's glucose levels are elevated, most of the glucose is metabolized through glycolysis, first to glucose -6-phosphate, then to fructose -6- phosphate and then on through the rest of the glycolytic pathway. But some of that fructose -6- phosphate is diverted into a signaling pathway, where it is converted to glucosamine-6-phosphate and then to uridine diphosphate (UDP) N acetyl glucosamine for the final step. This was added to the serine and threonine residues of transcription factors and other proteins. This over modification results in pathological changes in gene expression leading to elevation of specificity protein 1 (transcription factor), resulting increased expression of transforming factor β1 and plasminogen activator inhibitor-1. Litty *et al.,* demonstrated that hypoglycemia induces abnormalities in porcine kidney cells mediated through hexosamine pathway (72). Clark RJ *et al*., demonstrated that hyperglycemia impairs cardiomyocyte calcium cycling through increased N acetyl glucosamine (73).

### *2.8. Carbohydrates and diabetes*

Glucose is the primary source of energy for body cells. Glucose comes from the liver and the digestion of carbohydrates in food and drinks. The pancreas produces insulin, which acts like a key to unlocking the cells and allowing glucose into them. The body automatically regulates insulin levels in healthy individuals to maintain stable glucose levels. Insulin is released continuously throughout the day, with additional insulin released after meals. However, in type 1 diabetes, insulin must be taken periodically to keep blood glucose levels (74).

The digestion of carbohydrates starts immediately after chewing the food in our mouth. The inhibition of glucose absorption is one of the key strategies used to treat diabetes. The digestive enzyme α-amylase facilitates the hydrolysis process of starch, forming a mixture of oligosaccharides. These oligosaccharides include maltose, maltotriose, and several α-(l-6) and α-(1-4) oligo glucans (**Fig**.**2**). They are subsequently broken down to glucose by the action of α-glucosidases which, upon absorption, enter the bloodstream. The rapid breakdown of this dietary carbohydrate

results in increased blood sugar levels, a condition referred to as hyperglycemia. To control hyperglycemia, medical professionals frequently prescribe antidiabetic medications. One effective strategy is to slow down the digestion of carbohydrates. By slowing down the breakdown of carbohydrates, these inhibitors reduce glucose absorption, helping lower blood sugar levels after meals (75).

### *2.9 Role of phytoconstituents*

Phytoconstituents, which are bioactive compounds derived from plants, have been shown to exhibit various pharmacological properties that can contribute to the management and prevention of diabetes. Since ancient times, plant sources of antidiabetic compounds have been widely used since they are safer and less expensive than synthetic pharmaceuticals. These compounds include flavonoids, polyphenols, alkaloids, and terpenes, each possessing unique mechanisms through which they can influence metabolic processes. Research indicates that many phyto constituents can enhance insulin sensitivity, which is crucial for maintaining normal glucose levels in the body. Their incorporation into daily dietary practices not only offers promising avenues for prevention but also complements traditional medical approaches aimed at controlling this chronic disease.

In addition to exhibiting anti-inflammatory qualities, traditional herbal remedies and functional foods are thought to improve diabetic syndromes through six noteworthy mechanisms of action, such as increased insulin secretion and sensitivity, glucose uptake by muscle cells and adipose tissues, inhibition of intestinal glucose absorption, and inhibition of hepatocyte glucose production.

### *2.10. Lifestyle modification in diabetes*

In addition to lowering blood glucose with medications, lifestyle modifications should address any associated risk factors such as obesity, smoking, hyperlipidaemias and hypertension.

**Dietary guidelines:** Daily consumption of dietary fat 25-35 %, protein intake 10-15 %, carbohydrates 50-60%, fibre intake 35 grams per day will be beneficial in the management of diabetes (76).

**Exercise:** Daily 45-60 mins of exercise lowers cardiovascular risk factors, helps to lose weight, improves blood glucose control in T2DM. Insulin actions in the muscle and liver can be modified by the exercise and physical activity(77).

**Sleep:** insufficient sleep and poor sleep hygiene were linked to increased HbA1c levels in adults with T2DM. High fasting glucose concentrations are observed in below 5-6 hrs sleep and above 9-10 hrs sleep (78).

**Stress management:** People with diabetes experience higher levels of stress and anxiety. Stress also increases Hba1c and blood glucose. In a experiment conducted by araban *et al.,* on stress management training for 3 months successfully reduced HbA1c levels from 8.52 to 6.1 (79).

### 2.11. *Types of amylases*

Amylase is an essential digestive enzyme secreted mainly by the salivary glands and pancreas. However, it is also present in minute levels in other tissues in the body. Its scientific exploration dates to the early 1800s, when it was initially denoted as diastase before being renamed amylase in the early 20th century. The three fundamental variations of amylases (α, β, γ) exert distinct effects on carbohydrate molecules. α-Amylase, a ubiquitous protein, is present in microorganisms, plants, animals, and humans. In the meantime, β-amylase may be found in both plants and bacteria, and γ-amylase can be seen in both plants and animals (80).

There are two variants of the amylase enzyme: P-type and S-type. P-type amylase is produced by pancreatic acinar cells and released into the gastrointestinal system. S-type amylase activity, which starts the digestion of carbohydrates when food is in the mouth and esophagus, is most active in the salivary glands. S-type amylase is also present in striated muscle, lungs, adipose tissue, fallopian tubes, ovaries, semen, colostrum, tears, and testes (81). α-amylase hydrolyzes the -1,4 bonds of starch to glucose, maltose, and maltotriose, with dextrin as the predominant product.

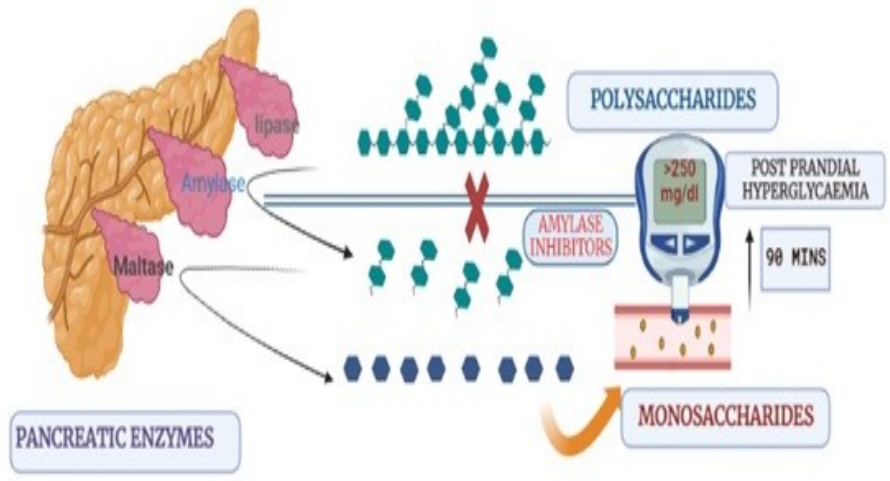



**Fig.2. Mechanism of α-*amylase* inhibitors**

The inhibition of α-amylase holds promise for effectively regulating blood glucose levels in individuals diagnosed with T2DM (**Fig.2.).** Inhibiting this enzyme can significantly lower the blood glucose level after a meal, assisting in controlling blood sugar. Acarbose (α-amylase inhibitor) helps manage T2DM by reducing carbohydrate absorption. However, it is important to note that several side effects, including abdominal distention, flatulence, diarrhoea, and meteorism, can accompany it (82).

The significant inhibition of α-amylase may cause these serious side effects. Previous research has suggested that phenolic compounds derived from food could delay glucose absorption by inhibiting carbohydrate-hydrolysing enzymes. This approach could be considered suitable for treating T2DM. Certainly, ongoing efforts are dedicated to exploring natural digestive enzyme inhibitors as potential means to counteract the symptoms of diabetes, with a focus on minimizing associated side effects. Extensive research has been conducted to discover effective and non-toxic inhibitors of α-amylase derived from natural products to find potential treatments for T2DM.

T2DM continues to be a significant issue in healthcare globally, even after decades. A key component of glycemic management and the prevention of complications from diabetes is glucose-lowering medication in combination with a healthy lifestyle. Due to the emergence of resistance and the unfavorable consequences

associated with the long-term usage of these drugs, significant research is now being done to increase the glucose-lowering effectiveness of future diabetic treatments (83).

## 2.12. Pharmacoinformatic studies

Drug discovery is an ongoing process, but many lead compounds fail to enter the market due to their poor pharmacokinetic parameters (35). The ideal oral medication will reach its site of action quickly, thoroughly, and specifically after entering the digestive tract. It won't interact or bind to similar receptors, and it won't inadvertently bind to passing serum proteins. The perfect substance may act as a substrate for liver enzymes and transporters that degrade or remove foreign substances from the body in a completely predictable manner. However, in the real world, lead compounds rarely exhibit ideal characteristics.

Drug-likeness was proposed as a valuable guide in early drug research to increase the chances of a chemical entering and succeeding in clinical trials (84). In pharmacokinetics, most drugs are easily absorbed, distributed, metabolized, and excreted without producing toxicity in our bodies. These parameters (ADMET) can be studied in computational studies using web servers. Computational methods do not provide confirmation; they provide information about the most likely drug-like compounds among various molecules. The methodologies have been widely established in medicinal synthetic chemistry; nevertheless, further exploration is needed to fully investigate their applicability in studying natural chemicals (85).

### 2.12.1. *In silico studies*

Docking has emerged as a critical tool in drug discovery. It enables scientists to gain valuable insights into the intricate interactions between chemical compounds and their molecular targets. By utilizing this technique, researchers can deepen their understanding of how these compounds function at a molecular level, paving the way for more efficient and effective drug development strategies.

### 2.12.2. *In silico evaluation*

Identifying an α- amylase inhibitor with fewer side effects is difficult because of the complexity of diabetes and its underlying mechanisms. Although it is a challenging task, significant progress is being made in diabetes research to develop more effective and safer treatment options for individuals with diabetes. The screening aims to identify potential therapeutic substances for further preclinical research in developing new

medicines. Some proteins and their protein data bank (PDB) IDs are mentioned in **Table 3.** below.

**Table 3. Examples of PDBs in the evaluation of antidiabetic activity**

| Sl.No | PDB ID | Target enzyme | Ref |
|---|---|---|---|
| **1.** | 1NOI | Glycogen phosphorylase | (86,87) |
| **2.** | 3G9E | PPAR γ | (88) |
| **3.** | 2P8S | Dipeptidyl peptidase 4 | (89) |
| **4.** | 5T19 | Protein Tyrosine Phosphatase 1B | (90) |
| **5.** | 5EQG | GLUT1 catalytic site | (91) |
| **6.** | 4RCH | Glucokinase | (92) |
| **7.** | 1OSE | α-amylase | (93) |
| **8.** | 2QMJ | α-Glucosidase | (94) |
| **9.** | 3BAJ | α-amylase | (87) |
| **10.** | 3SAY | Glycogen Synthase Kinase-3 | (89) |
| **11.** | 1EKO | Aldose Reductase | (89) |
| **12.** | 1VJT | α- Glucosidase | (89) |
| **13.** | 5NN8 | α- Glucosidase | (87,95) |
| **14.** | 2QOE | Dipeptidyl peptidase 4 | (90) |
| **15.** | 7TAA | α-amylase | (96) |

Before commercialization, it must show satisfactory drug likeness and pharmacokinetic parameters (97).

## 2.13. Induction of diabetes in animals

STZ is an antibiotic isolated from Streptomyces achromogenes in 1960. STZ can selectively target and destroy the β cells of the pancreas (98). This property makes it a powerful tool for studying diabetes and other conditions involving these specific cells' dysfunction or loss. Except for rabbits, animals like rats, mice, and monkeys are more sensitive to STZ and produce elevated blood glucose levels, more water intake, and frequent urination in laboratory animals, which are the characteristic features of diabetes in humans.

### 2.13.1. *Common methods to induce diabetes in rats*

#### 2.13.1.1. *STZ only*

This approach involves administering

a) Administration of a concentrated dose of STZ (200mg/kg). Within 48 hours, the blood glucose level surpasses 500 mg/dL.

b) Administration of a concentrated dose of STZ (65mg/kg). Within 3-7 days, blood glucose levels raise >500 mg/dL(99).

#### 2.13.1.2. *STZ+ Nicotinamide*

In this model, nicotinamide partially protects against the damaging effects on the pancreas and produces T1DM (insulin deficient) (100).

#### 2.13.1.3. *STZ+ HFD*

In this model, a high-fat diet produces insulin resistance in rats, a characteristic feature of T2DM in humans. Instead of a normal chow diet, rats are provided with High Fat Diet (60% of total recommended calories are from fat) for 2-3 weeks. Within 3-4 days of administering 35mg/kg STZ, diabetic animals exhibit blood glucose levels >350mg/dL (101).

#### 2.13.1.4. *Alloxan-induced diabetes*

Administration of 160 mg/kg alloxan produces diabetes with a minimal mortality rate (102).

- ❖ I phase 30 min: Decrease in blood glucose concentration.
- ❖ II phase: 1-2 hr, Increase in blood glucose concentration and low insulin level
- ❖ III phase: 4-8 hr, Decrease in blood glucose concentration.
- ❖ IV phase: 12-48 hr, Diabetic hyperglycemic phase.

#### 2.13.1.5. *Differences b/w Alloxan and STZ*

Alloxan follows 4 phases(I-IV) after administration, whereas STZ follows 2 phases (II-IV) [(No phase-I) &(phase-III)] (103).

### 2.14. α-amylase inhibitory potential of Isolated secondary metabolites

The development of medicinal plant extracts or their secondary metabolites as a reliable and supplemental natural medicine for treating diabetes is gaining interest due to their widespread availability and few negative side effects. The prospective inhibitors of the α-amylase enzyme are shown in **Table 4**. below

**Table 4. List of α-amylase inhibitors and their $IC_{50}$ values from various plant species**

| S.No | Botanical name of the plant | Bioactive compound isolated | secondary metabolite category | $IC_{50}$ (mean ±SD) | Acarbose $IC_{50}$ (mean ±SD) | Ref |
|---|---|---|---|---|---|---|
| **1.** | *Melilotus officinalis* | Isoquercitrin | Flavonoid | 9.65 ±0.43 µg/ml | 17.68 ± 1.24 µg/ml | (104) |
| **2.** | *Euryale ferox* | Epicatechin gallate | Flavonoids | 0.92 ±023 mg/ml | 01.08 ± 0.1 mg/ml | (105) |
| **3.** | *Punica granatum* | Tricetin | Flavonoid | 0.44± 0.12 mg/ml | 0.38 ± 0.018 µg/ml | (106) |
| **4.** | *Melilotus officinalis* | Rutin | Flavonoid glycoside | 11.42 ± 0.62 µg/ml | 17.68 ± 1.24 µg/ml | (104) |
| **5.** | *Tagetes minuta L.* | Quercetagetin 7-O-β-D-glucopyranose | Flavonoid glycoside | 7.8 µM | 7.1 µM | (107) |
| **6.** | *Phyllanthus debilis* | Glochidon | Triterpenoid | 38.15 ±1.4 µM | 33.68 ± 3.12 µM | (108) |
| **7.** | *Passiflora ligularis* | Ligularoside A | Triterpenoid saponin | 409.8 ±11.4 µM | 234.1 ±15.9 µM | (109) |
| **8.** | *Tamarindus indica L* | Xyloglucan | polysaccharides | 72.69 ± 0.84 µg/ml | 92.49 ±1.97 µg/ml | (110) |
| **9.** | *Quercus variabilis* | Ellagic acid | Phenolic acid derivative | 0.19 ± 002 µg/ml | 0.25 µg/ml | (111) |
| **10.** | *Rubus chingii hu* | Chingiitannin A | Ellagitannins | 4.52 ±0.30 µM | 35.71 ±4.93 µM | (112) |
| **11.** | *Rhus coriaria* | Penta-O-galloyl-β-D-glucopyranose | Gallotannins | 6.30 ±0.18 µM | 10.69 ±0.50 µM | (113) |
| **12.** | *Ocimum tenuiflorum* | Dehydrodieugenol B | Phenolic | 29.6 µM | 13.85 µM | (114) |

| | | | | | | |
|---|---|---|---|---|---|---|
| **13.** | *Cardiospermum halicacabum* | Berberine | Alkaloid | 72 % at 10 µg/ml | | (115) |
| **14.** | *Dregea volubilis* | Drevoluoside | Alkaloid | 51.3 ±2.1 µM | 36.3± 0.5 µM | (116) |

The values are expressed as mean ±SD. Acarbose is used as a standard drug

## 2.15. Ayurvedic medication for diabetes

Ayurveda is a traditional practice of the Indian medical system traced back to 1000 BC. It connects physiological, psychological, and spiritual processes to live healthily. The primary goal of Ayurveda is to balance five basic elements such as air, water, fire, earth, and vacuum. Currently, the Indian government has brought all the traditional systems under one roof, implemented regulations and framing policies, and is looking for growth of the Indian medical system across the globe (117).

Ayurveda has several formulations for the management of diabetes. Commonly used Indian herbs in the treatment of obesity and diabetes includes Triphala choornam, kolakulathadi choornam, dhanyamala, mahashivagarba taila, balaguduchyadhi taila, yashtimadhu panta, madanaphala yoga, avipatti choorna, K.V erandamoola Kashaya, tilataila, honey, shatapushpa kalka, madanaphala, shilajit, A. V nimbamritadi erandam. The top 10 plants used in the treatment of diabetes include Pomegranate, Ivy guard, Holy fruit tree, Margosa tree, Tinospora, Turmeric, Madhunashini, Indian kino tree, fenugreek, and bitter melon.

Despite the availability of existing antidiabetic medicines, looking for new antidiabetic sources from nature appears desirable, as they may contain plant constituents that have a safe and alternative impact on DM. Alternatives are required due to the failure of existing treatments to treat all unwanted effects of diabetes, their high cost, and reduced availability. Even though the availability of several insulin formulations and many synthetic antidiabetic medications, there is still a need to discover and develop innovative antidiabetic therapies. Traditionally, humans have used plant parts or crude extracts to cure many diseases. More than 800 plants have been studied scientifically for their antidiabetic activity (16).

**Table 5. List of Ayurvedic preparations available in India for the management of diabetes**

| S. No | Trade name/generic name | Manufactured by | Ingredients |
|---|---|---|---|
| **1** | Diabetes Care kit | Search wellness | Jamun Beej, Kusumakar Ras, Gurmar, Shilajit |
| **2** | Diabetex strong capsule | Multani Pharm. LTD | *Syzygium cumini* seeds, *Gymnema sylvestre*, *Embelica officinalis*, *Trigonella foenumgraecum*, Tinospora cardiofolia, coccinia grandis, Bombex ceiba, *Asphaltum punjabianum* |
| **3** | SMB capsules | Sai health care | *Syzygium cumini*, *Berberis Aristata*, *Gymnema Sylvestre*, *Momordica Charantia*, *Curcuma longa*, *Pterocarpus Marsupium*,*Azadirachta indica*, *Asphaltum punjabianum* |
| **4** | Suga Heal | Trigonella labs | *Trigonella foenumgraecum*, *Embelica officinalis*, *Tribulus terrestris*, *Camellia sinensis, Gymnema Sylvestre, Linum usitatissimum, Piper nigrum* |
| **5** | Amree plus | Aimil Pharmaceuticals Ltd | *Momordica charantia, Trigonella foenumgraecum, Withania somnifera, Tinospora cordifolia, Prunus dulcis* |
| **6** | Sanyasi Diab Controller Tablet | J. A. Pharma | *Azadirachta indica, Momordica charantia, Syzygium cumini, Tinospora Cordifolia, Citrus limon. Gymnema Sylvestre, Withania somnifera, Swertia chirayita,* |
| **7** | Vansar Karela Jamun Juice | Shree Baidyanath Ayurved Bhawan Pvt. Ltd. | *Momordica charantia* and *Syzygium cumini* |

| | | | |
|---|---|---|---|
| **8** | Diabiant Sugar Care Tablet | Ambic Ayurved India Pvt Ltd | *Withania somnifera, Gymnema Sylvestre, Momordica charantia, Syzygium cumini, prameha gaj kesari, Pterocarpus marsupium, Azadirachta indica* |
| **9** | Good health sugar knocker | Sushrut Ayurved Industries | *Ocimum sanctum, Momordica charancia, Eugenia jambolana, Emblica officinalis, Tinospora cordifolia, Salacia retriculata, Pterocarpus marsupium, Gymnema Sylvestre, Curcuma longa, Cinnamomum zeylanicum, Lagerstroemia speciosa* |
| **10** | Tri-Origin Ayurveda | JB's Natural Panchratna Products | *Gymnema Sylvestre, Enicostema axillare, Holarrhena pubescens, Centratherum anthelminticum, Andrographis paniculata, Terminalia chebula, Tinospora cordifolia* |
| **11** | Daitrin Tablet | Jiva Ayurveda | *Curcuma longa, Syzygium cumini, Aegle marmelos, Cucumis sativus, Phyllanthus emblica, Momordica charantia, Gymnema Sylvestre, Trigonella foenumgraecum, Azadirachta indica, Acacia nilotica* |
| **12** | Diabza_Sugar Controller | Jantayu Panchgavya Research Ayurveda | *Terminalia arjuna, Ficus racemose, Ficus religiosa, Mangifera indica, Cinnamomum cassia, Pterocarpus marsupium, Trichosanthes dioica, Catharanthus roseus, Moringa oleifera, Annona squamosa, Salacia chinensis,* |

## 2.16. *Dalbergia latifolia*

*Dalbergia latifolia* (DL) (synonyms: Dalbergia emarginata Roxb, *Amerimnon latifolium* Roxb Kuntze), also known as Indian rosewood, is a member of the Dalbergia genus in the Fabaceae (Leguminosae) family. It is also known as Black Rosewood, Blackwood, Indian palisander, Java palisander, Rosetta rosewood, Bombay blackwood, East Indian rosewood, and Elite rosewood (118). It is exported to Europe under the name of Bombay blackwood or rosewood. Genus *Dalbergia* is named in honor of two brothers, Nicholas Dahlberg (Botanist) and Carl Gustav Dahlberg (119).

### 2.16.1. *Ecology*

DL is native to India and Indonesia. It is growing throughout India except for Jammu &Kashmir, Himachal Pradesh, and Sikkim, as extreme weather conditions may be one of the reasons (120). The genus consists of approximately 274 species across the world. In tropical Africa (Nigeria, Kenya, Uganda, and Tanzania), it is planted on a small scale as an aromatic plant. DL is famous for its fragrance and is grown as a decorative plant in tropical Asia. It was also used as a shade tree in agroforestry areas (121).

### 2.16.2. *Biology*

DL grows up to 40 meters long and 180 centimeters in diameter, slightly twisted and branchless up to 12-24 meters. In many parts of tropical Asia, DL grows in deciduous forests at altitudes of up to 900–1500 meters, with annual rainfall ranging from 750 to 5000 mm. It can thrive for up to 6 -7 months in low rainfall areas (below 40mm) and survives in areas where relative humidity is 40-100%, max temp 37°C - 50°C, and min temp 15°C. In dried areas, the tree undergoes leaf shedding in January, and new leaves emerge in April-May. Flowering starts in December and lasts until March. Conversely, the trees maintain their green foliage throughout the year in humid areas. They grow on vertisols and well-drained gneiss, trap, alluvial, and laterite soils. In India, DL is reported to be scattered in forest areas, and optimum conditions for this species are reported in the Bombay region. It is a good shade tree for coffee plants (118).

### 2.16.3. *Morphological features*

DL consists of a single-stemmed deciduous tree that grows up to 20-40 meters. Different plant parts are shown in **Fig.3**. from A-F. The bark is thin, irregular, and grey. Leaves are odd-pinnate, alternate, 5-7 unequal leaflets generated from the same rachies. Flowers are white and 0.5-1 cm long. The pods are brown with 1-4 seeds. It takes 240 years for a tree to attain a max diameter of 220-250 cm and a height of 30-35 meters. The life span of heartwood is over 120 months (122).


**Fig.3. Morphological features of DL**

According to the Indian Forest Act of 1927, the exportation of DL wood was illegal. It was included in the red list (vulnerable) of the International Union of Conservation of Nature (IUCN)(90) and the "Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES)" annexure II B (123).

Due to the low viability of seeds and indiscriminate cutting, stock growth in India was reduced, creating a crisis in the timber industry. To overcome the situation, tissue culture methods (Micropropagation) are utilised to speed up the production of plant stocks. The studies reported that the DL plant can be regenerated from a young shoot callus (124) and immature embryos without a callus (125). It was also demonstrated that callus-mediated shoot bud formation and rooting of shoot bud formation of DL in a suitable media. (126). Single and multiple shoots were produced from mature rosewood trees *in vitro* (127)The Kerala Forest Research Institute in Thrissur, Kerala, India, has maintained this plant's germplasm resources (128)Due to overexploitation, Kerala, Tamil Nadu, Gujarat, and Karnataka restrict the export of DL (129).

### 2.16.4. *Uses of DL Heartwood*

Among all species of *Dalbergia*, DL produces the most valuable timber. The wood of DL is well known for its applications in the wooden industry. It is used in making passenger ships, cabinets, high-class bentwood furniture, umbrella handles, musical instruments, handles of heavy-duty hammers, and axes. It was used in agriculture to make wheel rims, spokes, poles, and shafts. In India, DL is planted as a roadside tree, a soil nitrogen enhancer, and provides mulch (118). The DL trees are a great source of forage for honeybees, yielding dark amber-coloured strong honey (130).

