ISOLATION, CHARACTERIZATION AND ANTI-DIABETIC EVALUATION OF AQUEOUS EXTRACT OF *INULA HELIANTHUS AQUATICA LEAVES*

Dissertation submitted to

THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY, CHENNAI.

In partial fulfillment for the award of degree of

MASTER OF PHARMACY

(PHARMACEUTICAL CHEMISTRY)

Submitted By

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Under the supervision of

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MAY– 2012

Nandha College of Pharmacy
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CERTIFICATES

CERTIFICATE

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This is to certify that the work embodied in this thesis entitled "**ISOLATION, CHARACTERIZATION AND ANTI-DIABETIC EVALUATION OF AQUEOUS EXTRACT OF *INULA HELIANTHUS AQUATICA LEAVES*"** submitted to The Tamilnadu Dr. M.G.R. Medical University Chennai, was carried out by **Mr. Cyril Mathew Jacob.** (Reg.No.26104231) in the Department of Pharmaceutical Chemistry, Nandha College of Pharmacy, Erode-52 in partial fulfillment for the award of degree of **MASTER OF PHARMACY** in Pharmaceutical Chemistry under my direct supervision and guidance.

This work is original and has not been submitted in part or full for any other degree or diploma of any university.

EVALUATION CERTIFICATE

This is to certify that the work embodied in this thesis entitled, **"ISOLATION, CHARACTERIZATION AND ANTI-DIABETIC EVALUATION OF AQUEOUS EXTRACT OF *INULA HELIANTHUS AQUATICA LEAVES*"** submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, was carried out by Reg. No. **26104231** in the Department of Pharmaceutical Chemistry, Nandha College of Pharmacy and Research institute, Erode-52 for the partial fulfillment for the award of degree of **MASTER OF PHARMACY** in Pharmaceutical Chemistry under the supervision and guidance of **Mr. K Srinivasan, M Pharm.,** Assistant professor, Department of Pharmaceutical Chemistry, Nandha College of Pharmacy and Research Institute, Erode- 52.

This work is original and has not been submitted in part or full for any other degree or diploma of this or any other university.

Internal Examiner **External Examiner**

DECLARATION

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I hereby declare that the work presented in this thesis entitled "**ISOLATION, CHARACTERIZATION AND ANTI-DIABETIC EVALUATION OF AQUEOUS EXTRACT OF *INULA HELIANTHUS AQUATICA LEAVES*"** was carried out by me in the Department of Pharmaceutical Chemistry, Nandha College of Pharmacy, Erode, under the direct supervision and guidance of **Mr. K Srinivasan, M Pharm.,** Assistant professor, Department of Pharmaceutical Chemistry Nandha College of Pharmacy, Erode - 52.

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ACKNOWLEDGEMENT

ACKNOWLEDGEMENT

I submit my sincere and heartful gratitude to my respectable guide ***Mr. K. Srinivasan****,* ***M. Pharm****., Assistant Professor, Nandha College of Pharmacy, Erode whose guidance was unforgettable and incomparable. The inspiration, impressive and innovative ideas as well as his constructive suggestions, untiring efforts and timely help have made the materialization of my research work.*

It is proud privilege to express my sincere thanks to ***Dr. T. Sivakumar, M. Pharm., Ph. D., Principal,*** *Nandha college of Pharmacy, Erode, with a deep sense of gratitude for his cooperation, kind suggestions and providing the best facilities during this work.*

I am extremely grateful to ***Dr. R. Rajavel, M. Pharm., Ph. D.,*** *Head of Department of Pharmaceutical Chemistry, Nandha College of Pharmacy, Erode for his support and efforts in the form of suggestion, guidance, encouragement throughout the course of this thesis.*

I express my loyal thanks to ***Thiru. V. Shanmugan, B.Com.,*** *Chairman and* ***Mr. Nandha Kumar Pradeep, M.B.A.,*** *Secretary, Nandha College of Pharmacy, Erode for providing all the facilities to make this work a success.*

I would like to express my gratitude to ***Dr.S. Sengottuvelu, M.Pharm., Ph.D.,*** *Head of Department of Pharmacology, Nandha College of pharmacy, Erode, for his kind help and inspiration to carry out the work.*

I would like to express my gratitude to ***Dr.Duraisamy, M.Pharm., Ph.D.,*** *Head of Depratment of Pharmacognosy, Nandha College of pharmacy, Erode, for his kind, advices and motivation to carry out the work.*

It is my privilege to express my sincere thanks to ***Mr.S. Haja sheriff, M.Pharm.,*** *and* ***Mr. G.Thamotharan, M.Pharm.,*** *Asst.prof, Department of Pharmacology, Nandha College of pharmacy, Erode , for their immense help and suggestions throughout the work.*

I hereby also take this opportunity to give my sincere thanks to ***Mrs. S.Ruby, M.pharm., and Mrs. N.Kiruthika, M.Pharm .,*** *Asst, Prof. Department of*

Pharmaceutical Chemistry, Nandha College of Pharmacy, Erode for their timely and valuable guidance.