### 2.16.5. *Pharmacological actions*

Traditionally, the decoction of bark was given in diarrhoea since ages (22), bark and leaves were used for leprosy (23,24), and oil was used for skin diseases (25). In recent years, DL has been in the limelight due to its pharmacological actions (Table 6). Recent studies have shown that DL has antitermitic action (26), anthelminthic activity (27), antibacterial activity (25), antifungal activity (26), cerebroprotective effects (28), antioxidant activity (29), hemolytic activity (30), immunomodulatory activity(31), nootropic, anxiolytic activity (32), *in vitro* anticancer activity (33) and antidiabetic activity (34). Recent studies have shown that various extracts of plant parts consist of alkaloids, glycosides, flavonoids, phenols, resins, tannins, steroids, and carbohydrates

(32,37,38). The bark extract of DL exhibited antidiabetic activity (34), while the leaf extract showed *in-vitro* α-glucosidase activity(39). A few compounds, like Isoliquirigenin, Latifolin, and all-E lutein (**Table 7.**) have been isolated and evaluated for pharmacological activity. Products (**Table 8.**) from DL were also used in preparations for anti-aging and essential oils.

**Table 6. Different pharmacological actions of DL extracts**

| Sl.No | Pharmacological activity | Plant part | Extract | References |
|---|---|---|---|---|
| **1** | Antibacterial activity against *E.Coli, Staphylococcus epidermidis, Bacillus subtilis, –Bacillus brevis. Pasedomonas aeruginosa, Proteus mirabilis, Enterococcu faecalis, Staphylococcus aureus, Acinetobacter baumannii, Citrobacter freundii, Enterobacter aerogenes.* | Bark | Methanolic | (25,38, 131,132) |
| | | | Methanolic/Ethanolic/Chloroform | |
| | | Root | | |
| | *Staphylococcus aureus.* | | | |
| | *Antibacterial activity against E.Coli and Staphylococcus aureus* | Heartwood | Chloroform extract | |
| **2** | Antifungal activity against *Candida Albicans* | Root | Methanolic/Ethanolic/ Chloroform, Hot light petroleum | (133,134) |
| | *Fomitopsis palustris, Cladosporium cladosporioides* | Heartwood | | |
| **3** | Anti-termite activity against *Reticulitermes speratus Kolbe* | | Hot light petroleum, | (135,136) |
| | | Heartwood | Acetone extract/ether soluble fraction | |
| | *Reticulitermes speratus Kolbe* | | | |

| | | | | |
|---|---|---|---|---|
| **4.** | Antioxidant activity | | | |
| | DPPH | Leaves | Methanolic | (137–139) |
| | DPPH, SOD | Wood of root | Methanolic | |
| | DPPH ACTIVITY | Bark | | |
| | Nitric oxide scavenging activity, reducing potential activity, Ferric thiocyanate scavenging activity | | Ethanolic | |
| **5** | Anti hyperglycaemic activity | leaves | Methanolic | (140) |
| **6** | Anti-obesity activity | Bark | Hydroalcoholic | (29) |
| **7** | Anticancer activity | Whole | Methanol | (138,141) |
| | MTT assay against L6, EAC, MCF 7, HEP G2, and Hela cell lines | plant | fraction of hydroalcoholic extract | |
| | MCF10A (breast cancer cells) | Root wood | Methanolic | |
| **8** | Antioxidants (catalase, SOD, MDA, MPA) and cerebroprotective action | bark | Methanolic | (142) |
| **9** | Inhibitor activity on nitric oxide production | leaves | Methanolic | (137) |
| **10** | DPPH | heartwood | Ethanol-toluene extract | (143) |
| **11** | Nephroprotective action | leaves | Methanolic extract | (144) |

| | | | | |
|---|---|---|---|---|
| **12** | Antimutagenic activity | leaves | Methanolic extract | (145) |
| **13** | Antihemolytic action | leaves | Methanolic extract | (30) |
| **14** | Nootropic activity, anxiolytic, and locomotor activity | roots | Ethanolic extract | (146) |
| **15** | Immunomodulatory activity | bark | Hydroalcoholic extract | (147) |

Thus, this study was conducted to study the pharmacoinformatic parameters of previously identified compounds from DL, their isolation, and evaluation for antidiabetic activity using *in vitro* and *in vivo* methods.

### 2.16.6. *Active Constituents of DL*

Many active constituents like latifolin(136,148), O-dimethyl Latifolin(149), (R)-Dalbergione (150), β-sitosterol, Dalbergin (151), lupeol (152), Dalbinol (153), Dalbin (154), Latinone (155), Dalcriodain (156), Dalbergiphenol, 4-methoxy dalbergione (157), Obtusafuran, Isoparvifuran (158) These were reported from various parts of DL. Some essential oils, spathulenol, α-Berga motene, β elemene from DL wood were also reported (159).

**Table 7. Pharmacological activities of isolated compounds of DL**

| S. No | Active constituent | Plant part | Reported pharmacological activity | Ref |
|---|---|---|---|---|
| **1** | Isoliquiritigenin | heartwood | Cytotoxic activity | (138) |
| **2** | Latifolin | heartwood | Antitermitic action | (136) |
| **3** | All-E lutein | leaves | Anti haemolytic | (160) |

**Table 8. DL as an ingredient in marketed products**

| S. No | Trade name/generic name | Purpose | Mfg by |
|---|---|---|---|
| **1** | Rosewood oil | Anti-ageing | Crysalis India |
| **2** | Veda earth bathing soap | Essential oil | S.K Enterprises |

# CHAPTER-3

## 3. RATIONALE OF THE STUDY

### 3.1 HYPOTHESIS

**Prevalence and Challenges of Diabetes Treatment**

Diabetes is a metabolic disorder characterised by disturbances in carbohydrate, fat, and protein metabolism (161). T2DM is the most prevalent form of diabetes. The treatment of diabetes can be challenging due to uncontrolled postprandial hyperglycaemia and genetic factors. Elevated blood glucose levels in the blood may cause different types of complications in the body (162). Effective treatment is challenging due to uncontrolled postprandial hyperglycemia, genetic factors, and adverse effects of current pharmacological therapies. One practical therapeutic approach to control hyperglycaemia is inhibiting the conversion of polysaccharides to monosaccharides by α-amylase and α-glucosidase enzymes in saliva and the small intestine (163).

**Limitations of Existing Therapies**

Acarbose, Voglibose, and miglitol are clinically approved α-amylase inhibitors for controlling diabetes in many patients. These drugs prolong the release of carbohydrates from the stomach and effectively control the elevation of postprandial blood glucose (164). Approximately twenty to thirty percent of the population experiences a variety of adverse reactions to these medications, including abdominal discomfort, bloating, diarrhoea, and flatulence (165). These limitations underscore the need for safer and more effective therapeutic options. Due to the lack of adequate and safer treatment options, there is a need to identify potential candidates capable of overcoming the above-mentioned limitations.

**Potential of Plant-Derived Compounds:**

In recent years, compounds derived from plants have been researched for their potential to treat diverse chronic diseases, including diabetes. One of such teeming plants identified recently was Dalbergia species, i.e., DL. Many DL extracts have been reported to have utility in traditional systems of medicine worldwide in treating various diseases. DL have shown promise due to their traditional medicinal uses for various conditions including body aches (166), leprosy (167), skin diseases (25), and their reported hypoglycemic properties (140).

**Knowledge Gap in Current Research**

While DL extracts have demonstrated hypoglycemic activity, the specific compounds responsible for this activity have not been identified or thoroughly studied.

These observations led to the design of the current work, which aims to conduct *In silico* studies to identify lead compounds against alpha amylase enzyme (1HNY), their isolation and evaluate its pharmacological activity (*in vitro* and *in vivo*) toward diabetes **(Fig.4).**

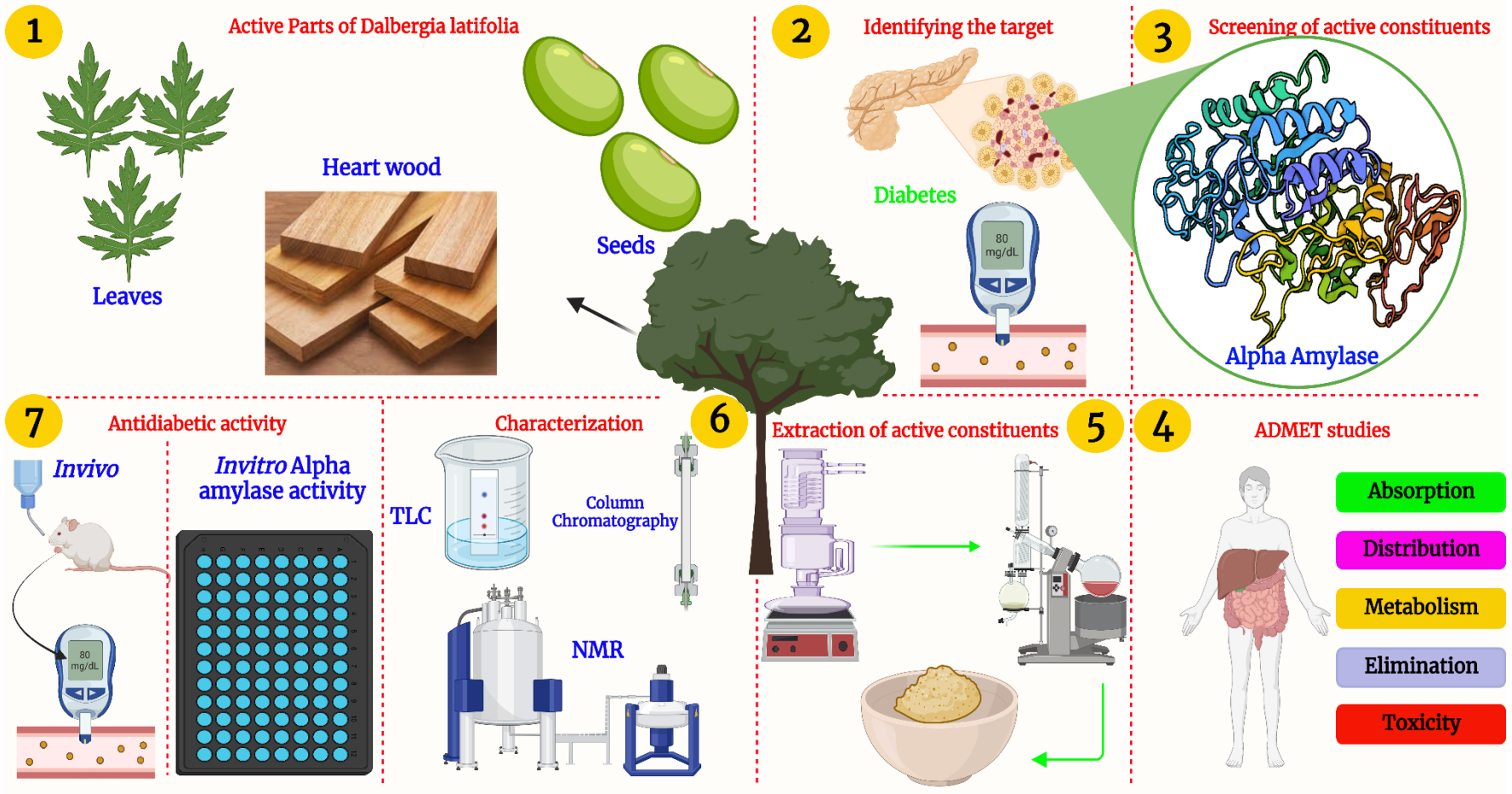



**Fig.4. Diagrammatic representation of the hypothesis of the study**

## 3.2 AIM

Extraction, Pharmacological, And Pharmacoinformatic Evaluation of Active Constituents of DL as Potential Antidiabetic Agents

## 3.3 OBJECTIVES

The objectives of the proposed research work are enlisted as:

1. Isolation of active constituents of DL by using different extractive methods
2. Structural analysis of isolated compounds by using IR, NMR, and MS spectral methods
3. Molecular docking of active constituents with α-amylase enzyme (PDB: 1HNY) and study of pharmacokinetic parameters using different computational techniques. Identification of hit molecules based on virtual screening among all isolative constituents.
4. *In vitro* Evaluation of enzymatic inhibitory activity against the α-amylase enzyme.
5. *In vivo* evaluation of hypoglycaemic activity

# CHAPTER-4

# 4. MATERIALS AND METHODS

## 4.1 Materials and Equipment

**Table 9. List of chemicals used and manufacturer/supplier**

| S.No | Chemical name | Manufacturer/Supplier |
|---|---|---|
| **1.** | Sodium carboxy methyl cellulose | Loba Chemie, Mumbai, India |
| **2.** | Methanol | Molychem, Mumbai, India |
| **3.** | Ethanol | Hi-Media Laboratories Pvt Ltd, Mumbai, India |
| **4.** | Chloroform | Sisco Research Laboratories Pvt Ltd, India |
| **5.** | Diethyl ether | Loba Chemie, Mumbai, India |
| **6.** | Benzene | ACS Chemicals, Gujarat, India |
| **7.** | Light petroleum Ether | Ranchem Pvt Ltd, India |
| **8.** | n-hexane | ACS Chemicals, Gujarat, India |
| **9.** | chloroform | Merck International, Gujarat, India |
| **10.** | Sodium dihydrogen phosphate | Loba Chemie, Mumbai, India |
| **11.** | Sodium citrate | Hi-Media Laboratories Pvt Ltd, Mumbai, India |
| **12.** | TLC Plates | Merck, USA |
| **13.** | Sephadex LH20 | Merck, USA |
| **14.** | Silica gel 100-200 mesh size | Loba Chemie, Mumbai, India |
| **15.** | DMSO | Loba Chemie, Mumbai, India |
| **16.** | STZ | SRL Diagnostics, India. |
| **17.** | Acarbose | TCI (India) |

**Table 10. List of equipment used and manufacturer/supplier**

| S.No | Equipment name | Manufacturer/Supplier |
|---|---|---|
| **1** | Centrifuge | REMI, RM-12C, Mumbai, India |
| **2** | pH Meter | PerkinElmer, USA |
| **3** | FTIR | PerkinElmer, USA |
| **4** | Electronic weighing balance | CY360, Shimadzu Co Ltd, Japan |
| **5** | Soxhlet apparatus | Borosil, India |
| **6** | Rotavapor | R-100, Buchi ,mumbai |
| **7** | Bath sonicator | Loba Life, Lobachemie, Mumbai, India |
| **8** | Melting point apparatus | Popular, India |

### 4.2. Plant material .

Heartwood of DL was collected from the Suresh Agroforestry Network (Bangalore, Karnataka) and was authenticated by Prof. MD. Mustafa, Department of Botany, Kakatiya University, Warangal. The plant materials were washed, dried, powdered, and stored until further use.

### 4.3. Pharmacoinformatic Methods

The active constituents of DL were identified by performing an extensive literature survey, and the pharmacoinformatic study of identified compounds was performed.

#### *4.3.1. Smiles of the compounds*

The smiles of active constituents of DL were obtained from the PubChem database and structure file generator, a free online tool accessible on the SwissADME website (168).

#### *4.3.2. ADME*

Predictive ADME studies provide useful insights into how chemical compounds function in the human body, including metabolism, distribution patterns, and their interaction with proteins. Early ADME computation during the discovery phase has been shown to substantially decrease the percentage of clinical failures in clinical trials (169). Smiles of each compound are required to run the Swiss ADME tool

(http://www.swissadme.ch). The software computes an array of parameters, including molecular weight (MV), rotational bonds (nRB), hydrogen bond donors (nHBD), hydrogen bond acceptors (nHBA), inhibition of cytochrome isoforms (CYP), skin permeability (LogKp), gastrointestinal absorption (GIA), permeability, p-glycoprotein substrate, water solubility, blood-brain barrier (BBB) and skin permeability (cLogP). The compounds were primarily assessed for their drug-like properties using Lipinski's rule of five. The following properties are required of drug-like compounds as per the rule: molecular weight (MV) ≤ 500 daltons, nHBA ≤ 10, nHBD ≤ 5, and clogP ≤ 5. Additionally, substances that exhibit multiple violations of these set limits have not been accepted (168).

#### 4.3.3. *Determination of bioactivity scores*

The bioactivity score values of a compound can be used to assess its potential as a drug candidate. Canonical SMILES strings of the compounds were acquired from PubChem and converted into mol or. sdf files with the help of open Babel software (139), and then added to the Molinspiration program (97). The Molinspiration web application (https://www.molinspiration.com) was employed to forecast the effects of bioactive synthetic compounds on various human receptors, such as ion channels, nuclear receptors, kinases, proteases, and G-Protein coupled receptors (GPCRs) (170).

#### *4.3.4. Determination of toxicity*

The toxicity prediction of newly designed therapeutic molecules before their utility in humans has a significant role. Using combinatorial toxicity predictions can effectively reduce the necessity for animal testing, offering a swifter alternative to ascertaining harmful doses on animals. The compounds' toxicity was studied using ProTox software (https://tox-new.charite.de/protox_II). ProTox-II software predicts better than other commercial software, such as Discovery Studio's TOPKAT (171). To forecast several toxicity endpoints, such as toxicity targets, immunotoxicity, carcinogenicity, mutagenicity, hepatotoxicity, cytotoxicity, acute toxicity, and adverse outcome (Tox21) pathways (**Fig.5**). The toxicity of the compounds was measured in terms of the $LD_{50}$ (lethal dose in 50% of the population). The $LD_{50}$ values for toxic dosages are frequently expressed in mg/kg body weight. As per the GHS, an internationally recognized system for chemical classification and labeling globally, toxicity classes are defined (172)

- 1. Fatal ($LD_{50} \leq 5$)
- 2. Fatal ($5 < LD_{50} \leq 50$)
- 3. Toxic ($50 < LD_{50} \leq 300$)
- 4. Harmful ($300 < LD_{50} \leq 2000$)
- 5. May be harmful ($2000 < LD_{50} \leq 5000$)
- 6. Nontoxic ($LD_{50} > 5000$).

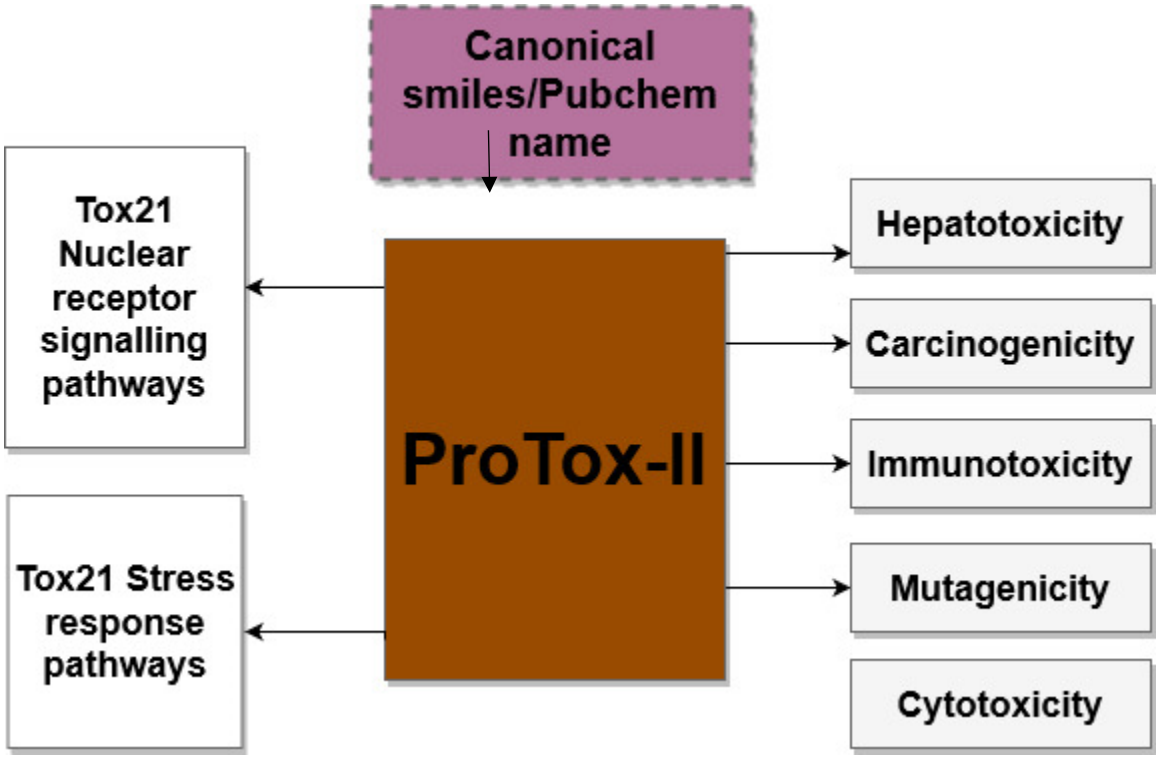



**Fig.5. Study of different toxicities using the Protox-II web tool**

### *4.3.5. Docking studies*

#### *4.3.5.1. Molecular docking*

Molecular docking, a virtual screening method, is a computational approach for identifying bioactive interactions between ligands and target proteins through their structure and orientation to the binding site (173). However, pyrx is an innovative program that combines multiple open-source applications, such as open Babel, auto dock, and vina, for performing docking-based virtual screening (DBVS) seamlessly (174).

#### *4.3.5.2. Protein preparation*

Protein Data Bank (PDB) ID: 1HNY is the three-dimensional crystal structure of human pancreatic α-amylase obtained from the website (https://www.rcsb.org). Biovia Discovery Studio Visualizer was used for protein preparation.

#### *4.3.5.3. Ligand preparation*

Chem Bio 3D ultra-12.0.2 was used to minimize energy, while Chem Draw Pro 12.0.2 was used to draw the structures of ligands. Energy minimization aids in determining the ligand's bioactive conformer form (175).

### *4.3.6. Extraction of Active Constituents*

The active constituents having greater potential towards α-amylase inhibition (from docking) were extracted from DL.

#### *4.3.6.1. Extraction of DGN from DL Heartwood*

DGN was extracted from DL heartwood using the method demonstrated by Dervilla M. X. Donnelly et al. (176) With slight modifications. Briefly, DL heartwood powder (2.5 kgs) was subjected to soxhlation using benzene (2L) as a solvent for three days to extract the active constituents of DL. The evaporation of extract obtained after soxhlation processes resulted in a green-coloured paste (212 gms). Then, the paste was dissolved in acetone (500 ml) and evaporated, which produced dark red oil (157 gms). Furthermore, the red oil was subjected to column chromatography using (60-120) silica gel. Elution was carried out by using chloroform and chloroform-ethyl acetate mixtures (9.5:0.5, 9:1, 3:1, 1:1, and 1:3), and nine fractions (Fr:1-9) were obtained. All the obtained fractions were rechromatographed using benzene and ethyl acetate (5:1) to get nine subfractions (Fr:1A-9A). Again, fractions Fr: 3A (35 gms), 4A (27 gms), and 5A (16 gms) were eluted successfully with methanol: water (1:3). The resultant extractive content from fractions Fr: 3A, 4A, and 5A were collected and evaporated. The obtained powder 3A1(902mg), 4A1 (650 mg), and 5A1 (110 mg) was analysed by NMR (Bruker Avance 400 MHz). The results were compared with the chemical structures of various active constituents of DL to confirm the specific active constituents. The structure of fraction 3A1 was matched with Dalbergin.

#### **4.3.6.2.** ***Extraction of ISG from DL heartwood.***

Similarly, with some modifications, ISG was extracted from DL heartwood according to the method described in section 4.3.6.1. Briefly, DL heartwood powder from the central trunk (1.5 kgs) was subjected to Soxhlation for 96 h using ethyl alcohol (4L, 99.5 % pure) as the solvent. The resultant extract was concentrated using rotavapor

(Rotavapor® R-300, 15 min) at bath temperature between 50-53 ℃. Subsequently, the extract (176gms) was subjected to chromatography on a Sephadex LH 20 column employing varying ratios of methanol: water (30:70, 50:50, and 70:30 v/v), and four distinct fractions were collected ( Fr:1 (3 gms), Fr:2 (15 gms), Fr:3 (4 gms) and Fr:4 (1 gm). Fr-2 was chromatographed on a silica gel column using CHCl3: MeOH (10:1) as the solvent system (1.5L). As a result, two subfractions were obtained and named Fr-2A (760 mg) and Fr-2B (333mg), as shown in **Fig.6.** Furthermore, Fr-2A and Fr-2B were analysed by NMR, FTIR and compared with the structures of reported active constituents of DL (177–180)

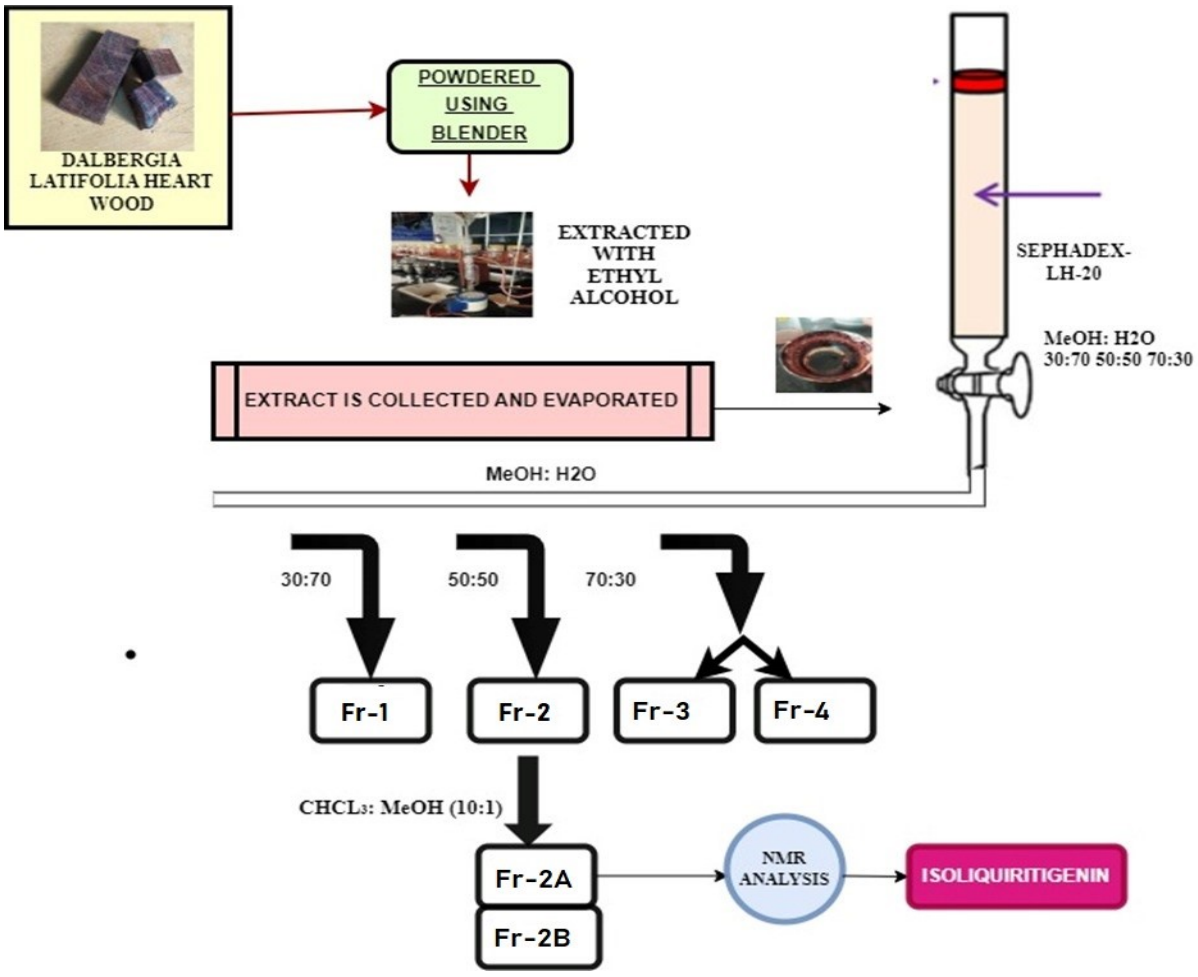



**Fig.6. Isolation of ISG from the heartwood of DL**

### *4.3.7. Characterization of isolated compounds*

#### *4.3.7.1.NMR Spectral analysis ( $^{13}C$ and $^{1}H$)*

Before spectrum analysis, the solubility of the resulting fractions was examined in CHCL3, DMF, and Dimethyl Sulfoxide (DMSO). Five milligrams of Fr 3A1 and

five milligrams of FR-2A were dissolved in 0.4 ml of deuterated chloroform-D and DMSO-D6, respectively.

#### *4.3.7.2.FTIR*

The mortar was filled with two to three milligrams of fraction 3A1, KBr powder was added, and the mixture was ground thoroughly with a pestle. The sample was placed between two stainless steel discs and put in an IR spectrum holder to prepare the disk for spectral analysis. A similar procedure was performed for Fr.2A.

### *4.3.8. In vitro studies*

#### *4.3.8.1. α-amylase inhibitory activity*

Determination of α-amylase activity was carried out by the GOD-POD method(181). Final values are expressed in terms of $IC_{50}$. Sample dilutions of DGN, ISG, and acarbose [0.1, 0.5, 1.0, 2.5, 5.0, 10.0, and 25.0 µg/mL in sodium phosphate buffer (pH 6.9)] were prepared. Enzyme solution (10 µL) containing 20 mg/mL pancreatic α-amylase was placed in the defined well of a 96-well plate. Each dilution (0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0 µg/mL) was added in a volume of ten microliters, and the resulting mixture was incubated at 37°C for 10 minutes. The reaction was started by adding 50 µL of substrate (0.1% soluble starch), and the mixture was further incubated at 37°C for 15 minutes. After 15 minutes, 100 µL of GOD-POD Reagent was added to the mixture. The plate was then incubated at 37°C for 10 minutes. Subsequently, the absorbance of the mixture was measured at a wavelength of 490 nm using a microplate reader (iMark, Bio-Rad). Acarbose, a known standard α-amylase inhibitor (25 µg/mL final concentration), was taken as a positive control. The experiment was repeated thrice. The α-amylase activity of DGN and ISG was determined, and their $IC_{50}$ values were calculated.

#### *4.3.8.2. In vitro glucose uptake assay*

The glucose uptake assay using L6 cell lines was carried out using the GOD-POD method (182). L6 cells, obtained from NCCS Pune, were placed at a density of 8 × 104 cells per well in 24-well cell culture plates and cultured in standard medium for 24-48 hours. After the cells had fully differentiated, they were washed twice with KRPH buffer, which has 20 mM HEPES, 5 mM KH2PO4, 1 mM CaCl2, 136 mM NaCl, 4.7 mM KCl, and a pH of 7.4. Subsequently, the cells were placed in glucose-free DMEM and incubated at 37°C for 18 hours. The medium was disposed of, and the cells were rinsed once with KRPH buffer. Different concentrations of metformin (1 mM), DGN (313.5 µg/mL, 627 µg/mL, and 1254 µg/mL), and ISG (336 µg/mL, 672 µg/mL, and

1344 µg/mL) were added to the cells. Then, 1-methoxy glucose (2-DG) was introduced and left to sit for 30 minutes. After that, the cells were incubated for 30 minutes in KRPH buffer containing 2% (v/v) bovine serum albumin and 10 mM 2-DG. The liquid part of the cell contents was collected to estimate the amount of glucose present. To remove any extra 2-DG, the cells were washed three times with KRPH. Next, they were broken down using extraction buffer, frozen, thawed, and heated to 85°C for 40 minutes to break down endogenous nicotinamide adenine dinucleotide phosphate (NAD(P)). Finally, they were centrifuged for two minutes at 500 rpm. The resulting supernatant was analysed for 2-deoxyglucose-6-phosphate (2-DG6P) content by a GOD-POD Enzyme Assay Kit. Ten microliters of the sample were combined with 1 mL of reagent (GOD-POD reagent) and incubated at 37°C for 10 minutes. Using a microplate reader, the absorbance of the test compound (At) and standard (As) relative to the blank was measured at 505 nm. To determine the blank value, lysates of cells not exposed to 2-DG were examined. By comparing the data to standards, nanomoles of 2-DG were computed. The standard was 2-DG6P, authentic. Metformin (1 mM) was utilised as the negative control, and insulin (0.1 U/mL) as the positive control. The concentration of glucose was calculated by using a given formula

$$Glucose\ concentration\ (mg/dl)\ =\ (As/At) * 100$$

where As= Absorbance of standard, At=Absorbance of test

#### *4.3.8.3. In vitro cytotoxicity assay*

The neutral red uptake assay is a cell viability assay that can assess compound-induced cytotoxicity (183). In 96-well plates, the cells (5000–8000 cells/well) were cultured for 24 hours at 37°C with 5% CO2 in DMEM (AT149-1 L) supplemented with 10% FBS (HIMEDIA-RM 10432) and 1% antibiotic solution. The media was taken out the following day, and each plate well was filled with fresh culture medium. The designated wells were filled with five microliters of treatment dilutions of DGN and ISG (0, 1, 10, 50, 100, 250, 500, and 1000 µg/mL), and the treated plates were then incubated for an entire day. The specified wells were then filled with 100 µL of NRU (40 µg/mL in PBS) and incubated for one hour using a Heal Force-Smart cell CO2 Incubator-Hf-90. After 1 hr, the medium was removed, and Neutral red dye was extracted using 100 µL of destaining solution, and the absorbance was measured at 540 nm using a plate reader.

### *4.3.9. Antidiabetic activity*

#### *4.3.9.1. Experimental animals*

After isolation of compounds, Form-B for conducting animal experiments was submitted. 80 SD/Wistar rats ,either sex were approved by IAEC members with approval number LPU/IAEC/2023/25. 80 Sprague Dawley (SD) rats (160-300 gms) of either sex were purchased from the central animal facility, National Institute of Pharmaceutical Education and Research (NIPER), Mohali, Punjab, India. Rats were acclimatised and housed in standard propylene cages and maintained at 55±5% humidity and 22±2° C temperature with a 12:12 h light and dark cycle. Before dietary manipulation, a commercially available normal pellet diet and water ad libitum were provided. Briefly, a total of 80 rats (either sex) were taken and divided into 10 groups (GPs), as shown in **Table 11.** A normal pellet diet (NPD) was given to the first three GPs ($GP_1$, $GP_2$, and $GP_3$), and the remaining GPs ($GP_4$ – $GP_{10}$) were given HFD (58% fat, 25% protein, and 17% carbohydrate, as a % of total kcal) and water ad libitum.

#### *4.3.9.2. Induction of diabetes*

A single, low dose of STZ (35 mg/kg) was used to induce Diabetes in SD rats using the method reported by Srinivasan et al., 2005 (50). After two weeks, a single dose of STZ was injected into each rat present in the GPs ($GP_4$ – $GP_{10}$) intraperitoneally (*i.p.*). The control GP ($GP_1$) was given a phosphate buffer at 1 mL/kg *i.p.* (pH 4.4).

**Table 11. Animal experiment design**

| GP. No. | GP Name | Diet type, Doses and route of drug treatment | Animals |
|---|---|---|---|
| **$GP_1$** | Vehicle control | NPD# + 0.5% CMC (*p.o.*) (Vehicle) | 8 |
| **$GP_2$** | DGN *per se* | NPD + 10 mg/kg | 8 |
| **$GP_3$** | ISG *per se* | NPD + 10 mg/kg | 8 |
| **$GP_4$** | Negative control | HFD##+ 35 mg/kg of STZ (single dose, *i.p.)* | 8 |
| **$GP_5$** | Positive control | HFD + 35 mg/kg of STZ (single dose, *i.p.*) + 20 mg/kg of acarbose (*p.o.*) | 8 |
| **$GP_6$** | DGN low dose | HFD + 35 mg/kg of STZ (single dose, *i.p.*) + 5 mg/kg (*p.o.)* | 8 |

| | | | |
|---|---|---|---|
| **GP7** | DGN high dose | HFD + 35 mg/kg of STZ (single dose, *i.p.*) + 10 mg/kg (*p.o.*) | 8 |
| **GP8** | ISG low dose | HFD + 35 mg/kg of STZ (single dose, *i.p.*) + 5 mg/kg (*p.o.)* | 8 |
| **GP9** | ISG high dose | HFD + 35 mg/kg of STZ (single dose, *i.p.)* + 10 mg/kg (*p.o.)* | 8 |
| **GP10** | Combination (DGN+ISG) | HFD + 35 mg/kg of STZ (single dose, *i.p.*) + DGN 5 mg/kg (p.o.) + ISG 5 mg/kg (*p.o.)* | 8 |

[#]Normal Pellet Diet, [##] High Fat Diet, GP = group, STZ= streptozotocin, DGN= Dalbergin, ISG= isoliquiritigenin, *i.p* = intra peritonial, *p.o* = per oral

The doses of DGN 5mg/kg and 10 mg/kg are selected based on the recent study on osteoporotic effect the DGN conducted by Choudary D et al., (184). The dose of ISL 5mg/kg and 10 mg/kg was based on recent study conducted by zhan c et al.,(185) on protective effect of ISL on cerebral ischemia. To compare the effect of DGN and ISG in HFD fed rats , the dose of 10 mg/kg of DGN and ISG were given to NPD fed rats for easy comparison. By utilizing these specific doses, the impact of DGN and ISG on metabolic parameters, including body weight, blood glucose levels, lipid profile, liver profile and kidney profile were assessed in 14 days of the study.

The rats showing non-fasting blood sugar ≥300 mg/dL after 3 days of STZ administration were considered for evaluation of antidiabetic activity **(Fig.7).** An assessment was made on the impact of treatments on rats' body weight and biochemical parameters. Additionally, the study included the measurement of both feed and water intake, and the corresponding diet was maintained throughout the study.

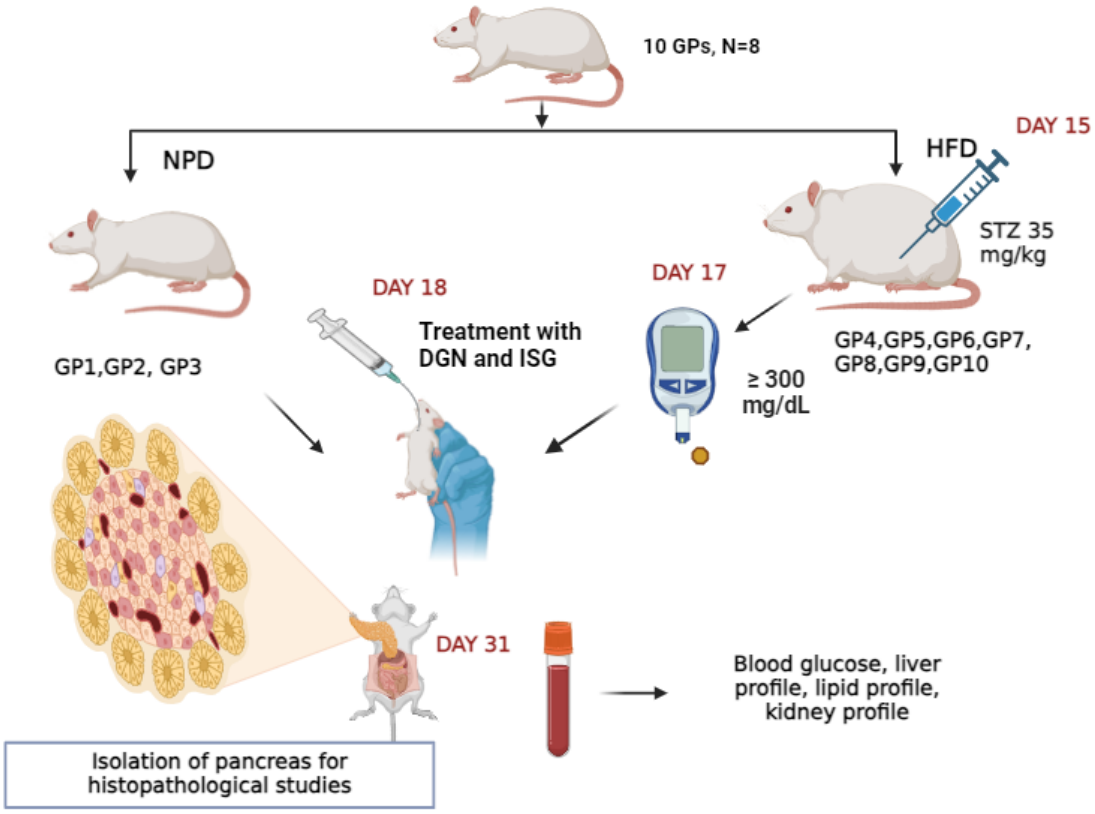



**Fig.7. Schematic representation of *in-vivo* antidiabetic activity. GPs-Groups, N-No. of animals per group, NPD- Normal Pellet Diet, HFD-High Fat Diet, STZ-Streptozotocin**

#### *4.3.9.3.Composition of HFD*

The required ingredients for preparing 1 kg of HFD include normal pellet diet powder (365 gms), lard (310 gms), casein (250 gms), cholesterol (10 gms), vitamin and mineral mix (60 gms), DL-methionine (3 gms), yeast powder (1 gm), and sodium chloride (1 gm) (50).

#### *4.3.9.4.Preparation of HFD*

The normal pellet diet powder was taken to make the laddus for daily consumption, and the required quantities of all the ingredients were added and mixed well. A little water was added to the preparation, and 30-50 gms of laddus were made. The freshly prepared diet mixture was stored at 4°C to maintain its freshness.

### *4.3.9.5. Estimation of Biochemical Parameters*

Upon completion of the study, all the animals underwent an overnight period of fasting and were subsequently euthanised by decapitation. Blood was collected from the retroorbital plexus of the rats while they were under light ether anesthesia. The blood was collected using a capillary tube and transferred into Eppendorf tubes. The collected blood was centrifuged (5 min, 5000 rpm) to separate the serum. The lipid, renal profile, and liver function tests were performed (Roche Cobas C 111 Biochemistry analyser) using commercially available kits (Rapid diagnostics pvt ltd, Delhi).

**Lipid profile:**

The assay measures both free cholesterol and cholesterol esters using an enzyme driven reaction. cholesterol esterase hydrolyses cholesterol esters into cholesterol, cholesterol oxidase converts cholesterol into cholest-4-ene-3-one plus hydrogen peroxide. The hydrogen peroxide is then detected with a highly specific colorimetric probe. Samples are compared to a known concentration of cholesterol standard in a 96 well plate reader. Samples and standards are incubated for 45 mins at 37℃ and read the absorbance at 540-570 nm. (The same kit provided the reagents for separation and quantification of LDL,HDL and VLDL).

**Liver profile :**

**SGPT/ALT:**

At 37℃ and pH 7.04 , alanine and α-ketoglutaric acid react to form pyruvic acid and glutamic acids, which are catalyzed by alanine amino transferase (ALT). Next, pyruvic acid and phenylhydrazine were combined to produce phenylhydrazone. Phenylhydrazone is a reddish brown solution under alkaline conditions. ALT activity can be calculated by measuring the OD values at 510 nm.

**SGOT:** The reaction between α-ketoglutarate and L-aspartate, which yields glutamate and oxaloacetate, is catalyzed by aspartate aminotransaminase. In the presence of malate dehydrogenase, oxaloacetate and NADH react to produce malate and NAD. At 340 nm, absorbance decreases as NADH is changed into NAD. The rate of decrease in absorbance is measured and is proportional to AST in the sample.

**Alkaline phosphatase (ALP):** in alkaline conditions, 4-nitro phenol phosphate is hydrolyzed by the enzyme alkaline phosphatase to yield 4-nitrophenol. 4-nitrophenol formed is detected spectrophotometrically at 405 nm to give a measurement of alkaline phosphatase activity in the given sample.

**Kidney profile:**

Serum creatinine: In an alkaline environment, creatinine reacts with picric acid to form an orange red color complex. The development of this orange red color was read in colorimeter at 500-520 nm.

Blood urea: The test utilizes a metabolic enzyme urease to measure the concentration of urea. Urease converts urea to ammonia, which is measured calorimetrically at 620nm.

***4.3.9.6. Blood glucose determination, body weight changes, and water intake***

The blood glucose levels were determined by collecting the blood from the tail vein using a glucometer (Dr Morepen, BG-03). The body weight of each rat was carefully measured using an analytical balance (Shimadzu) before and after treatment by DGN and ISG, respectively. The consumption of water by experimental rats present in each GP was measured and provided *ad libitum* access to water to ensure proper hydration(186)

***4.3.9.7. Histopathological studies of the pancreas***

After decapitation of the animal, the pancreas was isolated carefully and preserved in 10% formalin solution until further histopathology study.

# CHAPTER-5

## 5. RESULTS AND DISCUSSION

### 5.1. *statistical analysis*

**Various groups were compared using one-way analysis of variance (ANOVA) with Tukey's posthoc test for multiple comparisons.**

#### *5.1.1. Lipinski's Rule of Five*

Lipinski's Rule of Five was often misused and misinterpreted in most cases. It was originally designed to facilitate the development of bioavailable oral drugs and was not intended to control the medicinal chemistry of all small-molecule drugs. Oral administration is an acceptable goal for many treatments but is not an absolute requirement. To be clear, the rule of five is not a quantitative estimate of oral absorption, and compounds that do not meet any of the five criteria are not necessarily "absorbed" orally. This rule does not classify all well-absorbed and poorly absorbed compounds, but it easily and quickly provides a good level of classification (187,188).

**Table 12. Physicochemical properties of selected compounds**

| Compound | Name of the compound | Properties | | | | | Lipinski rule |
|---|---|---|---|---|---|---|---|
| | | M. Wt | RB | HBA | HBD | CLog P | y/n |
| **1.** | Dalbergin | 268.26 | 2 | 4 | 1 | 2.83 | yes |
| **2.** | Dalbergione | 224.25 | 3 | 2 | 0 | 2.58 | yes |
| **3.** | Dalbergichromene | 254.28 | 2 | 3 | 1 | 2.99 | yes |
| **4.** | 4-Methoxy Dalbergione | 254.28 | 4 | 3 | 0 | 2.54 | yes |
| **5.** | Methyl dalbergin | 282.29 | 3 | 4 | 0 | 3.17 | yes |
| **6.** | Latifolin | 286.32 | 5 | 4 | 2 | 3.10 | yes |
| **7.** | Dalbin | 588.56 | 7 | 13 | 5 | 0.59 | no |
| **8.** | Dalbinol | 426.13 | 4 | 8 | 2 | 2.12 | yes |
| **9.** | Sisafolin | 342.30 | 4 | 7 | 2 | 2.22 | yes |
| **10.** | Obtusafuran | 256.30 | 2 | 3 | 1 | 3.06 | yes |
| **11.** | Isoparvifuran | 254.28 | 2 | 3 | 1 | 3.45 | yes |
| **12.** | β-sitosterol | 414.71 | 6 | 1 | 1 | 7.19 | yes |
| **13.** | Isoliquiritigenin | 256.25 | 3 | 4 | 3 | 2.37 | yes |

Following the Lipinski Rule of Five, it was observed that all compounds demonstrated a molecular weight within the specified range (molecular weight ≤ 500), except for compound 7, which exhibited a molecular weight of 588.56 g/mol. The remaining 12 compounds with a mol weight ≤500 had good absorption, transport, and diffusion potential. Lipinski's rule also states that compounds with HBA ≤ 10 and HBD ≤5 will have more excellent permeability when administered. Compounds with RBs≤15 have greater molecular flexibility. Except for compound 7 (Dalbin), all the compounds had better HBA (≤10) and HBD (≤5) values. In the case of nRB, all 13 compounds were present (nRB≤ 15), improving oral bioavailability. Several recommendations have been proposed to evaluate drug-likeness: a partition coefficient (log P) in the -0.4 to and +5.6 range, a molar refractivity ranging between 40 and 130, a molecular weight ranging from 160 and 480, and a few heavy atoms ranging from 20 and 70 (189).

Drug metabolism, pharmacokinetics, pharmacodynamics, and toxicological profiles are all impacted by the key physicochemical feature of lipophilicity (log P), which is part of what makes a drug have ADMET properties (190). The unionized solute's partition equilibrium between an immiscible organic solvent and water is represented by the molecular parameter log P. CLogP values are calculated and shown in **Table 12**. Except for compound 12, all the compounds obeyed the rule of ClogP (≤5). When compounds in the early stages of research were compared with commercialized oral medications, it was shown that A high lipophilicity(>5) often results in molecules with limited solubility, poor absorption, and rapid metabolic turnover. In general, a medication with low lipophilicity will have poor ADMET characteristics. High lipophilicity will cause adverse effects by binding to the undesired site on the receptors (190).

### *5.1.2. Solubility*

In the drug discovery process, the drug's solubility is a key element for checking drug permeability across membranes. The aqueous solubility of all the compounds was estimated using the Swiss ADME computational tool (http://www.swissadme.ch). This web tool determines the physicochemical properties, lipophilicity, water solubility, pharmacokinetics, drug likeliness, and medicinal chemistry of the selected compounds. All the results are shown in **Table** 13. The values were calculated on the Log S scale. All the compounds except compound 1 exhibited better aqueous solubility.

**Table 13. Comparison of aqueous solubility using 3 different classes**

| Compound | LogS (ESOL) Class | | LogS (Ali) Class | | LogS (SILICOS-IT) Class | |
|---|---|---|---|---|---|---|
| | **Value** | **solubility** | **Value** | **solubility** | **Value** | **solubility** |
| **1.** | -3.66 | ++ | -3.61 | ++ | -5.68 | - |
| **2.** | -2.91 | ++ | -2.92 | ++ | -3.63 | ++ |
| **3.** | -3.67 | ++ | -3.53 | ++ | -4.79 | + |
| **4.** | -3.12 | ++ | -3.32 | ++ | -3.97 | ++ |
| **5.** | -3.86 | ++ | -3.72 | ++ | -6.38 | + |
| **6.** | -4.03 | + | -4.61 | + | -4.51 | + |
| **7.** | -3.57 | ++ | -3.93 | ++ | -3.27 | ++ |
| **8.** | -3.84 | ++ | -3.93 | ++ | -5.06 | + |
| **9.** | -3.64 | ++ | -4.20 | + | -5.15 | + |
| **10.** | -3.91 | ++ | -3.89 | ++ | -4.69 | + |
| **11.** | -4.37 | + | -4.57 | + | -5.75 | + |
| **12.** | -7.90 | + | -9.67 | + | -6.19 | + |
| **13.** | -3.70 | ++ | -4.48 | + | -3.23 | ++ |

- no solubility, + mild soluble, ++ more soluble

### *5.1.3. Pharmacokinetic properties*

Following the earlier procedure, the pharmacokinetic parameters of all 13 compounds were calculated using the Swiss ADME tool. **Table** 14 displays the estimated absorption of each drug through the gastrointestinal tract. A substance is considered well absorbed when at least 90% enters the bloodstream. All the compounds except compounds 7 and 12 exhibit improved gastric absorption if taken through the oral route(191). After oral administration of substances, the ADMET-blood- brain barrier model predicted blood-brain barrier (BBB) penetration. Only chemicals that target the central nervous system require penetration across the BBB(192). The capacity of all the compounds to cross the BBB was assessed and shown in **Table** 14. It shows that compounds 7, 8, 9, and 12 are less likely to cross the BBB and may cause fewer CNS side effects. By pushing toxins and xenobiotics from cells as a biological barrier, P-glycoprotein is the most studied ATP-binding cassette (ABC) transporter. Numerous *in vitro* and *in vivo* studies have demonstrated the critical role of p-glycoprotein in the drug distribution and absorption process(193). Drug-drug interactions can be attributed

to the inhibition or activation of P-glycoprotein respectively. Monitoring solely the plasma concentration may lead to a substantial underestimation of the potential risk of p-glycoprotein mediated medication interaction. According to animal research, tissue distribution is substantially more affected by P-glycoprotein inhibition than plasma concentration, especially regarding the brain. Therefore, it is imperative to carefully consider the danger of P-glycoprotein-mediated medication interactions and drug resistance. Among all the compounds, compounds 7 and 10 are the substrates for P-glycoprotein. Therefore, fluctuations in the concentrations of compounds 7 and 10 are possible at the receptor site (194).