*I take this opportunity to thank Mr.**M. Jagadeeswaran, M.Pharm.,** Asst, Prof. Department of Pharmaceutical Analysis, Nandha College of Pharmacy, Erode, for all the support in Analytical studies.*

At this stage, I express my sincere thanks to my dear friends and colleagues S.Akshay, Bimal, K.Bindu, Joel, K.Kavitha, Murali, R.Niranjan ,K.Pavan, G.Prasad P.Preethy, Preysingh, G.Rajasheka ,Ramanjaneylu, Ramjith, Ruby, Sanju, K.Shahu,K. Sibu, Sudhir, Swati, L.Vivek, for their valuable help and constant encouragement throughout my P.G course, and I appreciate their hard work and effort in bringing this project a success.

I whole heartedly thank our Lab assistants and Librarians for their kind help and support during my project work.

I am extremely pleased and filled with indebtedness to my beloved parents and all my family members for their encouragement and good wishes which enabled me to go through all the hardships.

I take this opportunity with pride and enormous gratification to express my bunch of embedded feelings of thanks and thanks giving to all the persons backed me throughout the modeling of this research work to this magnitude.

Above all, I bow my work into the hands of Almighty who led me to the actualization of this research work.

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INTRODUCTION

INTRODUCTION

Natural world constantly stands as a golden mark to illustrate the exceptional phenomenon of symbiosis. Natural products from plant, animal and mineral source have been the basis of treatment for a variety of human diseases throughout the world. About 80 % of people in developing countries rely on traditional herbal medicines based mainly on species of plants and animals. Studies have showed that regarding 800 plants have been used in native systems of medicine.

India is a measureless storehouse of medicinal plants that are used in traditional medical treatments. The various native systems such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat different ailments. Herbal medicines as a major remedy in traditional system of medicine have been used in medical practices since antiquity. The use of herbal medicines have become renowned due to increased toxicity and side effects produced by allopathic medicines that paved its way to a swift increase in the number of herbal drug manufacturers. The practice continues these days because of its biomedical reimbursement as well as place in intellectual beliefs in many parts of world and has made a great input towards maintaining human health. In India around 20,000 medicinal plant species have been recorded newly but more than 500 traditional communities use about 800 plant species for curing diverse diseases. At present 80% of the world population depends on plant-derived medicine as the first line of principal health care for human mitigation due to no side effects. 25% of pharmaceutical prescriptions in the United States contain at least one plant-derived component. In the last century, roughly 121 pharmaceutical products were formulated based on the long-established information obtained from various sources.

- **HERBAL MEDICINES OBTAINED FROM PLANTS**

India has one of the richest plants medical traditions in the world. There are estimated to be around 25,000 effective plant-based formulations, used in folk medicine and known to rural communities in India. There are over 1.5 million practitioners of traditional medicinal system using medicinal plants in preventive, promotional and curative applications. It is estimated that there are over 7800 medicinal drug-manufacturing units in India, which consume about 2000 tonnes of herbs annually.

- **MARKET VALUE OF HERBAL MEDICINES**

For ayurvedic medicine, the demand is estimated to be expanding at a rate of 20% yearly. The sales of medicinal plants have grown-up by nearly 25% in India in past ten years (1987-96), the highest rate of growth in the world. But the per capita expenses in India on medicines per annum is amongst the lowest in the world. The largest users of medicinal plants are China and India. Conventional Chinese Medicine uses over 5000 plant species; India uses about 7000. According to Export Import Bank, the international market for medicinal plant related trade having a growth rate of 7% per annum. China's share in world herbal market is US$ 6 billion and India's share is only US$1 billion. The yearly export of medicinal plants from India is valued at Rs. 1200 million. All the main herbal-based pharmaceutical companies are showing a constant growth of about 15 per cent. Conventional medicine has served as a source of alternative medicine, new pharmaceuticals, and healthcare products. Medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as preparatory materials for the synthesis of drugs or as models for pharmacologically active compounds. A considerable number of modern pharmaceutical drugs are derived from medicinal plants. The derivatives of medicinal plants are non-narcotic with little or no side effects.