Metabolic liability is a significant safety concern for pharmaceutical research because it can result in a variety of problems, including poor bioavailability due to increased clearance, toxic effects from drug accumulation, and interactions between drugs (195). Cytochrome P450 monooxygenase is a key player in drug metabolism and elimination in living organisms. The results of the drug metabolism prediction against the cytochrome P450 (CYP) monooxygenase family isoforms are shown in **Table** 14. Compounds 5 and 6 inhibit all the CYP isoforms. Compounds 7 and 9 did not inhibit any of the isoforms. Specifically, majority of compounds (N=9) inhibiting CYP1A2, 69.23% of compounds are inhibiting CYP1A2, 53.84% of compounds are inhibiting CYP2C19, 46.15% compounds are inhibiting CYP2C9, 53.84% are inhibiting CYP2C9, 53.84% compounds are inhibiting CYP2D6, and 46.15% compounds are inhibiting CYP3A4. Inhibition or activation of CYP isoenzymes leads to toxicity or decreased therapeutic activity. Metabolites are compounds generated after the completion of phase I or phase II reactions in the liver. Metabolite prediction is essential in drug discovery. Web tools such as Nexus Meteor, BioTransformer, $GLORY_{x,}$ XenoSite server, MetScore, and SMARTCyp are widely used in the drug discovery process for metabolite detection(196).

Kp is commonly used to quantify the dermal absorption potential of chemical and naturally derived compounds. However, the lack of experimentally measured Kp values under standardized and consistent processes frequently makes it difficult to use Kp for regulatory purposes when developing standards. (197). **Table 14.** shows the LogKp values of 13 selected compounds. All the compounds are impermeable, as they have negative log values. Seven had a high negative log p value (-9.52 cm/s).

**Table 14. Results of pharmacokinetic properties**

| Properties | Compounds | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** |
| **GIA** | H | H | H | H | H | H | L | H | H | H | H | L | H |
| **BBB** | Y | Y | Y | Y | Y | Y | N | N | N | Y | Y | N | Y |
| **P-GP** | N | N | N | N | N | N | Y | N | N | Y | N | N | N |
| **CYP1A2** | Y | Y | Y | Y | Y | Y | N | N | N | Y | Y | N | Y |
| **CYP2C9** | N | Y | Y | Y | Y | Y | N | N | N | Y | Y | N | N |
| **CYP2C9** | N | N | N | Y | Y | Y | N | Y | Y | N | N | N | Y |
| **CYP2D6** | Y | N | Y | N | Y | Y | N | Y | N | Y | Y | N | N |
| **CYP3A4** | N | N | Y | N | Y | Y | N | Y | Y | N | Y | N | Y |
| **LogKp** | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **(cm/s)** | 6.02 | 5.85 | 5.69 | 5.90 | 5.87 | 5.43 | 9.52 | 7.40 | 6.73 | 5.45 | 5.03 | 2.20 | 5.63 |

CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4- cytochrome p isoforms H-high, L-low, N-no, Y-yes. GIA- gastrointestinal absorption, BBB- blood-brain barrier, P-Gp-P-glycoprotein.

When substances are delivered orally, bioavailability refers to the degree and rate at which they are absorbed into the body and eventually reach the desired areas(198). The different oral bio scores used were as follows: 1) 0.85 PSA 0≤ 75 $A^{o}2$, 2) 0.56 75<PSA<150 $A^{o}2$, 3) 0.55 Pass rule of five, 4) 0.17 fail rule of five, and 5) 0.11 PSA≥150 $A^{o}2$ (199). Gastrointestinal absorption of all the compounds is shown in **Table 15**. Except for compound 7, all the compounds had a bioavailability score of 0.55, and compound 4 had an oral bioavailability score of 0.85. These findings also indicated that all the compounds, except compound 7, obeyed the Lipinski rule. Compound 7 is not necessarily of use. However, it can be modified to improve its bioavailability.

### *5.1.4. Lead likeness and synthetic accessibility (SA)*

Lead likeness is characterized by the physical and chemical similarity of the compound with the reference or standard compound. It was calculated based on the following criteria: 1) 250≤Mol. Weight ≤350, 2) XLOGP≤ 3.5, and 3) No of Rotable bonds ≤7 (200). Compounds 1, 3, 4, 5, 9, 10, and 13 are likely lead-like compounds.

The SA of a lead candidate is an essential factor in lead discovery, regardless of how the lead candidate was identified. When many molecules need to be ranked

according to their synthetic accessibility, such as when buying samples for screening, choosing hits from high-throughput screening for further investigation, or ranking molecules produced by various de novo design approaches, the calculated SA score may support multiple drug discovery processes. The SA scores of all the compounds are shown in **Table 15.** A score of 1 is very easy, whereas a score of 10 is very difficult to use to synthesize a molecule. Compounds 1, 11, and 13 had lower SA scores than the other compounds (201).

**Table 15. Lead likeness and SA of compounds**

| Compounds | Bioavailability score | Lead likeness | Synthetic accessibility (1 Easy- 10 Difficult) |
|---|---|---|---|
| **1.** | 0.55 | yes | 2.96 |
| 2. | 0.55 | no | 3.05 |
| 3. | 0.55 | yes | 3.13 |
| 4. | 0.85 | yes | 3.41 |
| 5. | 0.55 | yes | 3.08 |
| 6. | 0.55 | no | 3.08 |
| 7. | 0.17 | no | 6.14 |
| 8. | 0.55 | no | 4.64 |
| 9. | 0.55 | yes | 3.29 |
| 10. | 0.55 | yes | 3.33 |
| 11. | 0.55 | no | 2.97 |
| 12. | 0.55 | no | 6.30 |
| 13. | 0.55 | yes | 2.52 |

### *5.1.5. Bioactivity score*

The oral bioavailability of a potential medicine is what Lipinski's rule of five measures, however, as this is the desired feature for a molecule with drug-like characteristics. Then, using a different strategy, similar searches in the chemical space of substances with comparable structures to those under study and known pharmacological effects were considered. The bioactivity scores, which are a measure of a potential drug's capacity to interact with various receptors, that is, to function as GPCR ligands or kinase inhibitors, to act as ion channel modulators, or to interact with enzymes and nuclear receptors—were calculated using the same software. The

matching SMILES notations were entered into the easily accessible online Molinspiration software to determine the oral bioavailability prediction (202). The bioactivity scores of the compounds were calculated based on the interactions of the GPCR ligand, ion channel modulator, nuclear receptor ligand, kinase inhibitor, protease inhibitor, and enzyme inhibitor and are shown in **Table 16**. These organic compound bioactivity scores can be translated into three categories: highly active (bioactivity score > 0), moderately active (bioactivity score between -5.0 and 0.0), and inert (bioactivity score -5.0). Among the GPCR ligands, compounds 7, 8, 10, and 12 are highly active in ion channel modulator activity, compounds 3 and 12 are highly active in kinase inhibitor activity, all the compounds are moderately active in nuclear receptor activity, compounds 3, 8, 9, 11 and 12 are highly active in protease inhibitor activity, only compound 12 is highly active in enzyme inhibitory activity, except for compounds 1, 2, 4, 5 and 6, is highly active. These receptors are involved in many physiological responses, such as smell, vision, taste, immunity, mood regulation, hormonal regulation, and cell growth. Therefore, inhibition or activation of these receptors is pivotal in disease management.

**Table 16. Study of bioactivity score**

| Compound | Gpcr ligand | Ion channel modulator | Kinase inhibitor | Nuclear receptor ligand | Protease inhibitor | Enzyme inhibitor |
|---|---|---|---|---|---|---|
| **1.** | -0.50 | -0.54 | -0.25 | -0.02 | -0.69 | -0.07 |
| **2.** | -0.63 | -0.22 | -0.56 | -0.66 | -0.64 | -0.13 |
| **3.** | -0.25 | 0.07 | -0.26 | 0.63 | -0.35 | 0.40 |
| **4.** | -0.72 | -0.40 | -0.35 | -0.73 | -0.55 | -0.07 |
| **5.** | -0.49 | -0.57 | -0.25 | -0.08 | -0.62 | -0.00 |
| **6.** | -0.08 | -0.18 | -0.27 | -0.03 | -0.36 | -0.07 |
| **7.** | 0.06 | -0.48 | -0.46 | -0.15 | -0.13 | 0.24 |
| **8.** | 0.05 | -0.31 | -0.40 | 0.14 | -0.29 | 0.31 |
| **9.** | -0.39 | -0.37 | -0.19 | 0.22 | -0.51 | 0.07 |
| **10.** | 0.17 | -0.21 | -0.27 | -0.07 | -0.46 | 0.27 |
| **11.** | -0.33 | -0.51 | -0.18 | 0.02 | -0.58 | 0.03 |
| **12.** | 0.14 | 0.04 | -0.51 | 0.73 | 0.07 | 0.51 |
| **13.** | -0.13 | -0.11 | -0.32 | 0.00 | -0.32 | 0.09 |

### *5.1.6. Toxicity prediction*

Many pharmacokinetic, pharmacodynamic, and toxicological concerns need to be considered in virtual screening methods. The predicted oral toxicity ($LD_{50}$), toxicity, hepatotoxicity, carcinogenicity, immunity, and mutagenicity of all the compounds are shown in **Table 17.** Compounds 7 and 8 are highly toxic and belong to class 1, whereas compound 4 is the least toxic and belongs to class 5. Among the predicted toxicology classes, compounds 1, 4, and 13 belong to class 5, indicating that the $LD_{50}$ values range from 2500 to 4000 mg/kg. According to the toxicity prediction software, the degree of protox-II hepatotoxicity, carcinogenicity, immunotoxicity, and mutagenicity was calculated. All the responses are shown as inactive or active. All the compounds are safe because they are neither hepatotoxic nor mutagenic. Compounds 1 and 5 all exhibited carcinogenicities. Except for compounds 1, 5, and 6, all the other compounds were immunotoxins.

**Table 17. Pro Tox II software predicted organ toxicity, hepatotoxicity, carcinogenicity, immunotoxicity, and mutagenicity.**

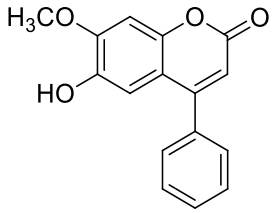
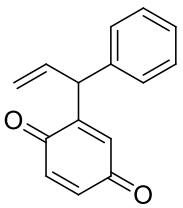
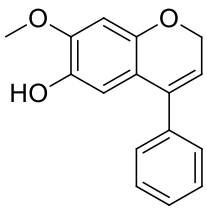
| Compound | Predicted LD 50 | Predicted toxicity class | Mutagenicity | Hepatotoxicity | Carcinogenicity | Immunotoxicity |
|---|---|---|---|---|---|---|
| **1.** | 2850 | 5 | Inactive | Inactive | Active | Inactive |
| **2.** | 1520 | 4 | Inactive | Inactive | Inactive | Active |
| **3.** | 500 | 4 | Inactive | Inactive | Inactive | Active |
| **4.** | 4000 | 5 | Inactive | Inactive | Inactive | Active |
| **5.** | 2000 | 4 | Inactive | Inactive | Active | Inactive |
| **6.** | 1000 | 4 | Inactive | Inactive | Inactive | Inactive |
| **7.** | 4 | 1 | Inactive | Inactive | Inactive | Active |
| **8.** | 4 | 1 | Inactive | Inactive | Inactive | Active |
| **9.** | 500 | 4 | Inactive | Inactive | Inactive | Active |
| **10** | 1743 | 4 | Inactive | Inactive | Inactive | Active |
| **11** | 2000 | 4 | Inactive | Inactive | Inactive | Active |

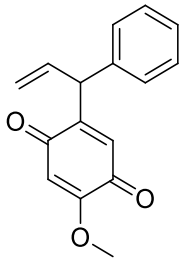
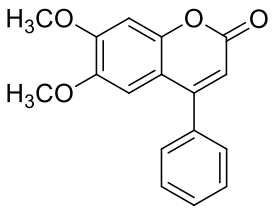
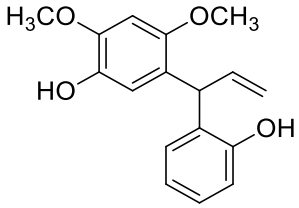
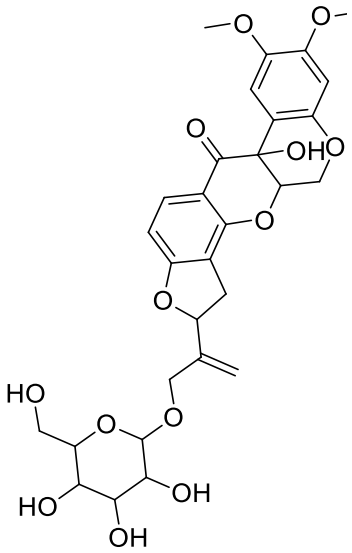
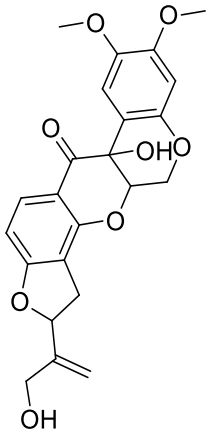
| | | | | | | |
|---|---|---|---|---|---|---|
| **12** | 890 | 4 | Inactive | Inactive | Inactive | Active |
| **13** | 3600 | 5 | Inactive | Inactive | Inactive | Active |

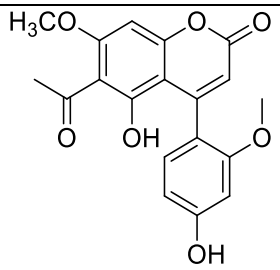
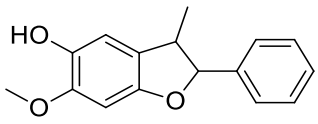
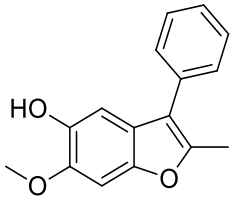
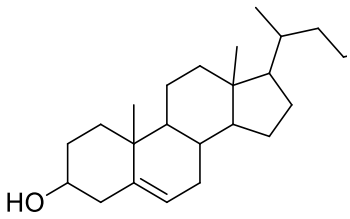
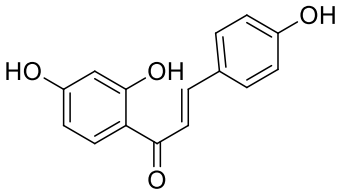
### *5.1.7. Validation and Analysis of Docked Target Human Pancreatic α-amylase-Ligand Complex Structures.*

Docking was done by using the PyRx virtual screening tool in which the protein human pancreatic α-amylase (PDB ID: 1HNY) and ligands were opened, energy minimised, converted the protein, ligands into pdbqt file and grid generated by giving X=155.646, Y=3.7682, Z=-74.5905 dimension of ligands and human pancreatic α-amylase (PDB ID: 1HNY, **Fig.8)**. The ligands docked 10 times, and the average binding affinities of ligands with zero rmsd were calculated and presented in **Table 18**. Among all, Dalbin, Dalbinol, Dalbergin, Dalbergione, sisafolin, and Isoliquiritigenin showed lower binding affinity in the range of -9.0 k/cal and -7.4 k/cal. Acarbose is shown binding affinity of -8.4 k/cal The Dalbergin and Isoliquiritigenin bind the amino acid residue of the target protein **(Fig.9 and Fig.10),** as shown in **Table 19.**

**Table 18. Docking scores of selected compounds**

| S.No. | Structure | Name of the compound | Binding Affinity k/cal |
|---|---|---|---|
| **1** |  | Dalbergin | -7.7 |
| **2** |  | Dalbergione | -7.5 |
| **3** |  | Dalbergichromene | -7.7 |

| | | | |
|---|---|---|---|
| **4** |  | 4-Methoxy Dalbergione | -7.2 |
| **5** |  | Methyl dalbergin | -7.2 |
| **6** |  | Latifolin | -6.9 |
| **7** |  | Dalbin | -9 |
| **8** |  | Dalbinol | -8.5 |

| | | | |
|---|---|---|---|
| **9** |  | Sisafolin | -7.7 |
| **10** |  | Obtusafuran | -6.8 |
| **11** |  | Isoparvifuran | -7.3 |
| **12** |  | β-sitosterol | -6.8 |
| **13** |  | Isoliquiritigenin | -7.4 |

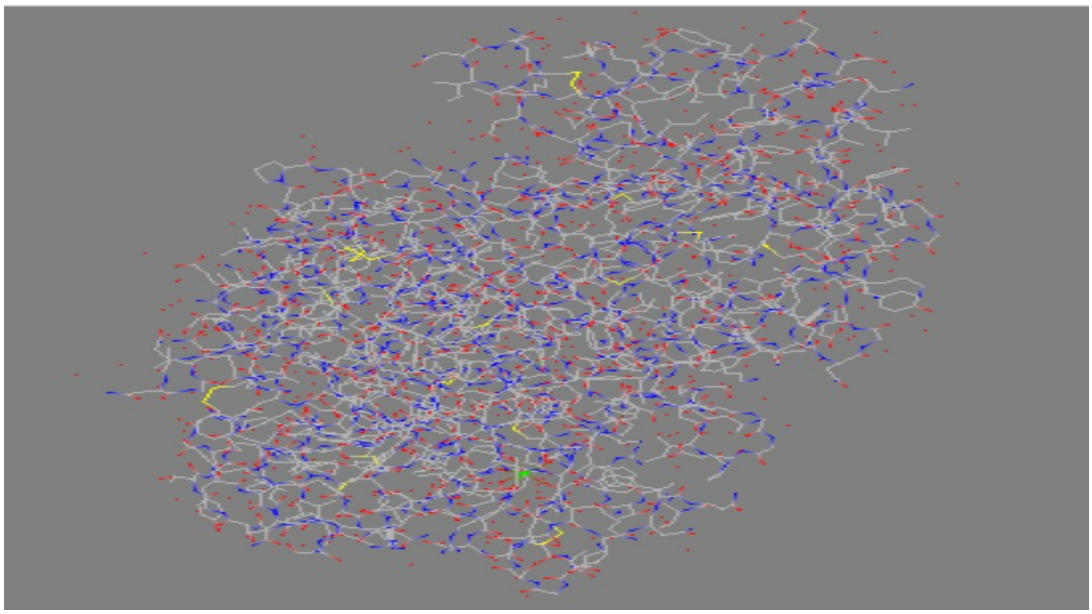


**Fig.8. Human pancreatic α-amylase protein structure (PDB ID: 1HNY).**

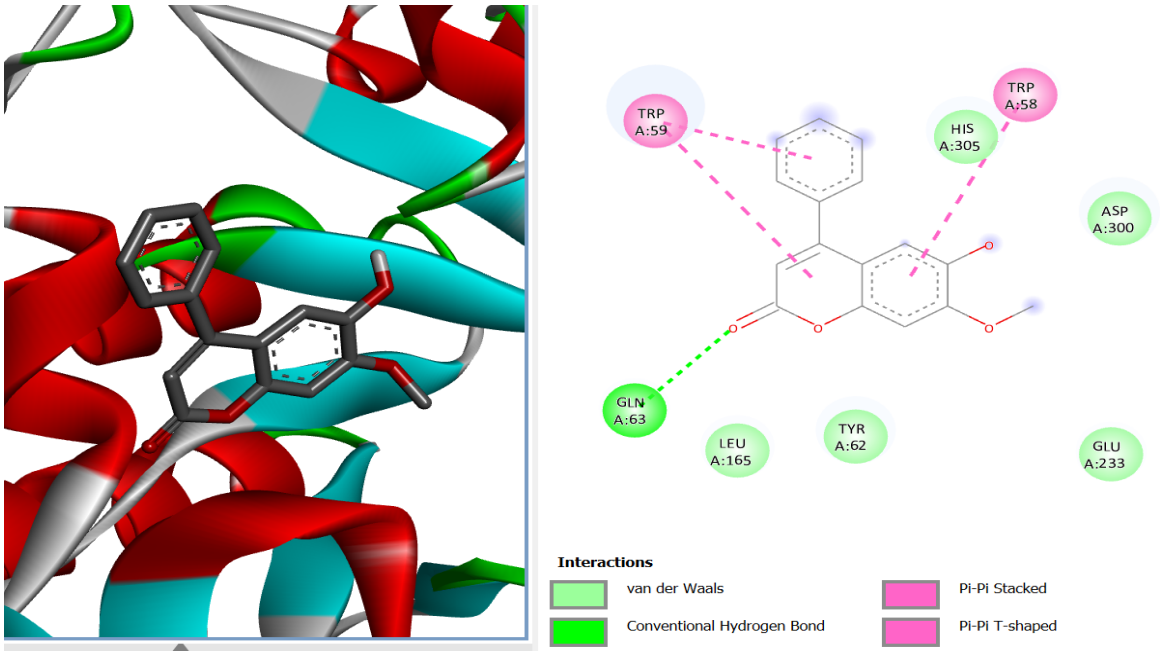



**Fig.9. 3D view of interactions of DGN with amino acids of 1HNY and 2D interaction of DGN with amino acids of 1HNY.**

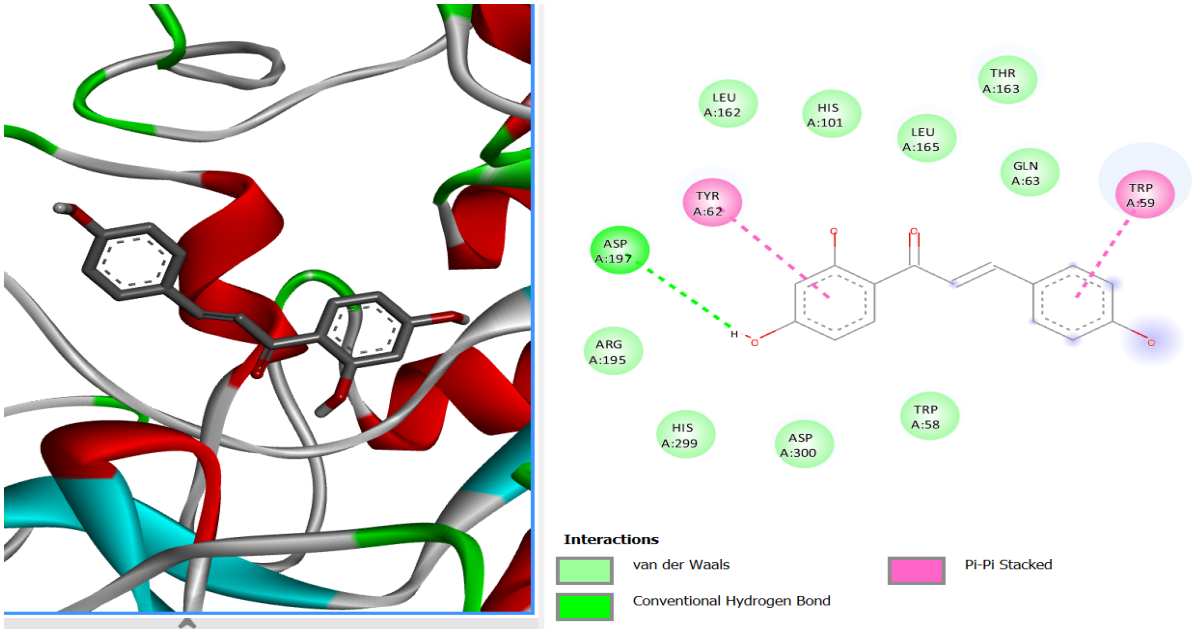



**Fig.10. 3D view of interactions of ISG with amino acids of 1HNY and 2D interaction of ISG with amino acids of 1HNY.**

**Table 19. Interaction of Dalbergin and Isoliquiritigenin with amino acids**

| Ligand | Interaction |
|---|---|
| **Dalbergin** | Conventional hydrogen bond with GLN A:63, Pi-Pi stacked with TRP A:58, Pi-Pi T-shaped with TRP A:59. |
| **Isoliquiritigenin** | Conventional hydrogen bond with ASP A:197, Pi-Pi stacked with TYR A:62, TRP A:59. |

### *5.1.8. Profiling of DGN and ISG*

The final compounds obtained from section **4.3.6.1** (Fr: 3A, 4A, and 5A) and section 4.3.6.2 (Fr-2A and Fr-2B) were analysed using NMR (Bruker Avance 400 MHz). According to the NMR (1H and C13), FTIR and mass results of Fr:5A and Fr:2B were identified as DGN and ISG, respectively. The NMR and FTIR results of Fr:5A and Fr:2B are represented in Fig 11-16. The results obtained were confined to those reported by Wangun e1. &l., and cheng jun ma et al., (180,203).

#### *5.1.8.1 DGN:*

Colour: Pale Brown; Melting point: 207-209 $^{0}$C (208-210 $^{0}$C); FTIR (KBr): 3248, 2924, 2853, 1691, 1145 cm$^{-1}$; 1H NMR (400 MHz, CDCl3) δ 7.43 (d, J = 2.7 Hz, 2H), 7.39 – 7.31 (m, 2H), 6.92 (s, 1H), 6.84 (s, 1H), 6.18 (s, 1H), 3.92 (s, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 161.49, 155.80, 150.07, 149.37, 142.44, 135.62, 129.58, 128.85, 128.33, 112.60, 110.54, 99.62, 56.48. Mass of the molecule:268.26 g/mol.

The FT-IR of compound DGN demarcated absorption bands at 3248 cm-1 for the O-H phenol group, 2924 cm-1, 2853 for C-H-aromatic, and the appearance of the carbonyl (C=0) groups at 1691 cm-1 conform the coumarin ring in the compound. The 1H NMR spectral data of compound DGN confirmed singlet peaks at δ: 3.92 ppm, consistent with -OCH3 protons attached to the 7$^{th}$ position of the coumarin ring. The 6 aromatic protons were observed as multiplet in the region δ 7.45-7.34, and a sharp singlet peak at δ: 7.48 ppm was attributed to the -CH (double bond) proton in the compound.

#### **5.1.8.2.** *ISG:*

Color: Yellow; Melting point:200-203 ℃ (198-200 ℃); FTIR (KBr): 3289, 1638, 1514, 1126; 1H cm-1; NMR (400 MHz, CDCL3+DMSO) δ 13.49 (s, 1H), 9.95 (s, 2H), 8.07 – 7.18 (m, 5H), 7.03 – 6.46 (m, 2H), 6.64 – 5.66 (m, 2H); 13C NMR (101 MHz, CDCL3+DMSO) δ 191.72, 165.61 (d, J = 150.0 Hz), 160.14, 144.36, 131.65, 130.51, 126.19, 116.94, 116.19, 113.47, 108.30, 103.42; Mass of the molecule:256.25 g/mol.

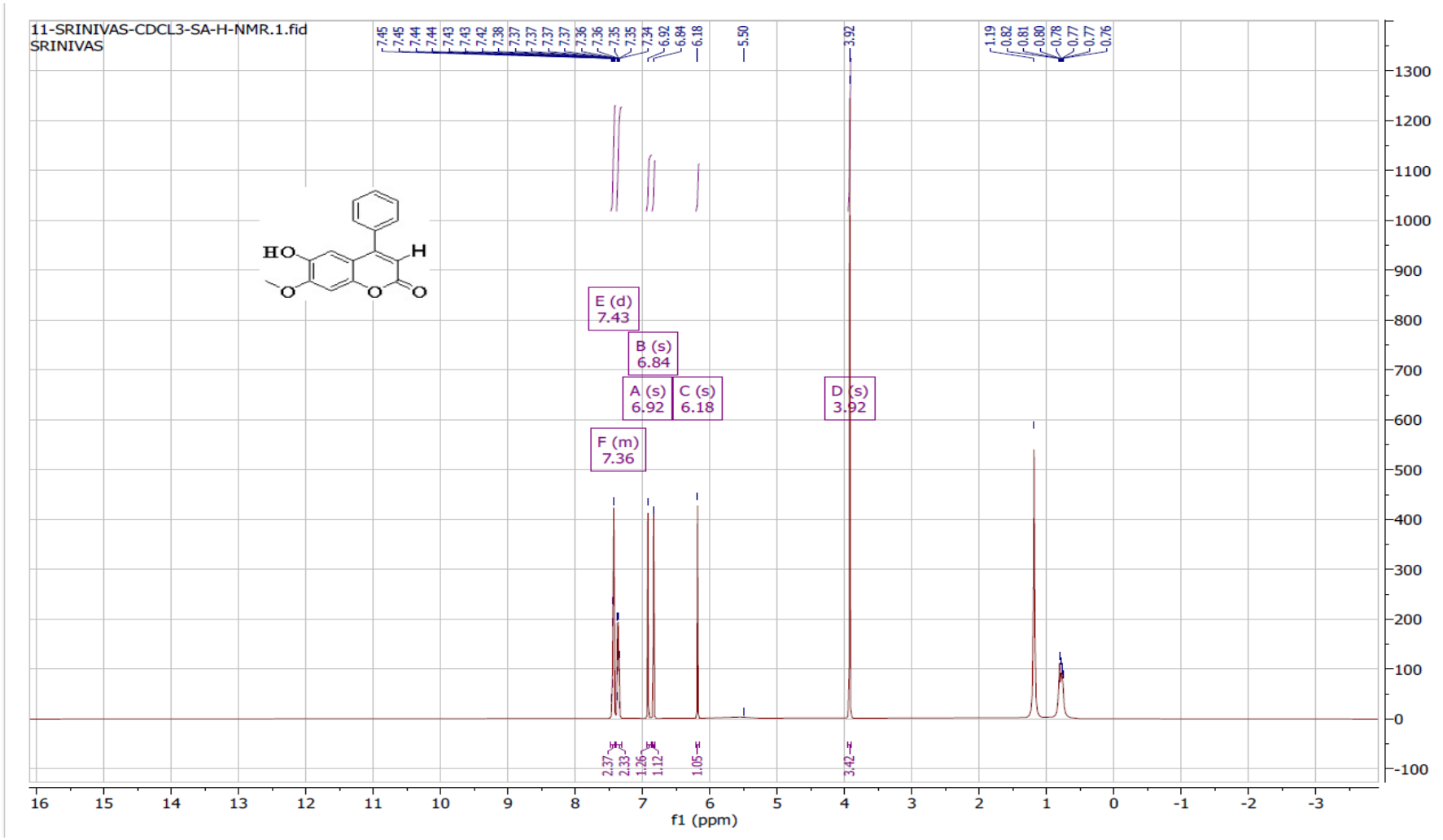



**Fig.11. $^{1}$H NMR OF DGN**

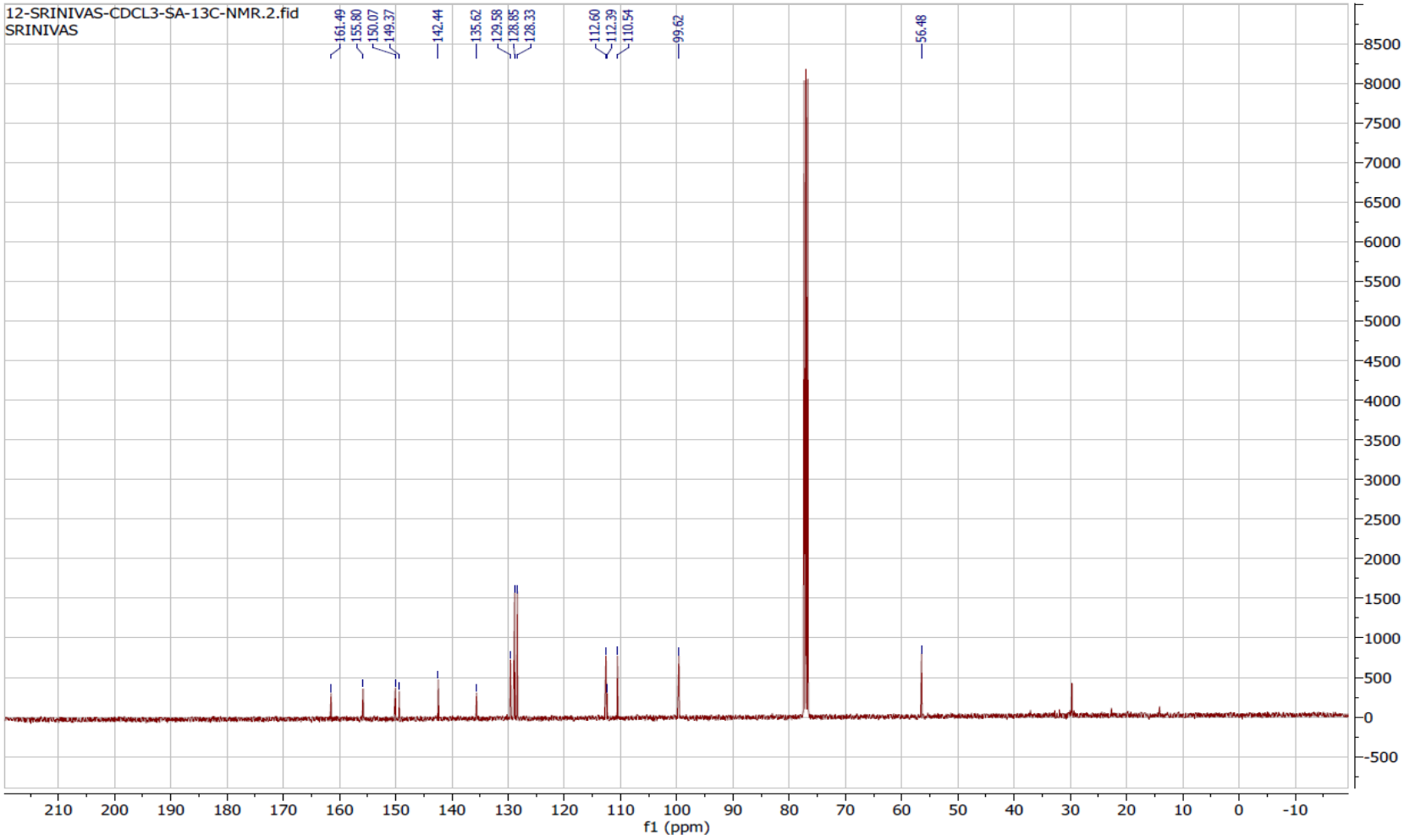



**Fig.12. $^{13}$C NMR OF DGN**

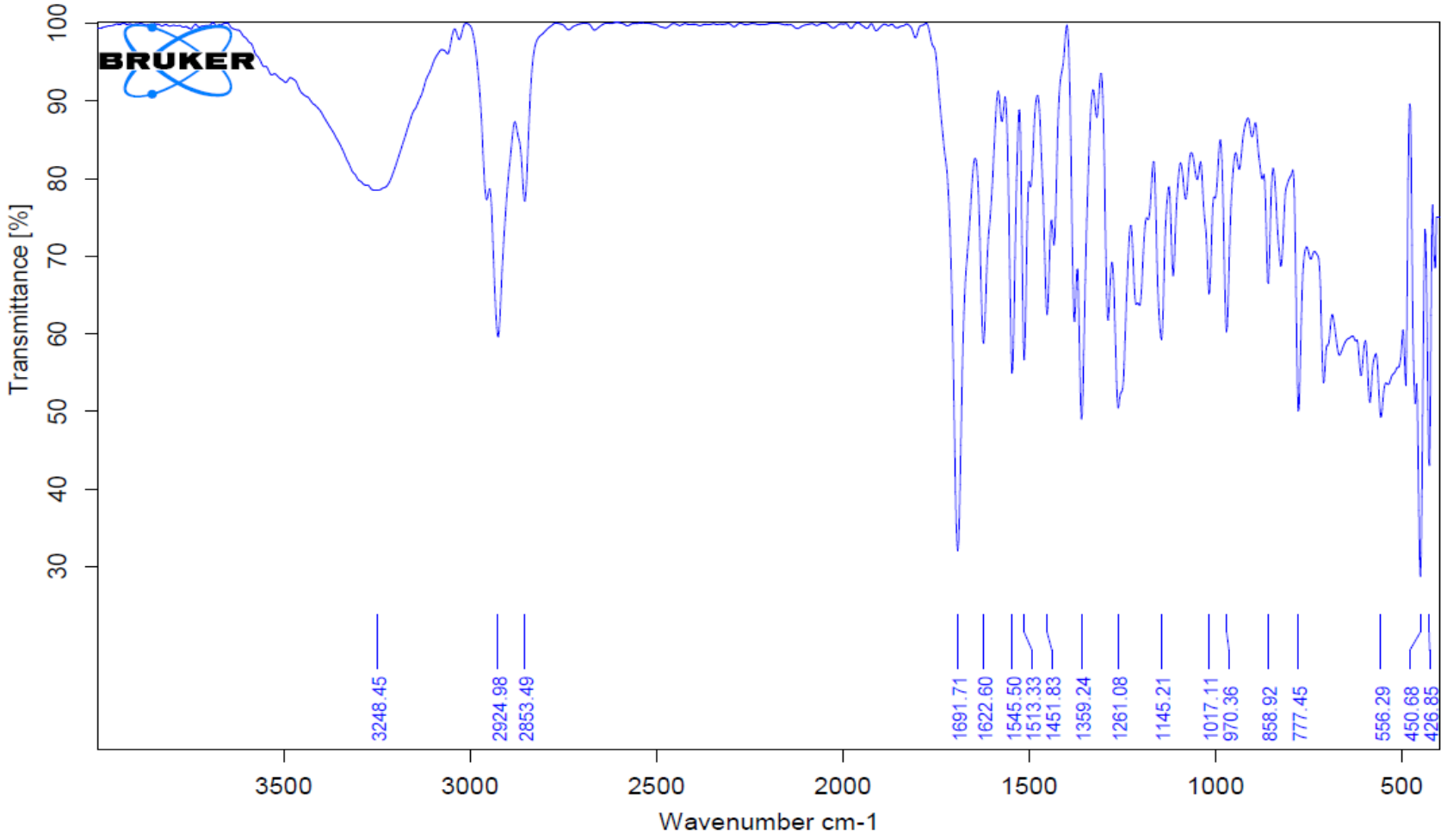



**Fig.13. FTIR OF DGN**

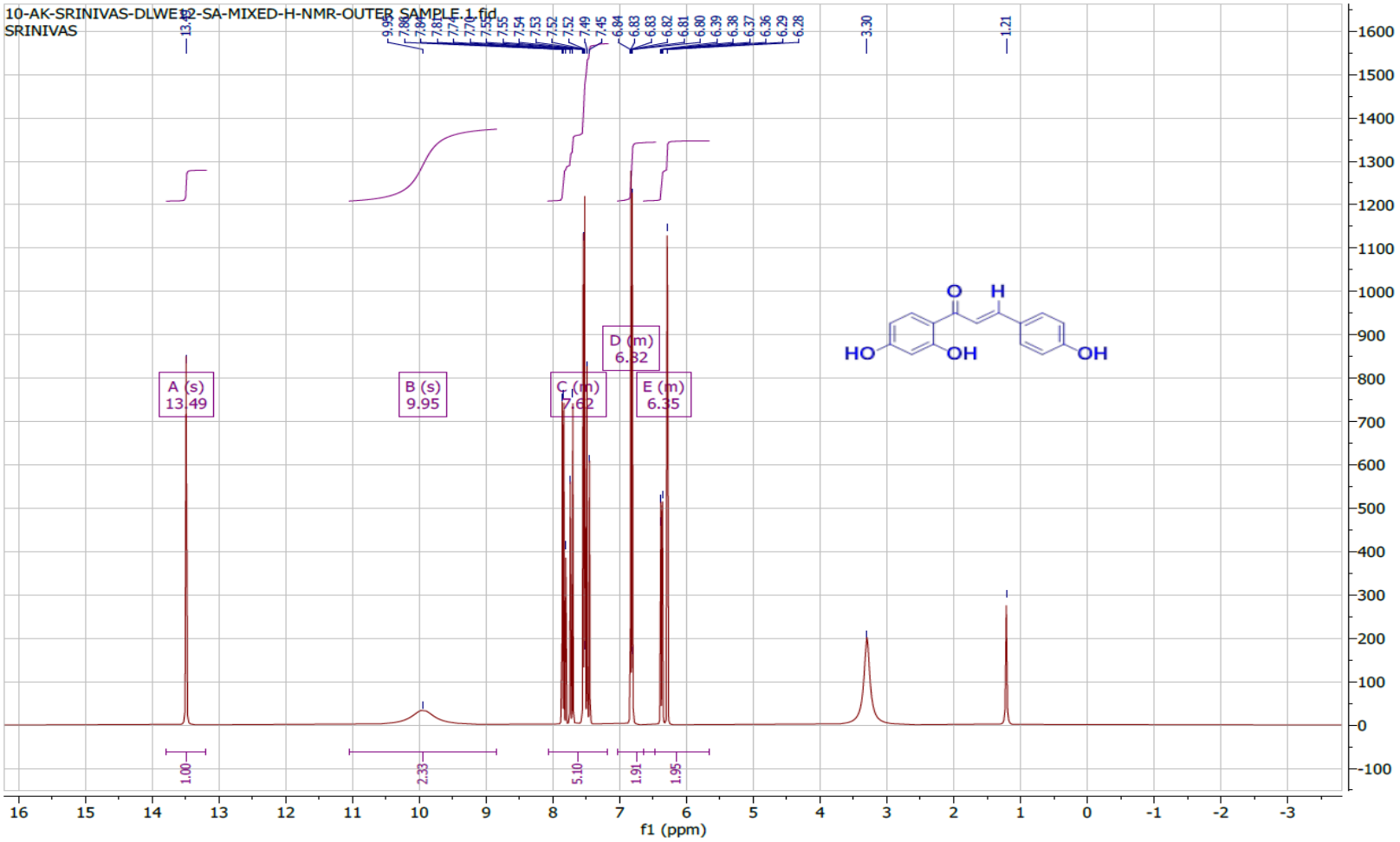



**Fig.14. $^{1}$H NMR OF ISG**

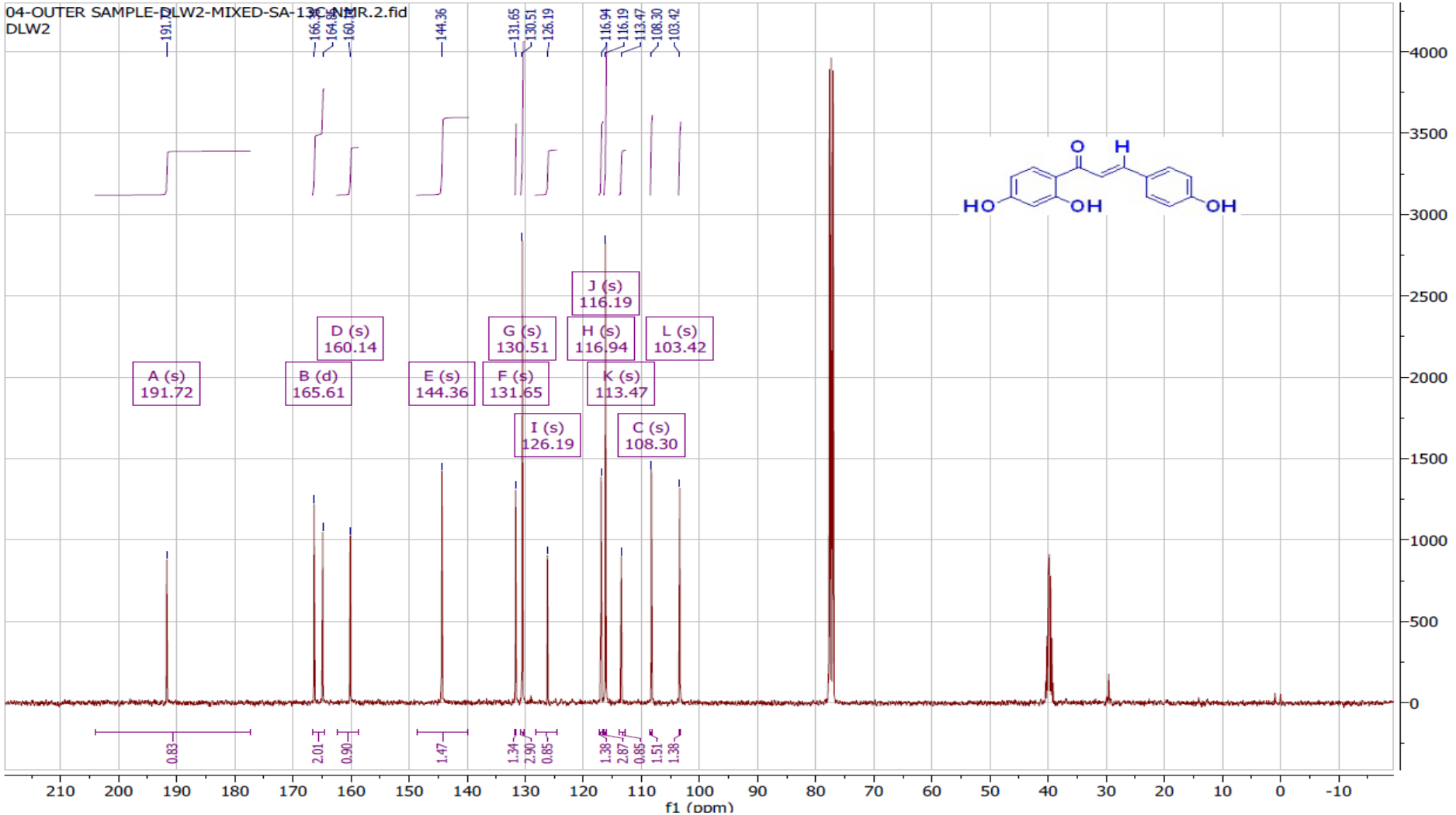



**Fig.15. $^{13}$C NMR OF ISG**

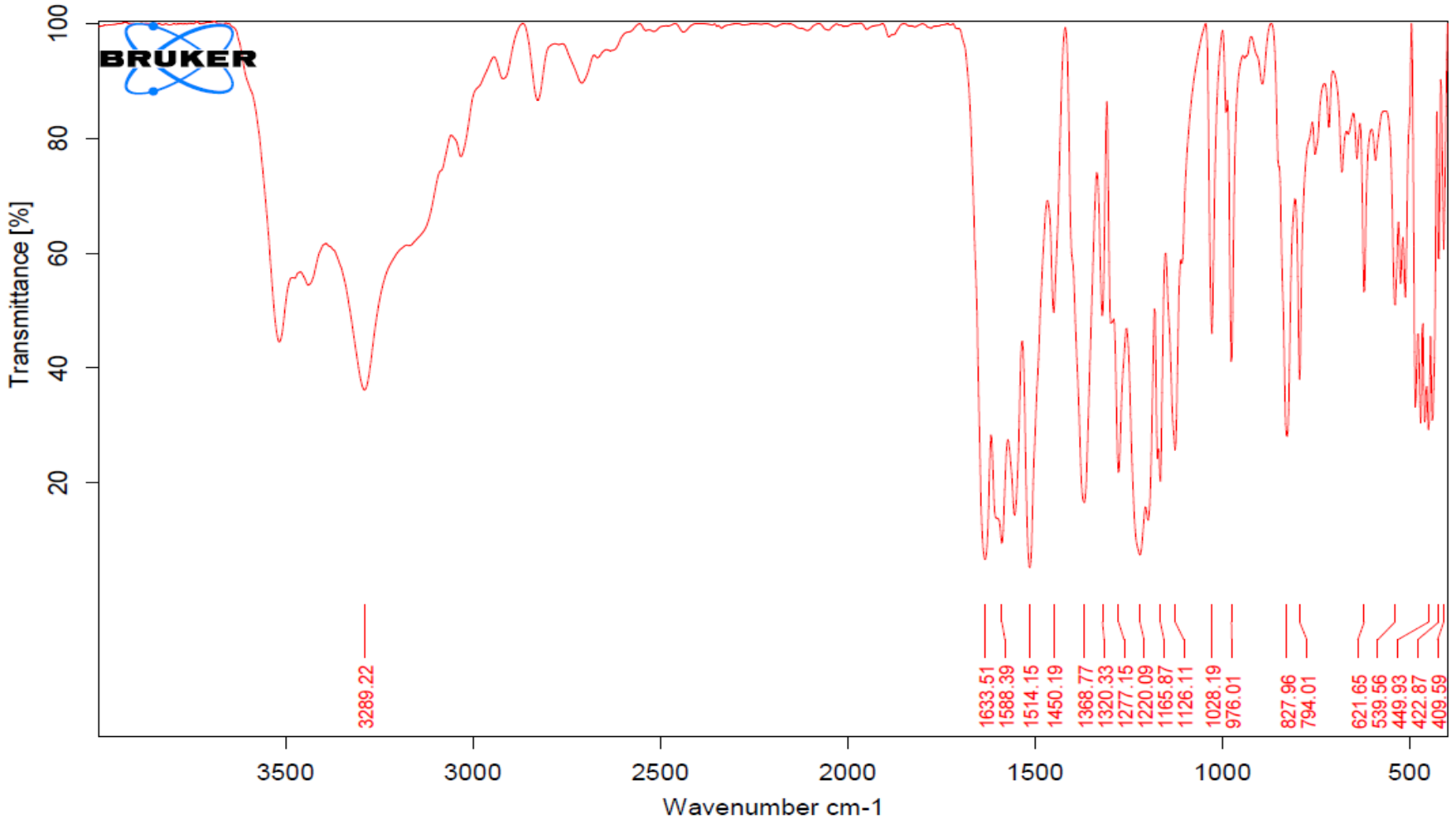



**Fig.16. FTIR OF ISG**

### 5.2. *In vitro* studies

#### 5.2.1. *α-amylase inhibitory activity*.

Flavonoids extracted from plant sources were reported to possess potent α-amylase inhibitory activity (204). DGN and ISG were investigated for their α-amylase inhibitory potential and compared with the standard (acarbose). The results revealed that both DGN and ISG exhibited α-amylase inhibitory potential. DGN showed a better $IC_{50}$ of α-amylase inhibitory action in a dose-dependent manner than ISG and acarbose. At a concentration of 25 µg/ml, ISG inhibited 99.05±8.54% of α-amylase activity with an $IC_{50}$ value 0.6025 µg/ml, DGN inhibited 84.68±5.2% of α-amylase activity with an $IC_{50}$ value 00.02169 µg/ml, and acarbose inhibited 99.21±1.21% of α-amylase activity with an $IC_{50}$ value 1.043 µg/ml. The order of α-amylase inhibitory potential of the compounds is as follows: DGN > ISG > acarbose compared at maximum and standard doses (**Fig.17)**. The α-amylase inhibitory activities of DGN and ISG are shown in **Table 20**.

**Table 20. Percent inhibition and $IC_{50}$ values of DGN, ISG and acarbose**

| Concentration (µg/mL) | % of inhibition (mean±SD) | | | $IC_{50}$ value (µg/mL) | | |
|---|---|---|---|---|---|---|
| | **DGN** | **ISG** | **Acarbose** | **DGN** | **ISG** | **Acarbose** |
| **0.1** | 58.67±3.97 | 18.38±4.75 | 5.02±1.15 | | | |
| **0.5** | 68.65±5.18 | 54.83±4.31 | 25.10±2.32 | | | |
| **1** | 75.18±3.14 | 58.80±6.60 | 40.66±3.99 | 0.02169 | 0.6025 | 1.043 |
| **2.5** | 76.37±6.25 | 64.66±3.03 | 67.39±1.08 | | | |
| **5** | 81.24±3.07 | 75.59±4.09 | 69.23±1.41 | | | |
| **10** | 81.59±4.72 | 96.20±1.72 | 78.70±1.25 | | | |
| **25** | 84.68±5.22 | 99.05±8.54 | 99.21±5.84 | | | |

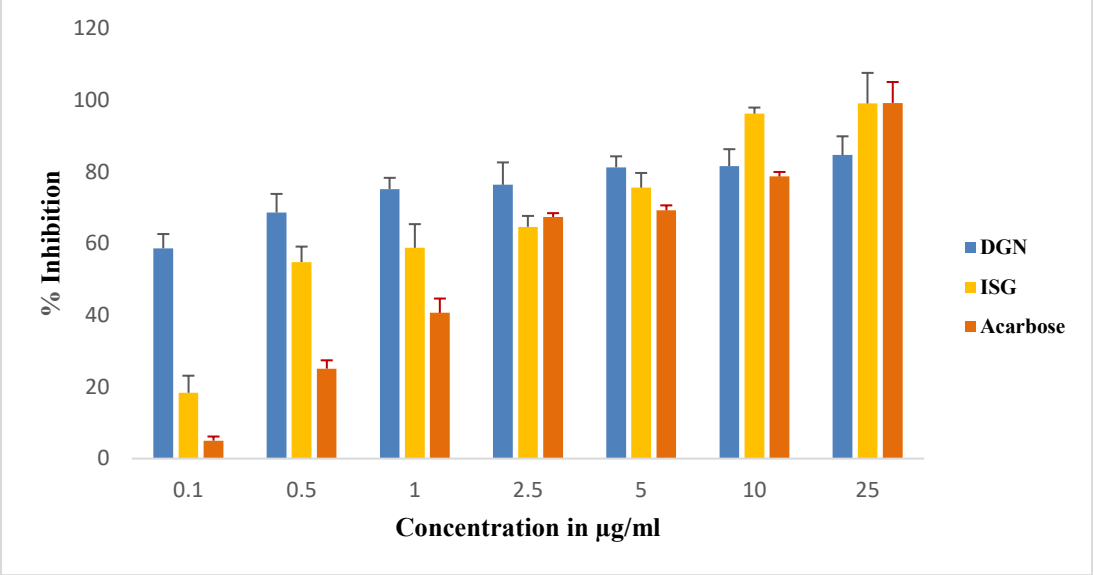



**Fig.17. α-amylase inhibitory activity of DGN, ISG, and acarbose. All the experiments were carried out in triplicate. α Amylase inhibitory potential was expressed in mean±SD values. (DGN: Dalbergin, ISG: Isoliquiritigenin).**

### *5.2.2. Cytotoxicity assay*

A neutral red dye uptake (NRU) assay developed by Rockefeller University was used to determine the cytotoxicity of DGN and ISG on L6 cells (205). The immunotoxicity and cytopathogenicity of infectious agents and the presence of toxic substances depend on the entry of neutral red into a cell. The results of % of cell viability and $IC_{50}$ values of DGN and ISG are shown in **Fig.18.** The % cell viability was found to be concentration-dependent in the case of both DGN and ISG. However, the cytotoxic effect of both compounds was observed to be minimal even after treatment with 1000 µg/mL concentration of DGN and ISG. Therefore, these were used for the glucose uptake assay with L6 cells. The $IC_{50}$ values of DGN and ISG in the NRU assay were greater than 100 µg/mL, indicating that both compounds have a minimal cytotoxic effect (206).

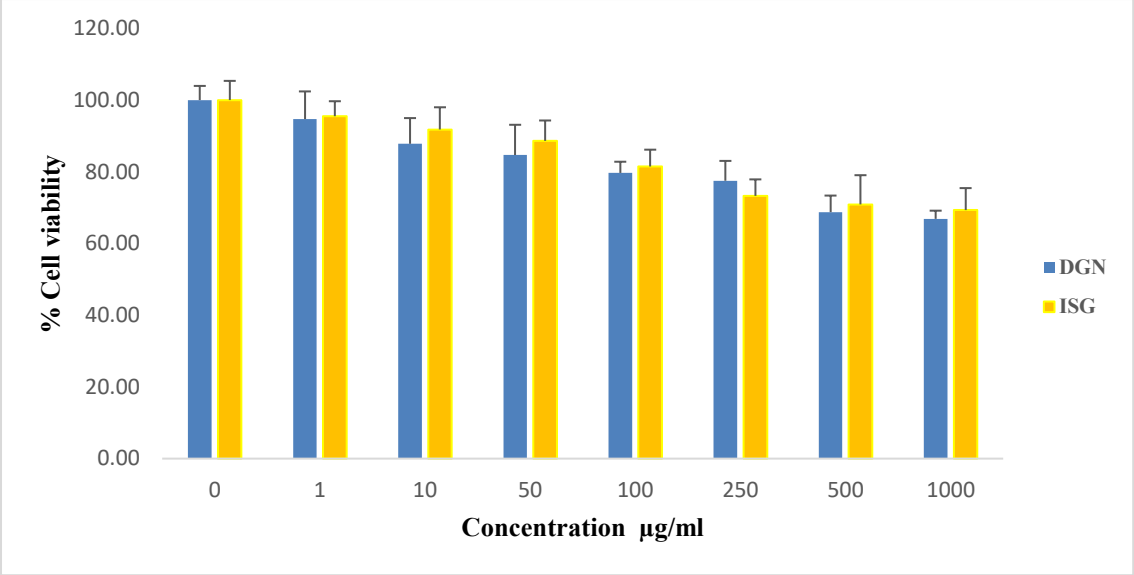



**Fig.18. Cytotoxicity assay of DGN, ISG, and acarbose. All the experiments were carried out in triplicate. Cell viability potential was expressed in mean±SD values. (DGN: Dalbergin, ISG: Isoliquiritigenin).**

### 5.2.3. Glucose uptake assay

Glucose uptake assay in cell lines is a widely used technique for evaluating *in-vitro* antidiabetic activity (207,208). Glucose uptake assay of DGN and ISG was carried out using L6 cell lines by taking metformin (oral antidiabetic drug) and insulin (injectable antidiabetic drug) as model drugs. In the present work, L6 cells were used because they express appropriate levels of GLUT4 (207). The results demonstrated that DGN has promoted maximum glucose uptake (158%) in cell lines at 1254 µg/mL, and ISG has promoted maximum glucose uptake (77%) at the dose of 1344 µg/mL over control. The glucose uptake assay for standard drugs and the ranking order for the potency of the glucose uptake assay was as follows: insulin > DGN > metformin > ISG compared at maximum and standard doses, as shown in **Fig.19**. The strong action of DGN may be due to the translocation of GLUT4 to the target site (209) At a concentration of 0.1U/mL, insulin exhibited glucose uptake of 193.42%, and metformin at a concentration of 1mM reduced glucose uptake by 97.95 % over the control.

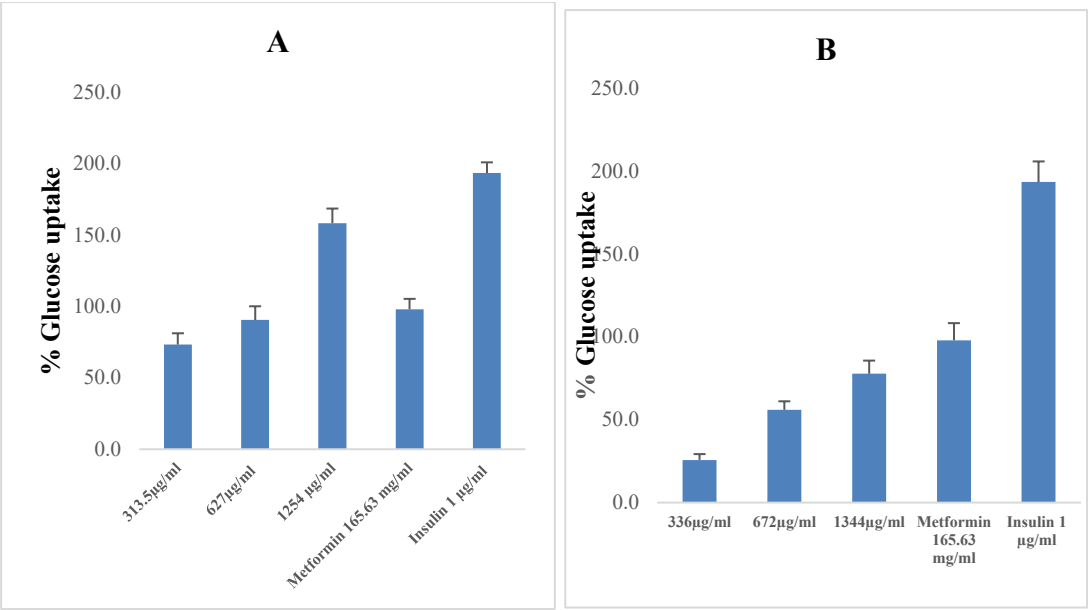



**Fig. 19. Glucose uptake potential of A) DGN and B) ISG. All the experiments are carried out in triplicate. Glucose uptake potential was expressed in mean±SD values in L6 cell lines. (DGN: Dalbergin, ISG: Isoliquiritigenin)**

### 5.3. *In-vivo* studies

One-way analysis of variance (ANOVA) with Tukey's post-hoc test for multiple comparisons was used to compare various groups.

#### 5.3.1. *Body weight measurement*

Obesity is known to be one of the contributing factors in the development of DM (35)(210). When treatment GPs ($GP_4$-$GP_{10}$) are fed with a high-fat diet, an increase in body weight is noted. On the 15$^{th}$ day, more than 100 grams of weight increase was observed in all the treatment GPs. In contrast, a reduction of 54 grams of weight was observed on the 17th day in the treatment GPs compared to the weight observed on the 15$^{th}$ day. The decrease in weight might be due to the administration of STZ on the 15$^{th}$ day ((50). Blood glucose levels were increased in all treatment GPs on the 17$^{th}$ day, which confirmed the induction of diabetes by STZ (blood glucose ≥300 mg/dL). The decrease in weight was attributed to an increase in glucose levels in the blood, which was the typical sign of type 2 diabetes (T2D)(211). After confirmation of diabetes in the rats (17$^{th}$ day), the treatment was started from the 18$^{th}$ day using DGN and ISG. The reduction of body weight in comparison to the body weight observed on the 15$^{th}$ day was noted on (the 31$^{st}$ day), and the order of decrease in weight was as follows: $GP_{10}$ > $GP_5$ > $GP_7$ > $GP_9$ > $GP_8$ > $GP_6$ > $GP_4$.

Additionally, no significant weight gain was observed in the control GP, DGN, and ISG-treated rats (GP1, 2, and 3, respectively) until the end of the study. The effect of DGN and ISG treatment on body weight is shown in **Fig. 20 and 21.** The obtained results were matched with the results reported by Nambirajan et al.(212).

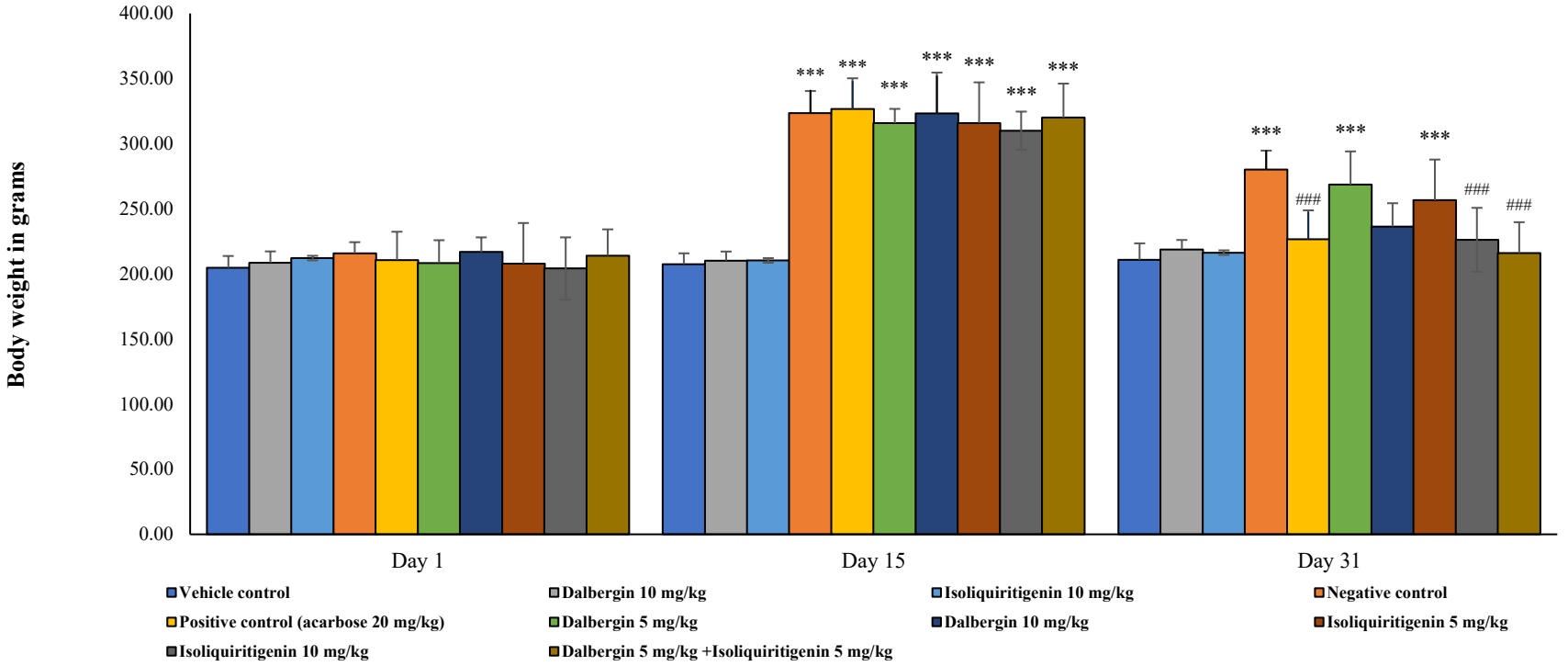



**Fig.20. Variations in body weight at different time points (1st day, 15th day, and 31st day). Effect of HFD diet and treatments on body weight on days 1, 15, and 31. Data expressed as mean ± SD of body weight of each group (N=8). '***' represents (P<0.001) compared to the vehicle control group with treatment groups. '###' represents (P<0.001) as compared to the negative control group with the treatment group. One-way analysis of variance (ANOVA) with Tukey's post-hoc test for multiple comparisons was used to compare various groups.**

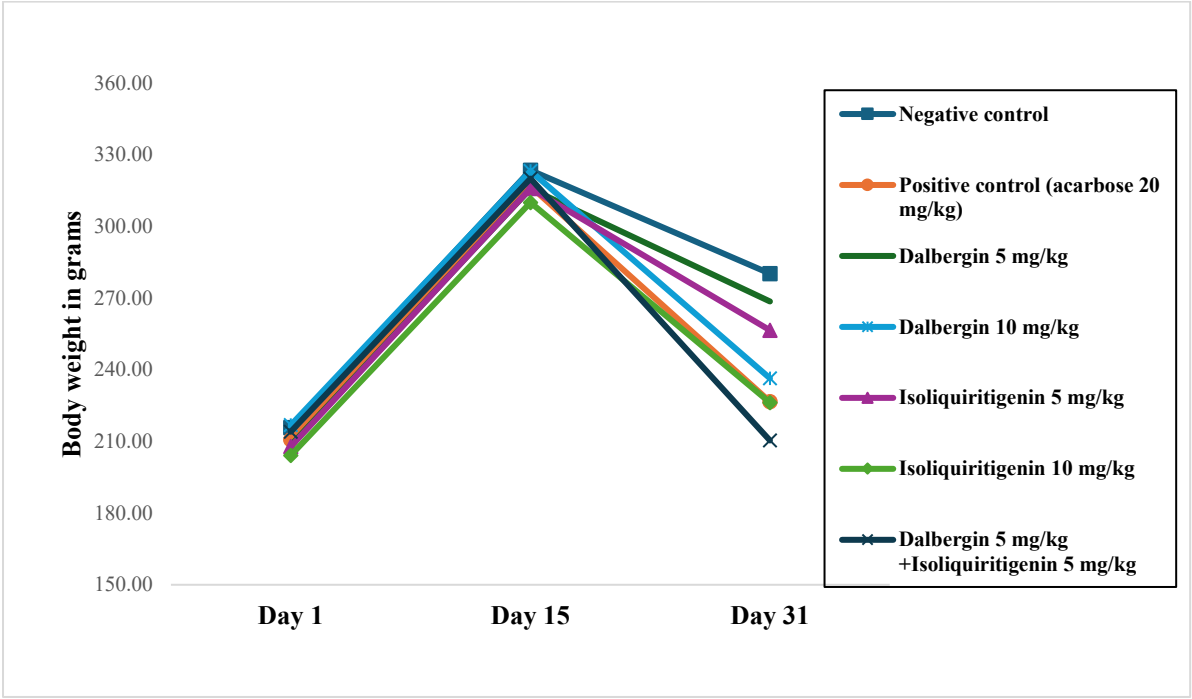



**Fig.21. Variations in body weight of treatment GPs(N=8) at different time points (1st day, 15th day, and 31st day). The weight of each animal was measured before and after the treatment.**

### 5.3.2 *Blood glucose estimation*

HFD induces obesity and triggers an elevation of animal blood glucose levels, which closely resembles the natural history and metabolic characteristics of human T2DM. To induce diabetes in obese rats ($GP_4$-$GP_{10}$), a low dose of STZ 35 mg/kg was administered through *i.p.* on the 15th day. STZ damages the beta cells of the pancreas, leading to increased blood glucose levels in all obese animals ($GP_4$-$GP_{10}$) after 3 days. To confirm diabetes in all the animals, blood glucose levels were recorded on the 17th day **(Fig.7.)**. The pharmacological evaluation of DGN and ISG was started (18th day) after the confirmation of diabetes (above 300 mg/dL) in all the animals and continued till 31st day. The rats in control GPs ($GP_1$, $GP_2$ and $GP_3$), which are on NPD, showed no significant difference in pharmacological parameters. After 14 days of continuous administration of DGN and ISG to treatment GPs ($GP_4$-$GP_{10}$), a decrease in blood glucose levels was observed in rats ($GP_6$-$GP_{10}$). The $GP_5$ (positive control) treated with acarbose (20 mg/kg) showed minimum antidiabetic potential than DGN and ISG (both individually and in combination) after the first seven days of treatment (25th day) **(Fig.21.)**.

The reduction of blood glucose levels after the first week was noted. The order was as follows $GP_9 > GP_7 > GP_6 > GP_{10} > GP_8 > GP_5 > GP_4$. Further, treatment continued until the 31$^{st}$ day, and the blood glucose levels were checked. A combination of DGN+ISG showed maximum reduction of blood glucose levels *(P*<0.001; 262.80 mg/dL) than DGN, ISG (individually) at both high and low doses than acarbose after two weeks (31$^{st}$ day) of treatment. This strong antidiabetic potential of ISG and DGN might be due to strong inhibitory action against the α-amylase enzyme, confirmed by *in-silico* docking studies. The order of antidiabetic potential of DGN, ISG, DGN+ISG, and acarbose was as follows: $GP_{10} > GP_5 > GP_9 > GP_7 > GP_8 > GP_6 > GP_4$. To compare the results, there is no report of antidiabetic evaluation of antidiabetic activity of DGN. However, the results obtained from this anti-diabetic evaluation of ISG was compared with the antidiabetic potential of ISG isolated from the *Glycyrrhiza glabra* plant reported by Gaur R, Yadhav K et al. (213).

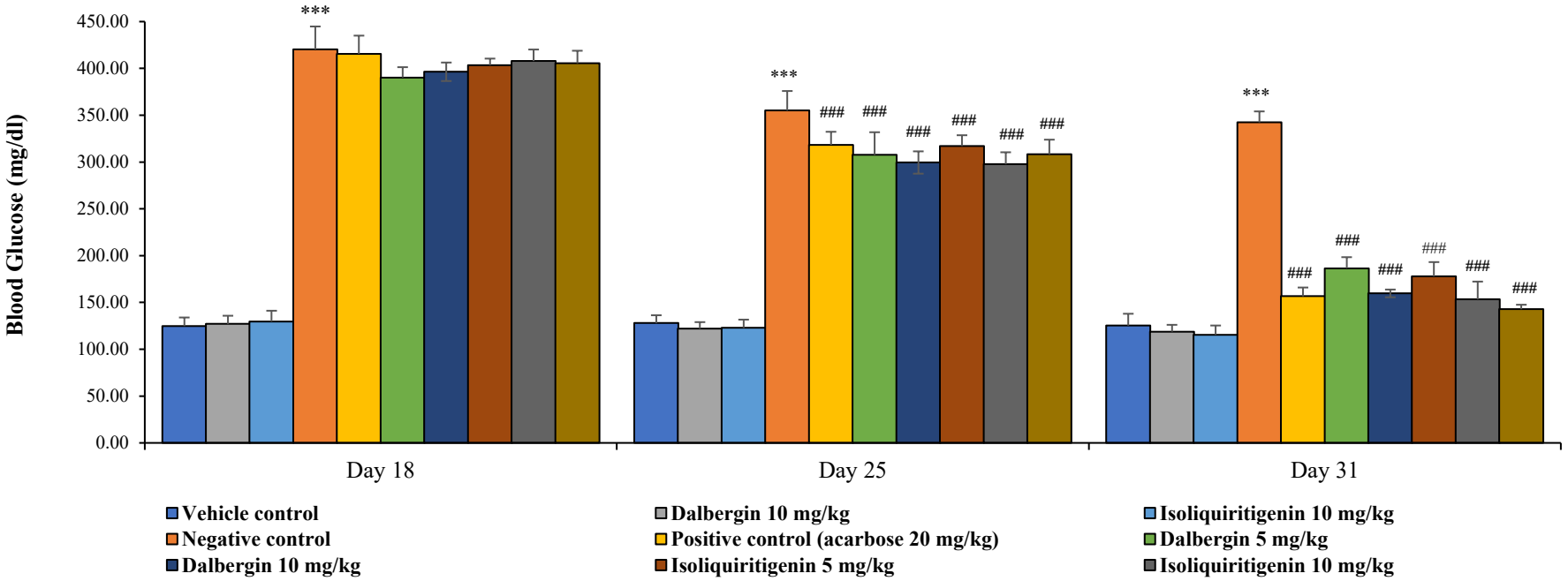


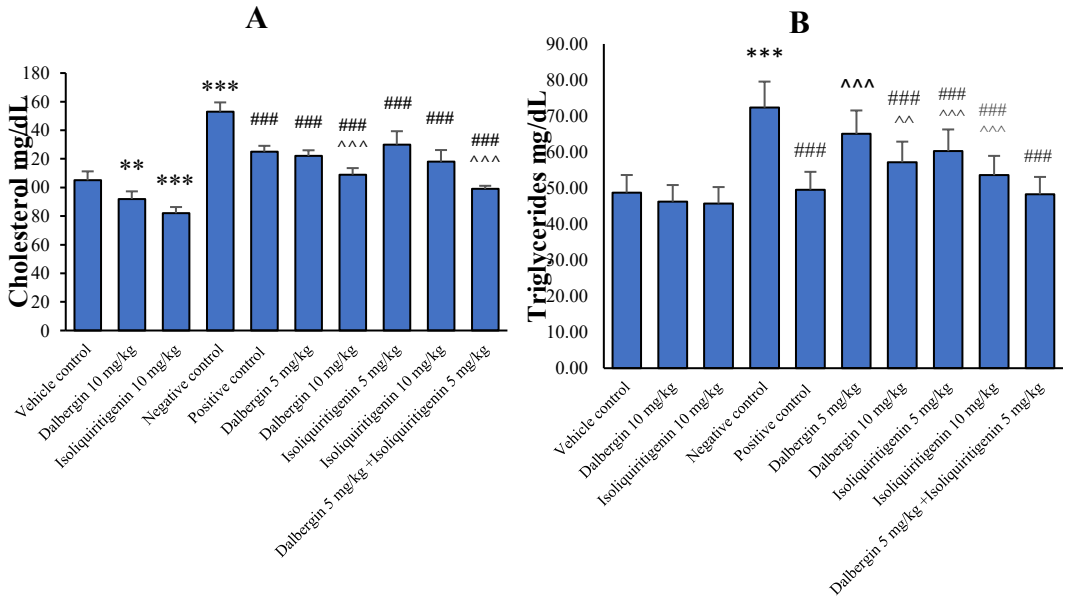

**Fig.22. Effect of treatments on blood glucose levels on 18th, 25th an 31st days. Effect of treatments on glucose levels on days 18, 25 and 31. Data expressed as mean ± SD of blood glucose levels of each group (N=8). '***' represents (P<0.001) as compared to the vehicle control group with the negative control group. '###' represents (P<0.001) as compared to the negative control group with the treatment group. One-way analysis of variance (ANOVA) with Tukey's post-hoc test for multiple comparisons was used to compare various groups.**

### 5.3.3. *Estimation of biochemical parameters*

In the present study, the HFD was given to treatment GPs ($GP_4$-$GP_{10}$) rats to induce obesity and hyperlipidemia, which leads to T2D. Blood samples were collected on the 15th and 31st days to study and compare various biochemical parameters, including total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), serum creatinine (SC), serum glutamic oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and alkaline phosphatase (ALP). As shown in **Fig.2.** and **Table 21**. It was noted that, except for HDL, all the lipid-based parameters were elevated in the treatment GPs, except for GP2 and GP3, confirming the successful induction of hyperlipidemia in all the obese rats (214).

### 5.3.4 *Lipid profile*

Blood samples were collected on the 17th and 31st days to study the effect of DGN and ISG on TC, TG, VLDL, HDL and LDL. It was observed that TC, TG, VLDL, and LDL levels were significantly ($p<0.001$) decreased in $GP_6$-$GP_{10s}$ after treatment with DGN and ISG than $GP_4$ (negative control), whereas HDL levels were significantly ($p<0.001$) increased in all treatments (HFD) GPs (215). The effect of DGN and ISG on the lipid profile is represented in **Fig.23. (A-E)** and **Table 21.**



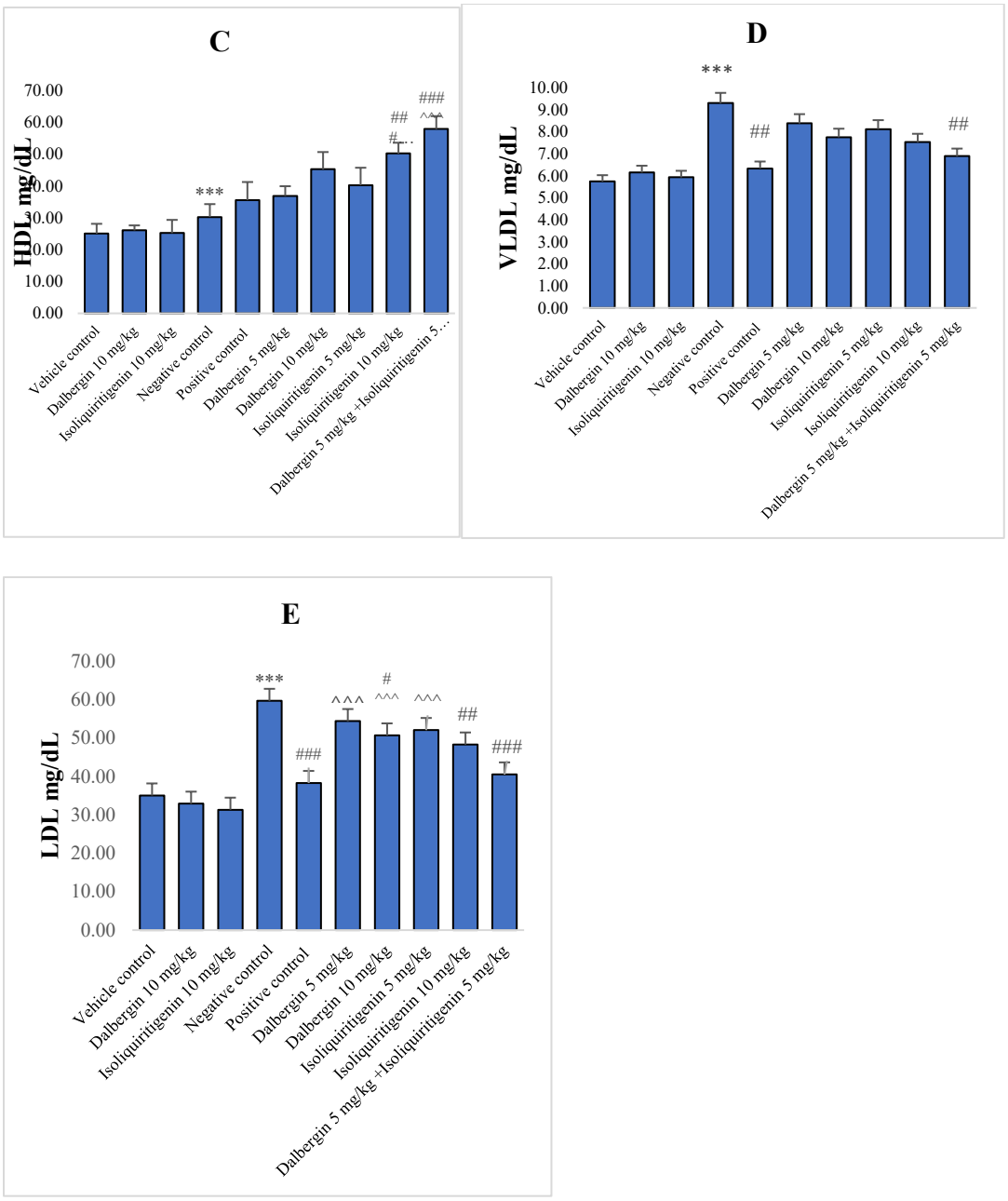



**Fig.23. Effect of treatments on lipid profile.** Data expressed as mean ± SD (N=8) of A) Cholesterol levels, B) Triglycerides, C) HDL, D) VLDL E) LDL. '***' represents (P<0.001) as compared to the vehicle control group with the negative control group. '###' represents (P<0.001) as compared to the negative control group with the treatment group. '##' represents (P<0.01) as compared to the negative control group with the treatment group, and '#' represents (P<0.05) as compared to the negative control group with the treatment group. '^^^' represents (P<0.001) as compared to the positive control group with the treatment group. '^^' represents (P<0.01) as compared to the positive control group with the

**treatment group. One-way analysis of variance (ANOVA) with Tukey's post-hoc test for multiple comparisons was used to compare various groups.**

### 5.3.5 *Kidney profile*

Elevated blood glucose levels alter the functioning of kidneys by altering blood urea and serum creatinine(216). The effect of DGN and ISG on serum creatinine and blood urea was evaluated to study kidney function. It was observed that there was an increase in blood urea and serum creatinine in all treatment GPs ($GP_6$-$GP_{10}$) after the induction of diabetes in obese rats with low doses of STZ than in $GP_4$. Whereas, after treatment with DGN and ISG for two weeks, these parameters were significantly ($p<0.001$) reduced in $GP_7$-$GP_{10}$ than $GP_4$ (negative control). No significant difference was observed with a low dose of DGN treatment in $GP_6$. The effect of DGN and ISG on kidney function was represented in **Fig.24. (A-B)** and **Table.21**.

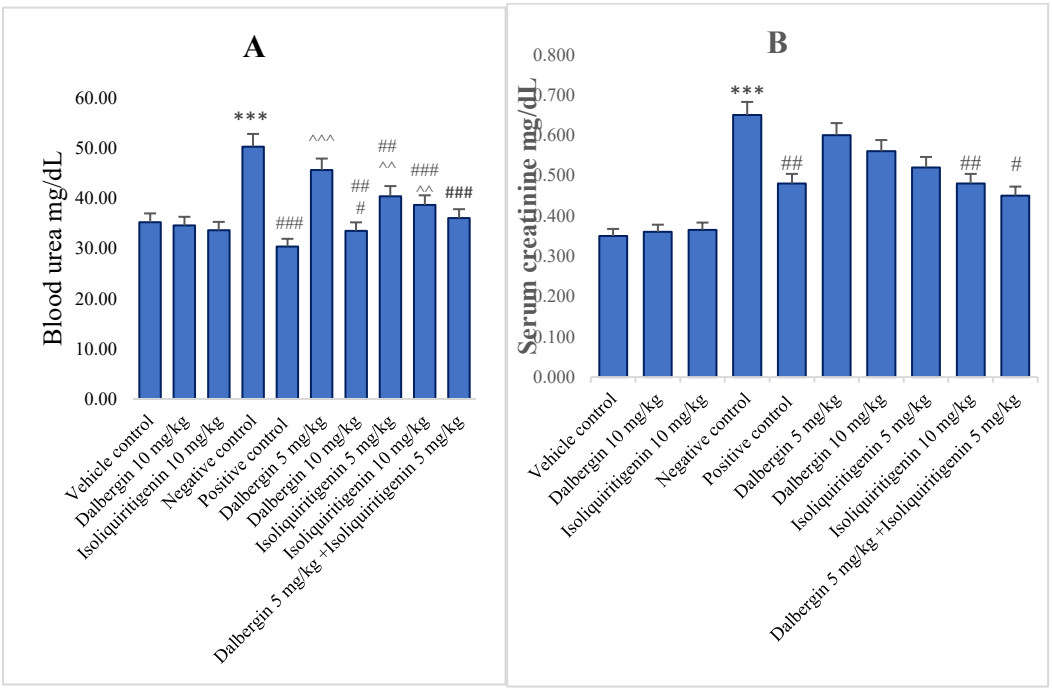
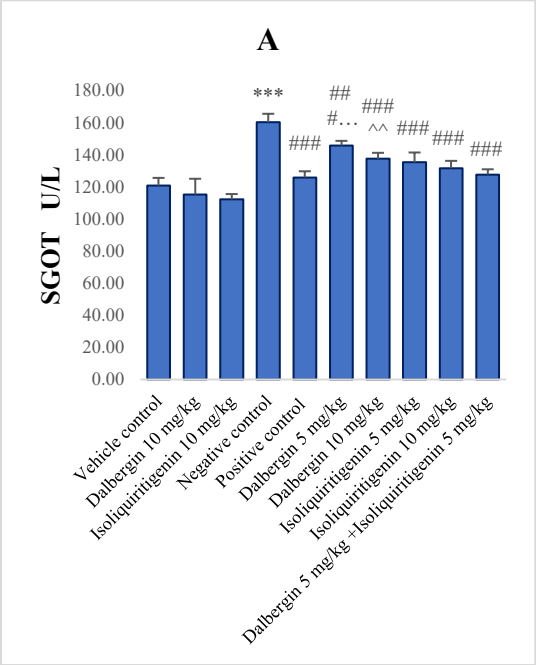
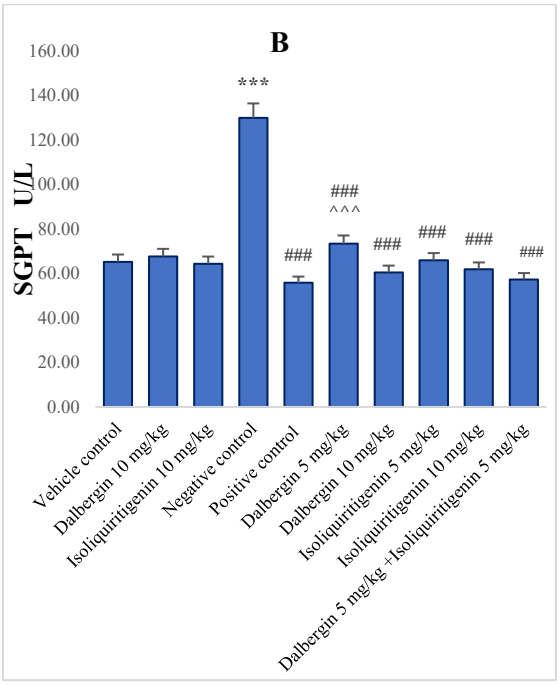



**Fig.24. Effect of treatments on kidney profile. Data expressed as mean ± SD (N=8) of A) Blood urea and B) serum creatinine. '***' represents (P<0.001) as compared to the vehicle control group with the negative control group. '###' represents (P<0.001) as compared to the negative control group with the treatment group. '##' represents (P<0.01) as compared to the negative control group with the treatment group, and '#' represents (P<0.05) as compared to the negative control group with the treatment group. '^^^' represents (P<0.001) as compared to the positive control group with the treatment group. '^^' represents (P<0.01) as compared to the positive control group with the treatment group. One-way analysis of variance (ANOVA) with Tukey's post-hoc test for multiple comparisons was used to compare various groups.**

### 5.3.6 *Liver function tests*

The liver is an organ essential for the continuous supply of glucose to various organs in the body (217). Continuous administration of HFD has been closely linked to various health issues, particularly hyperlipidemia and the development of fatty liver (218). Many researchers reported that liver parameters like SGOT, SGPT, and ALP are elevated after a continuous supply of HFD to obese rats. In diabetic rats SGOT, SGPT and ALP levels were found to be significantly higher than those in normal rats (218). However, after administering DGN and ISG to diabetic rats for 14 days, the levels of SGOT, SGPT, and ALP were significantly decreased **(Fig.25. (A-C), Table.21)**. These results suggest that DGN and ISG were effective in reducing diabetes-induced liver damage (219).



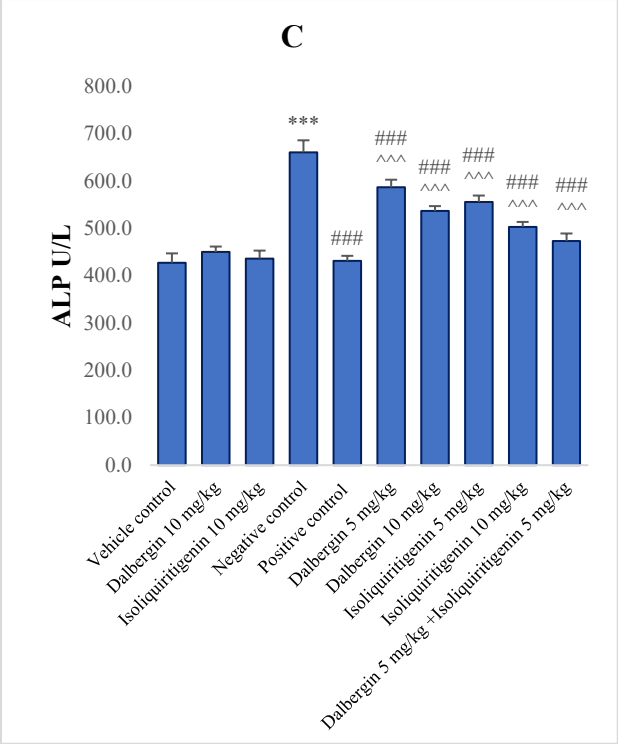



**Fig.25. Effect of treatments on liver profile.** Data expressed as mean ± SD (N=8) of A) SGOT: Serum glutamic oxaloacetic transaminase and B) SGPT: Serum glutamic pyruvic transaminase C) ALP: Alkaline phosphatase. '***' represents (P<0.001) as compared to the vehicle control group with the negative control group. '###' represents (P<0.001) as compared to the negative control group with the treatment group. '^^^' represents (P<0.001) as compared to the positive control group with the treatment group. '^^' represents (P<0.01) as compared to the positive control group with the treatment group. One-way analysis of variance (ANOVA) with Tukey's post-hoc test for multiple comparisons was used to compare various groups.

**Table 21. Effect of DGN and ISG on lipid, kidney, and liver parameters**

| GPs | Lipid parameters | | | | | Kidney parameters | | Liver parameters | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | TC (mg/dL) | TG (mg/dL) | HDL (mg/dL) | VLDL (mg/dL) | LDL (mg/dL) | BU (mg/dL) | SC (mg/dL) | SGOT (U/L) | SGPT(U/L) | ALP (U/L) |
| **$GP_1$** | 105.26±6.27 | 28.75±3.50 | 25.05±9.07 | 5.74±1.35 | 35.03±3.99 | 35.20±7.0 | 0.350±0.13 | 120.79±4.79 | 65.23±5.23 | 427.5±9.71 |
| **$GP_2$** | 92.19±5.29 | 41.22±4.08 | 26.07±6.55 | 6.15±2.02 | 32.89±3.78 | 34.55±2.8 | 0.360±0.02 | 115.23±9.80 | 75.65±9.63 | 450.1±11.5 |
| **$GP_3$** | 82.71±4.32 | 44.71±3.77 | 25.23±4.12 | 5.93±1.26 | 31.33±4.90 | 33.59±8.0 | 0.365±0.13 | 112.20±3.30 | 64.37±5.03 | 436.4±6.7 |
| **$GP_4$** | 153.28±6.48 | 72.32±4.90 | 30.25±8.06 | 9.30±1.40 | 59.65±8.66 | 50.27±2.6 | 0.650±0.10 | 160.27±5.23 | 129.91±8.76 | 660.6±25.3 |
| **$GP_5$** | 125.14±4.11 | 49.55±2.58 | 35.57±7.68 | 6.33±0.91 | 38.28±9.07 | 30.38±2.0 | 0.480±0.12 | 125.87±3.83 | 55.78±3.74 | 431.3±1.7 |
| **$GP_6$** | 122.27±4.00 | 65.04±2.94 | 36.89±8.04 | 8.38±1.28 | 54.38±5.84 | 45.60±7.3 | 0.600±0.10 | 145.74±2.89 | 73.38±2.94 | 586.6±11.2 |
| **$GP_7$** | 109.34±4.55 | 57.17±2.83 | 45.25±5.42 | 7.75±1.42 | 50.65±2.35 | 33.50±3.0 | 0.560±0.10 | 137.45±3.74 | 50.48±3.65 | 536.5±10.5 |
| **$GP_8$** | 130.88±9.35 | 60.25±1.71 | 40.23±5.51 | 8.12±1.26 | 52.06±3.38 | 40.38±1.0 | 0.520±0.05 | 130.25±6.14 | 55.00±8.60 | 555.8±7.5 |
| **$GP_9$** | 118.27±8.16 | 37.58±5.56 | 50.21±9.38 | 7.53±0.78 | 48.29±4.94 | 38.63±3.3 | 0.480±0.12 | 126.55±4.62 | 59.84±4.32 | 503.3±10.3 |
| **$GP_{10}$** | 99.00±2.16 | 45.27±4.99 | 57.89±6.06 | 6.89±2.24 | 40.50±1.28 | 36.00±1.6 | 0.450±0.12 | 132.65±3.27 | 57.29±8.52 | 473.5±4.7 |

Each value is expressed as mean±SD (N=8).

### 5.3.7 *Histopathological evaluation of the pancreas*

Pancreas from control and HFD-fed STZ induced diabetic rats demonstrated pathological changes in the cell components. Therefore, it is advantageous to access the efficacy of novel treatment medicines in enhancing the pancreatic architecture using a T2DM model that exhibits significant pathological alterations in the pancreatic architecture.

The administration of HFD primarily triggers an overproduction of insulin from the pancreatic β-cell. However, this excessive insulin release is subsequently decreased due to the partial destruction of working β-cells (220). As a result, rats experience frank hyperglycemia when given a low dose of STZ (221). After the termination of the study (14 days), the pancreas was carefully isolated and preserved in a 10% formalin solution for histopathological studies. A histopathological study of pancreatic tissue sections was performed at the Synergy Diagnostics laboratory in Jalandhar, Punjab. All tissue samples were fixed on glass slides and fixed with hematoxylin (H) & eosin and magnified under 40x. The pancreatic sections were examined (40X) for morphological changes which include acinar parenchyma, size, shape and loss of islets of Langerhans.

**5.3.7.1** Scoring of islets damage (222,223)

The criteria for scoring the islet cell destruction were as follows:

score 0 (normal): normal number of islet cells.

score 1 (mild): loss of 1/3 of islet cells.

score 2 (moderate): loss of 1/3 to 2/3 of islet cells.

score 3 (severe): loss of more than 2/3 of islet cells.

**5.3.7.2** Scoring of islet recovery (222,223)

The criteria for scoring the regeneration were as follows:

score 0 (none): no regeneration.

score 1 (mild): regeneration of 1/3 of islet cells.

score 2 (moderate): regeneration of 1/3 to 2/3 of islet cells.

score 3 (prominent): regeneration of more than 2/3 of islet cells.

In the nontreatment GPs ($GP_1$-$GP_3$), histological characteristics sections showed normal acinar parenchyma and islets of Langerhans. It was observed that islet cells of Langerhans were degenerated ($GP_4$-$GP_{10}$), with a reduction in the cell size and number of islet cells in the diabetic animals due to cytotoxic action of STZ. It was evident in

$GP_4$ that STZ injection resulted in severe loss of pancreatic islets and there is no recovery (score 0) was noted after 14 days.

After treatment with acarbose 20 mg/kg, DGN 5 mg/kg, DGN 10 mg/kg, ISG 5 mg/kg, ISG 10 mg/kg and DGN 5 mg/kg+ ISG 5 mg/kg ($GP_5$, $GP_6$, $GP_7$, $GP_8$, $GP_9$, and $GP_{10}$ (**Fig.26**.) islet cells of Langerhans recovered from degeneration, and proliferation was initiated. Regeneration scores of various groups: GP-5 (score-1), GP-6 (score-2),GP-7 (score-1),GP-8 (score-2),GP-9 (score-2),GP-10 (score-3). It is worth highlighting that combining both compounds has been proven to be significantly more potent compared to when they are used separately. Sections of ISG 5 mg/kg +DGN 5 mg/kg treated rats showed normal pancreatic acinar parenchyma with the increased number of islets of Langerhans, which are normal in size, shape and maximum recovery (score-3) noted with this treatment **(Table 22)**.Numerous histological investigations also showed that different phytoconstituents effectively increased number of islets of Langerhans in diabetic animals (224).

It was also observed that pancreatic acinar cells were damaged during the induction of diabetes compared to the normal GPs ($GP_1$-$GP_3$). Recovery of cells was noted when treatment was done with 5 mg/kg DGN, 10 mg/kg DGN, 5 mg/kg ISG, 10 mg/kg ISG and 5 mg/kg DGN+ 5 mg/kg ISG. Maximum recovery was noted with the combination of 5 mg/kg DGN+ 5 mg/kg ISG. This was attributed to the antidiabetic potential of DGN and ISG.

**Table 22. Histopathological evaluation of pancreatic tissue**

| GP. No. | Histopathology observation |
|---|---|
| $GP_1$ | Acinar parenchyma of pancreas along with ducts and islets of Langerhans |
| $GP_2$ | Acinar parenchyma of pancreas along with ducts and islets of Langerhans |
| $GP_3$ | Acinar parenchyma of pancreas along with ducts and islets of Langerhans |
| $GP_4$ | Acinar parenchyma of the pancreas, along with markedly reduced islets of Langerhans |
| $GP_5$ | Acinar parenchyma of the pancreas. Few islets of Langerhans noted |
| $GP_6$ | Acinar parenchyma of the pancreas. Few islets of Langerhans noted |
| $GP_7$ | Acinar parenchyma of the pancreas with very few and irregularly shaped islets of Langerhans |
| $GP_8$ | Acinar parenchyma of the pancreas with very few and abnormally shaped islets of Langerhans |
| $GP_9$ | Normal acinar parenchyma of the pancreas with an increased number of islets of Langerhans, which are larger in size and normal in shape |
| $GP_{10}$ | Normal acinar parenchyma of the pancreas with an increased number of islets of Langerhans, which are normal in size and shape. Maximum recovery noted |

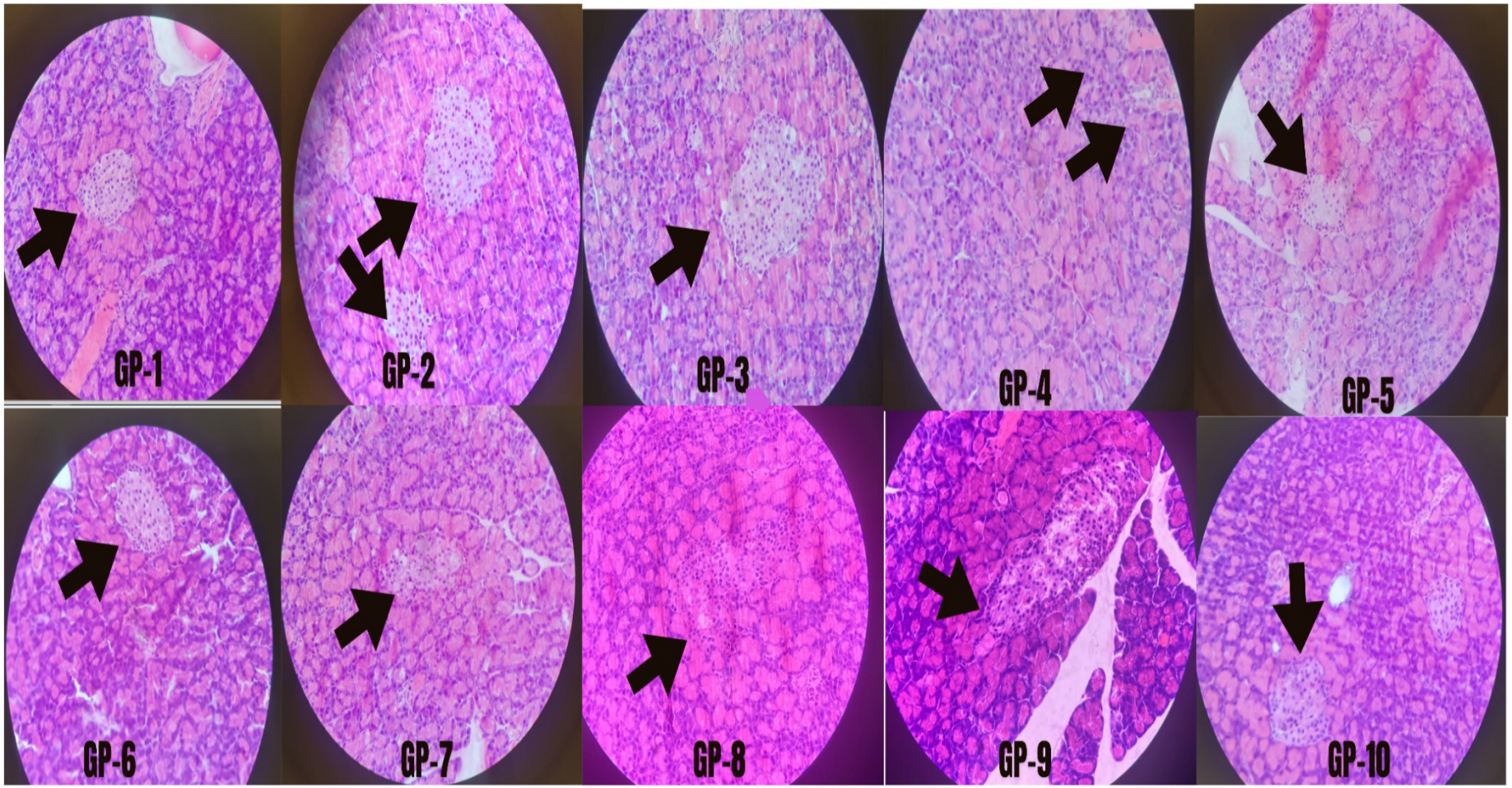



**Fig.26. The effect of DGN and ISG on the pancreas region (40X). The arrow indicates islets of Langerhans (GP=group). (DGN: Dalbergin, ISG: Isoliquiritigenin**

## 5.4 Discussion

It is crucial to take a proactive approach to maintain optimal health and prevent potential complications of diabetes. According to extensive research, α-amylase, which is an enzyme released by the pancreas, holds great potential as a therapeutic target because it plays a crucial role in breaking down carbohydrates. When this enzyme is blocked, the process that breaks down carbohydrates is hindered, affecting the absorption of glucose. As a result, the body's ability to efficiently absorb glucose becomes sluggish. By inhibiting the activity of this enzyme, the risk of developing diabetes and postprandial hyperglycemia can be lowered. Additionally, α-amylase enzyme inhibitors may also be used in conjunction with other chemotherapeutic medicines to treat diabetes.

Several herbal extracts are known for their anti-diabetic properties. Flavonoids in plants have potent α-amylase inhibitory activity. The key enzyme in controlling blood glucose is thought to be α-amylase, which primarily catalyses the hydrolysis of -1,4-glucan in carbohydrates to increase postprandial blood glucose. Therefore, blocking α-amylase activity would be a successful strategy to stop the absorption and utilisation of carbohydrates in the small intestine, further assisting in the management of postprandial blood glucose levels.

Animal studies have documented that DL extracts can reduce blood glucose levels. An assessment of the antidiabetic potential of ethanolic bark extract at a dose of 500 mg/kg was conducted on rats with diabetes induced by alloxan. However, the active constituent responsible for the antidiabetic activity has not been evaluated so far.

So, in this study, we focused on the utilisation of pharmacoinformatic tools for the identification of binding of active constituents with α-amylase (1HNY) first, then conducted ADMET studies to understand the pharmacokinetic and toxicity properties of each active constituent. By following various extractive procedures, we finally separated two compound DG and ISG. For these compounds, *in vitro* studies (α-amylase inhibitory activity, cytotoxicity assay and glucose utilisation assay using L6 cell lines) and *in vivo* (antidiabetic evaluation using STZ+HFD diet) are conducted.

DGN has shown better α-amylase inhibitory action in a dose-dependent manner compared with ISG and acarbose.

Glucose uptake assay in cell lines is used widely for the evaluation of in-vitro antidiabetic activity. Glucose uptake assay is generally carried out in L6, 3T3L1, C2C12, Caco-2, and RIE- cells (225). L6 cells offer an excellent model for glucose uptake since they express the insulin-sensitive GLUT4, have an intact insulin signaling pathway, and have been widely used to shed light on the mechanisms behind muscular glucose absorption. In the L6 glucose assay, all the results are dose-dependent, and insulin produces maximum glucose uptake in both cases. Numerous investigations have shown that the plasma membrane translocation of the glucose transporter GLUT4 is necessary for the stimulation of insulin-induced glucose uptake in muscle and fat tissues. A decrease in GLUT 4 translocation is one of the root causes of insulin resistance (226).

So, in this study, we have studied the glucose uptake assay in L6 cell lines with DGN and ISG. Our present study demonstrated that DGN and ISG promote glucose uptake in cell lines. We have performed the glucose uptake assay also to standard drugs, and the ranking order for the potency of glucose uptake assay follows insulin>DGN>metformin> ISG compared at maximum and standard doses as shown in **Fig. 18.** The strong action of DGN may be due to the translocation of GLUT 4 at the target site.

The neutral red dye was used in *in vitro* tissue culture experiments to test the immunotoxicity and cytopathogenicity of infectious agents and the presence of toxic substances. Rockefeller University created the neutral red absorption cytotoxicity assay as a cell viability chemosensitivity assay. It depends on living cells' ability to absorb and bind the neutral red dye. This cationic dye concentrates in lysosomes after permeating cell membranes via nonionic passive diffusion. It interacts hydrophobically and electrostatically with phosphate and anionic groups inside the lysosomal matrix. Once the dye has been dissolved, it is removed from the living cells using an ethanol solution that has been acidified. A spectrophotometer is then used to measure the dye's absorbance. The $IC_{50}$ values of DGN and ISG in NRU assay in cell lines are 1254 and 1344, indicating that both possess anticancer activity.

In rodent studies, HFD is often used to replicate the conditions of obesity, insulin resistance, and glucose intolerance commonly observed in prediabetes. Rats fed a high-fat diet (HFD) are often observed to develop insulin resistance, though not necessarily progressing to hyperglycemia or diabetes. HFD is considered an effective approach to initiating insulin resistance, a key feature of type 2 diabetes. Streptozotocin (STZ), on

the other hand, is commonly used to induce diabetes by causing β-cell death through DNA alkylation. While high doses of STZ lead to severe insulin secretion impairment resembling type 1 diabetes, low doses result in mild impairment of insulin secretion, mimicking the later stages of type 2 diabetes. Combining HFD with low-dose STZ has emerged as a widely used method to develop a rat model that replicates the progression from insulin resistance to β-cell dysfunction, closely resembling the natural history and metabolic characteristics of human type 2 diabetes.

In the present study, HFD and low-dose STZ 35mg/kg were given to rats. HFD induces insulin resistance, while STZ targets and destroys insulin-producing β-cells, resulting in impaired insulin secretion and hyperglycaemia. The weight of the animals decreased after a 14-day treatment with DGN and ISG, demonstrating the effectiveness of these treatments in combating obesity induced by a high-fat diet.

The hallmark of diabetes is an elevation in blood glucose (227). Administration of STZ at 35 mg/kg in HFD fed rats damaged the beta cells of the pancreas (GP 4 in **Fig.26**), resulted in increased blood glucose levels. Administering DGN and ISG resulted in a reduction in blood glucose levels and a reversal of histopathological changes (GP 5-10 in **Fig.26**). Major difference is not observed in quantity of water and food intake. In a recent study ISG was shown to stimulate AMPK and block the mTORC1 Pathway in diabetic mice indicating its antidiabetic potential (228). It was reported that ISG has anti-inflammatory and antioxidant properties, may contribute to enhanced pancreatic function (229). In recent studies DGN shown to exhibit the anticancer activity by changing mRNA levels of apoptosis related proteins (230,231) and anti-osteoporotic activity in ovariectomized rats (232).

The GP-10 exhibited more favorable outcomes. But islet cell regeneration in GP6-GP10 has attracted a lot of attention and has been proposed to compensate for the loss of $\beta$-cell mass in diabetes mellitus (233).

# CHAPTER-6

## 6. CONCLUSION

Even after identifying many compounds, treating diabetes effectively remains a challenge. Most of the available compounds for diabetes possess certain side effects. Since the last decade, many natural products have been identified and extracted from various plant sources and extensively explored to treat multiple chronic diseases with fewer side effects. DL is one of the plant sources that has been proven to have greater therapeutic potential for diabetes. However, there is no research available to date on the isolation of phytoconstituents of DL and the evaluation of their antidiabetic potential.

The current research study focused on assessing the antidiabetic potential of two isolated phytoconstituents, namely DGN and ISG, from the heartwood of DL. The evaluation involved studying *in silico* docking studies, ADMET, *in vitro* α- amylase inhibitory action, glucose uptake assays, and *in vivo* antidiabetic evaluation. Both compounds, ISG and DGN, exhibited effectiveness in all the areas of investigation: *in silico* docking studies, ADMET, *in vitro* α-amylase inhibitory action, glucose uptake assay, and in *vivo* antidiabetic evaluation. Insilco studies suggest that DGN and ISG specifically bind the active site of alpha amylase enzyme (1HNY), which is important in the control of post prandial hyperglycemia. Invitro and in vivo studies confirmed the antidiabetic potential of DGN and ISG. In the docking studies DGN formed conventional hydrogen bond with GLN, A:63 and ISG formed convention hydrogen bonds with ASP, A:197.

These findings suggest that both ISG and DGN hold promise as potential treatments for diabetes. Conducting a pharmacoinformatic study, isolating pure compounds, performing molecular docking, characterising the compounds, and performing *in vitro* and *in vivo* antidiabetic evaluations could all play a critical role in the development of an innovative drug in the future. These studies could help clarify the pharmacological mechanisms and aid in the development of medicinal formulas, nutraceuticals, and functional foods for diabetes and related symptoms.

It is important to acknowledge that more research is necessary to fully comprehend the mechanisms (wnt proteins, GSK-3β, PI-3K, akt or protein kinase B, PTP1B and IRS-1) underlying the antidiabetic properties of these phytoconstituents. Nevertheless, these preliminary findings offer promising evidence for the therapeutic potential of these plant-derived compounds.

# CHAPTER-7

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**Annexure-1**

LOVELY PROFESSIONAL UNIVERSITY

Centre for
Research Degree Programmes

*LPU/CRDP/PHD/EC/20201005/001264*

Dated: 25 Jun 2020

Srinivas Sutrapu
Registration Number: 41800404
Programme Name: Doctor of Philosophy (Pharmacology)

**Subject: Letter of Candidacy for Ph.D.**

Dear Candidate,

We are very pleased to inform you that the Department Doctoral Board has approved your candidacy for the Ph.D. Programme on 25 Jun 2020 by accepting your research proposal entitled: "EXTRACTION, PHARMACOLOGICAL AND PHARMACOINFORMATIC EVALUATION OF ACTIVE CONSTITUENTS OF DALBERGIA LATIFOLIA AS POTENTIAL ANTIDIABETIC AGENTS "

As a Ph.D. candidate you are required to abide by the conditions, rules and regulations laid down for Ph.D. Programme of the University, and amendments, if any, made from time to time.

We wish you the very best!!

In case you have any query related to your programme, please contact Centre of Research Degree Programmes.

Head

Centre for Research Degree Programmes

Note:-This is a computer generated certificate and no signature is required. Please use the reference number generated on this certificate for future conversations.

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**Annexure-2**

## ANIMAL HOUSE

**Lovely Institute of Technology (Pharmacy), Lovely Professional University**
**Ludhiana- Jalandhar G.T. Road, Phagwara (Punjab), 144411**
**Registration Number -954/PO/ReRcBiBt/S/06/CPCSEA**

## CERTIFICATE

This is to certify that the project titled *" Pharmacological evaluation of isolated compounds from Dalbergia latifolia for its antidiabetic activity "*
*(Thesis title: Extraction, pharmacological and pharmacoinformatic evaluation of active constituents of Dalbergia latifolia as potential antidiabetic agents) has* been approved by the IAEC.

**Name of Principal Investigator:** Dr. Rashmi Saxena Pal

**IAEC approval number:** LPU/IAEC/2023/25

**Date of Approval:** 29th April 2023

**Animals approved:** 80 SD/Wistar rats, either sex

**Remarks if any:** - NA

| Dr. Monica Gulati | Dr. Navneet Khurana | Dr. Bimlesh Kumar |
|---|---|---|
| Biological Scientist, Chairperson IAEC | Scientist from different discipline | Scientist In-Charge of Animal House, Member Secretary IAEC |

**Annexure-3**

**Awards:**

1. Got 2nd prize on title "***Insilico evaluation of potential alpha amylase inhibitors from Dalbergia latifolia plant***" in the National Symposium on Translation Research and Future Pharmaceuticals organised by JSS Academy of Higher Education and Research, Ooty. Chennai.
2. Got 2nd prize on the title ***"Insilico docking studies, isolation, and in-vitro antidiabetic evaluation of active constituents of Dalbergia latifolia***" in International Conference on Recent Advances In Health Sciences (ICRAHS- 2023) organised by LPU, Punjab.

**Presentations:**

1. Given poster presentation on ***"Insilico evaluation of potential alpha amylase inhibitors from Dalbergia latifolia plant"*** at "National symposium on translation research and future pharmaceuticals" organized by JSS academy of higher education and research, Ooty. Chennai. Tamilnadu.
2. Given oral presentation on "***Insilico docking studies, isolation, and in-vitro antidiabetic evaluation of active constituents of dalbergia latifolia"*** at International Conference on Recent Advances In Health Sciences (ICRAHS- 2023) organized by LPU, Punjab.

**Publications from Ph.D work:**

**Sutrapu S**, Pal RS, Khurana N, Vancha H, Mohd S, Chinnala KM, Kumar B,Pilli G. Diabetes Warriors from Heart Wood: Unveiling Dalbergin and Isoliquiritigenin from Dalbergia latifolia as Potential Antidiabetic Agents in-vitro and in-vivo. Cell Biochemistry and Biophysics. 2024 May 13:1-6.

**Publication from allied work:**

1. Pooja Agrawal, Vancha Harish, Sharfuddin Mohd, Sachin Kumar Singh, Devesh Tewari, Ramanjireddy Tatiparthi, Harshita, Sukriti Vishwas, **Srinivas Sutrapu**, Kamal Dua, Monica Gulati, "**Role of CRISPR/Cas9 in the treatment of Duchenne muscular dystrophy and its delivery strategies"** Life Sciences, Volume 330,2023,1220

2. Nihal PM, Mohapatra D, Manir AM, Mehra A, Sutrapu S, Harish V, Mohd S. Unveiling the Essence of Isoliquiritigenin: Exploring Its Chemistry, Pharmacokinetics, and Pharmacological Potential. Chemistry Africa. 2024 Dec 18:1-20.

**3.**Nihal P M, Mohapatra D, Manir AM, Harish V, Singh SK, Lad SU, Sutrapu S, Saini S, Mohd S. Reverse Phase-High-Performance Liquid Chromatography (RP-HPLC) Method Development and Validation Using Analytical Quality-by-Design Approach for Determination of Isoliquiritigenin in Bulk and Biological Sample. ASSAY and Drug Development Technologies. 2024 Dec 1;22(8):409-24.

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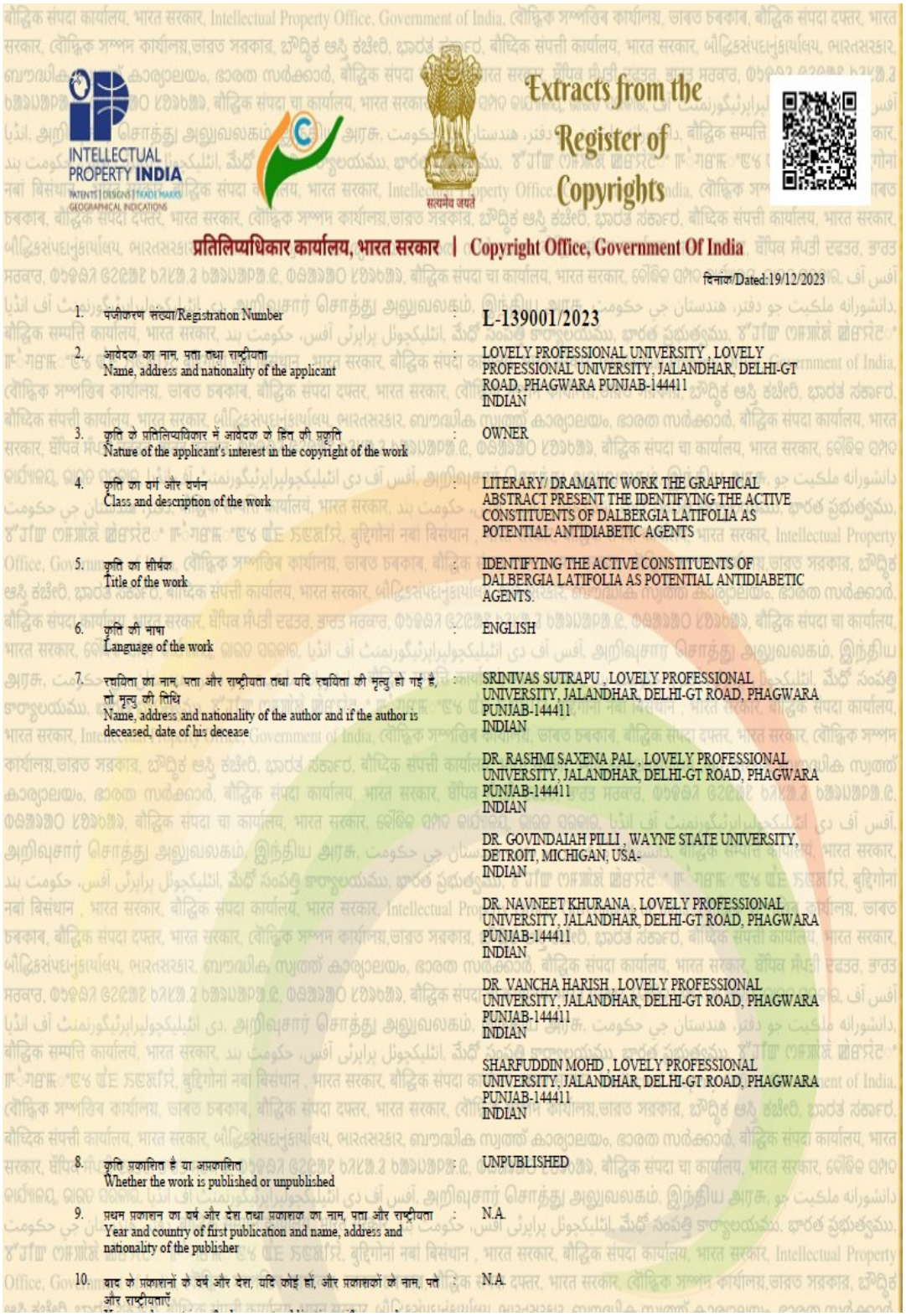

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ORIGINAL PAPER

# Diabetes Warriors from Heart Wood: Unveiling Dalbergin and Isoliquiritigenin from *Dalbergia latifolia* as Potential Antidiabetic Agents in-vitro and in-vivo

Srinivas Sutrapu[1] · Rashmi Saxena Pal[1] · Navneet Khurana[1] · Harish Vancha[1] · Sharfuddin Mohd[1] · Krishna Mohan Chinnala[2] · Bimlesh Kumar[1] · Govindaiah Pilli[1,3]

Accepted: 17 April 2024


**Abstract**
Diabetes mellitus is a serious and complex metabolic disorder characterized by hyperglycemia. In recent years natural products has gained much more interest by researchers as alternative sources for diabetes treatment. Though many potential agents are identified so far but their clinical utility is limited because of their adverse effects. Therefore, there is a keen interest in discovering natural compounds to treat diabetes efficiently with less side effects. *Dalbergia latifolia* is well explored because of its diverse pharmacological activities including diabetes. Therefore, the present research work aimed to identify and isolate the potential antidiabetic agents from the heart wood of *Dalbergia latifolia*. We successfully extracted DGN and ISG from the heartwood and evaluated their antidiabetic potential both in-vivo and in-vitro. Alpha amylase activity inhibition of ISG and DGN was found to be 99.05 ± 8.54% ($IC_{50}$ = 0.6025 µg/mL) and 84.68 ± 5.2% ($IC_{50}$ = 0.0216 µg/mL) respectively. Glucose uptake assay revealed DGN (158%) promoted maximum uptake than ISG (77%) over control. In vivo anti diabetic activity was evaluated by inducing diabetes in SD rats with the help of HFD and STZ (35 mg/kg body weight). After the continuous administration of DGN (5 mg/kg, 10 mg/kg) and ISG (5 mg/kg, 10 mg/kg) for 14 days, we observed the reduction in the blood glucose levels, body weight, total cholesterol, low density lipoprotein, very low-density lipoprotein, blood urea, serum creatinine, serum glutamate oxaloacetic transaminase, serum glutamate pyruvate transaminase and alkaline phosphatase levels than vehicle group indicates the potency of ISG and DGN against diabetes.

**Keywords** Dalbergia latifolia · Dalbergin · Isoliquiritigenin · *alfa-amylase* · Glucose uptake assay · Antidiabetic evaluation

## Introduction

Diabetes mellitus (DM) is a complex metabolic disorder characterized by hyperglycemia, polydipsia, polyphagia and polyurea [1]. The increase in glucose levels in blood is mainly due to inadequate release of insulin from the pancreas, degradation of the released insulin, lack of pancreatic β cell sensitivity, and excess glucose production from the liver [2]. The prevalence of hyperglycemia has a significant impact on the incidence of both nervous and vascular complications [3]. According to the latest data (2023 June) from the World Health Organization (WHO), 529 million people have diabetes, and it will be expected to project up to 1.31 billion cases worldwide by 2050. Among all types of DM, type-II diabetes is more prevalent (96%) in all age groups [4]. Recent studies by various researchers have suggested that maintaining tight control over blood glucose levels can have significant benefits in mitigating DM and its associated long-term complications [5]. The control of postprandial blood sugar levels not only helps minimize potential consequences but also promotes overall well-being

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Review article

# Role of CRISPR/Cas9 in the treatment of Duchenne muscular dystrophy and its delivery strategies

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REVIEW

# Unveiling the Essence of Isoliquiritigenin: Exploring Its Chemistry, Pharmacokinetics, and Pharmacological Potential

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**Abstract**

Isoliquiritigenin (ISL), a prominent chalcone-type flavonoid primarily derived from liquorice roots (*Glycyrrhiza* species), showcases remarkable therapeutic potential across a spectrum of pharmacological actions. Its diverse attributes include antioxidant, anti-inflammatory, anti-diabetic, cardioprotective, hepatoprotective, neuroprotective, and anticancer properties. This comprehensive review investigates the chemistry, structure-activity relationships (SAR), pharmacokinetics, derivatives, novel formulations, and mode of action of ISL. The intricate molecular structure of ISL and its intricate interactions with biological systems are meticulously elucidated. Insights into its pharmacokinetics, spanning absorption, distribution, metabolism, and excretion, are essential for discerning its in vivo behaviour and therapeutic effectiveness. Furthermore, the review delves into ISL's mode of action, revealing its modulation of oxidative stress, inflammatory pathways, glucose metabolism, and cancer progression, underscoring its pharmacological versatility. By consolidating existing knowledge and recent advancements in ISL research, the review emphasizes its pivotal role in managing various disease conditions. The integration of chemistry, pharmacokinetics, and mode of action enriches our understanding of ISL's therapeutic spectrum, facilitating its rational development and clinical application in the realm of medicine.

**Keywords** Isoliquiritigenin · Chemistry · Pharmacokinetics · Pharmacology · Cancer · Molecular targets

**Abbreviations**

| Abbreviation | Definition |
|---|---|
| AKT | Protein Kinase B |
| ALKP | Alkaline Phosphatase |
| AMP | Adenosine Monophosphate |
| AMPK | Adenosine Monophosphate-Activated Protein Kinase |
| AOM | Azoxymethane |
| AP-1 | Activator Protein 1 |
| ATF6 | Activating Transcription Factor 6 |
| ATM | Ataxia Telangiectasia Mutated |
| ATP | Adenosine Triphosphate |
| BAX | B-Cell Lymphoma-2 Associated X Protein |
| BBB | Blood-Brain Barrier |
| BCL-XL | B-Cell Lymphoma-Extra Large |
| CAM | Cell Adhesion Molecules |
| CCL | Chemokine (C-C Motif) Ligand |
| CDK | Cyclin-Dependent Kinase |
| CHK2 | Checkpoint Kinase 2 |
| C-IAP1/2 | Cellular Inhibitor of Apoptosis Protein 1 and 2 |
| COX | Cyclooxygenase |
| DAPK1 | Death-Associated Protein Kinase-1 |
| E2F | E2 Transcription Factor |
| EIF2 | Eukaryotic Initiation Factor 2 |
| ELAM-1 | Endothelial Leukocyte Adhesion Molecule-1 |
| ERBB3 | Erythroblastic Oncogene B Homolog 3 |
| ERK | Extracellular Signal-Regulated Kinase |
| FAS | Fatty Acid Synthase |
| FASL | Fatty Acid Synthase Ligand |
| GADD153 | Growth Arrest and DNA Damage-Inducible 153 |

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# Reverse Phase-High-Performance Liquid Chromatography (RP-HPLC) Method Development and Validation Using Analytical Quality-by-Design Approach for Determination of Isoliquiritigenin in Bulk and Biological Sample

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## ABSTRACT

The primary objective of the present investigation is to develop and validate a simple, robust, and cost-effective isocratic reverse phase-high-performance liquid chromatography (RP-HPLC) method for determining isoliquiritigenin (ISL) in both bulk and biological samples using an analytical quality-by-design (AQbD) approach. The central composite design was employed for method optimization using Design Expert® software, by taking mobile phase ratio and flow rate as independent variables and peak area, retention time, tailing factor, and theoretical plates as dependent variables. The design suggested the use of a mobile phase consisting of acetonitrile:0.2% ortho-phosphoric acid (75:25, v/v) and a flow rate of 0.9 mL/min as optimal chromatographic conditions. The detection of ISL was performed at 364 nm. The optimized method was validated in accordance with International Conference on Harmonization (ICH) Q2(R1) guidelines. The method showed excellent linearity, limit of detection, limit of quantification, accuracy, precision, robustness, and system suitability. All validation parameters fell within the acceptable limits set by ICH. Additionally, the applicability of the method in biological samples were analyzed. In conclusion, the results suggest that the developed and validated AQbD-based RP-HPLC method was well-suited for the estimation of ISL in bulk and biological sample.

**Keywords:** analytical quality-by-design, RP-HPLC, isoliquiritigenin, CCD, bioanalytical method, validation

## INTRODUCTION

Isoliquiritigenin (ISL) is one of the most abundant chalcone-type flavonoids isolated mainly from the roots of liquorice (*Glycyrrhiza* species), heartwood of *Dalbergia odorifera*,[1] *Dalbergia latifolia*[2] and roots of *Sophora flavescens*.[3] Chemically, ISL is 2',4',4-trihydroxychalcone (*Fig. 1*). ISL has garnered considerable attention in the fields of medicine and nutrition because of its potential health benefits and pharmacological properties such as antioxidant,[4] anti-inflammatory,[4] anti-microbial,[5] anti-diabetic,[6] hepatoprotective,[7] cardioprotective,[8] and anticancer activities.[9]

To harness the therapeutic advantages of ISL and enable its clinical use, the development of a suitable dosage form is essential. As of the latest available information, ISL has not been extensively marketed as an independent supplement or medication. Although research often explores its potential health benefits, the widespread availability of commercial

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