- **FUTURE PROSPECTS OF HERBAL MEDICINE MARKET**

Almost three fourths of the herbal drugs used worldwide were discovered following leads from local medicine. According to World Health Organization (WHO), about 25% of modern medicines are descended from traditionally used plants. Several others were synthetic analogues built on prototype compounds isolated from plants. Almost, 70% of current medicines in India are derived from natural products. The essential uses of plants in medicine will continue in the future, as a source of therapeutic agents, and as raw material base for the extraction of semi-synthetic chemical compounds such as cosmetics, perfumes and food industries. Reputation of healthcare plant-derived products has been traced to their increasing acceptance and use in the cosmetic industry as well as to increasing public costs in the daily maintenance of personal health and well being. In the twin role as a source of healthcare and income, medicinal plants make an important contribution to the larger development process. Though the effectiveness of herbal requires development of quality perception in respect of the evaluation related evidences,

supplying the demand for botanicals and herbals is a booming business. In recent times even developed countries, have implemented the use of herbal drugs and remedies which unquestionably increased the demand for plant derived products worldwide. This means that scientists, doctors and pharmaceutical companies will be looking at countries like China, India, etc. for their necessities, as they have the most number of medicinal plant species and are the top exporters of medicinal plants.

- **FUTURE INVESTIGATION OF TRIBAL MEDICINES**

The tribal healers in most of the countries, where ethno-medical healing is frequently used to treat cut wounds, skin infection, swelling, aging, mental illness, cancer, asthma, diabetes, jaundice, scabies, eczema, venereal diseases, snakebite and gastric ulcer, provide instructions to local people as how to prepare medicine from herbal. They keep no report and the information is mainly passed on vocally from generation to generation. World Health Organization (WHO) has shown great attention in documenting the use of medicinal plants used by tribals from different parts of the world. Various developing countries have intensified their efforts in documenting the ethno-medical data on medicinal plants. Investigation to find out scientific evidence for claims by tribal healers on Indian herbs has been intensified. Once these local ethno-medical preparations are systematically evaluated and disseminated properly, people will be better informed regarding efficacious drug treatment and improved health status.

- **ALLOPATHIC SYSTEM OF MEDICINE**

Allopathic system refers to the practice of usual medicine that uses physiologically active agents or physical interventions to treat or contain the symptoms or patho-physiologic processes of diseases or conditions. It was coined by Samuel Hahnemann (1755–1843) a homeopath, in 1810. Allopathic medicine often refers to "the broad category of medical practice that is so called Western medicine, bio-medicine, evidence-based medicine, or modern medicine"**.**

- **TRADITIONAL SYSTEM OF MEDICINE**

For the human beings disease threatens not only the well being of victims and their fellows, but also the integrity of the society. Illness and death are troublesome events that impose high economic, social and psychological costs wherever they occur. For that reason it is of

primary significance to the members of every group to try to maintain their health and to restore to health those who fall ill.

All human society has responded to this challenge by developing a medical system that can be used to restore health. A system of medicine or medical organization can be defined as the pattern of social institution and cultural traditions that evolved from purposeful behavior to enhance health. Numerous traditional medical systems exist along with present-day scientific medicine system. The available accounts of world's medical system make the subject matter of ethno-medicine which is concerned with the beliefs and practices connecting to disease that are products of aboriginal cultural developments and are not derived from conceptual framework of modern medicine.

- **AYURVEDA INDIAN SYSTEM OF MEDICINE**

The Ayurvedic system originated in India long back in pre-vedic era from *Vedas*, which is the most ancient manuscript and gives more information on the health and diseases than any other documented information. Ayurveda born out of instinct and revelation, developed in due course into a complete system of medicine. The term Ayurveda means '*Science of Life'*. The basic theories of Ayurveda arise from the conception of *Tridosha* that embraces the process of creation and evolution of Universe and laws of life. The function of the body is considered to be the harmonizing work of body, sense organs, mind and soul. These are classified into *Vata, Pitta* and *Kapha***.** These correspond principally to elements of air, fire and water.

The body consist of seven *Dhatus* or tissues . These are: *Rasa*- body fluids, *Rakta*-blood, *Mansa*-muscular tissue, *Meda*- adipose tissue, *Asthi*- bone tissue, *Majja*- nerve tissue and bone marrow, and *Shukra*- generative tissue. There are also waste products (*Malas*). There are many *malas* in the body- stool, urine, sweat, nails, hair etc. Health depends on balanced state of all *dhatus*, *doshas* and *malas* both in quantity and quality. According to Ayurveda all objects in the universe including human body are composed of five basic elements called *Panchamahabhutas* namely, earth, water, fire, air and sky.

- **UNANI SYSTEM OF MEDICINE**

The Unani system of medicine may be traced to that system of Greek medicine that was developed during the Arab civilization. It was the Greek philosopher-physician Hippocrates on

whose teachings the theoretical structure of medicine is based. After him a number of other Greek scholars enriched the system noticeably. Of them Galen stands out as the one who stabilized its underpinning, on which Arab physicians like Razes and Avicenna constructed the imposing edifice. It is now practiced in the Indo-Pakistan subcontinent after being introduced by Arabs. The basic viewpoint of *Tibb* is that the body is composed of matter and spirit. Human body is taken as a entirety because pleasant-sounding life is possible only if there is a proper equilibrium between bodily and spiritual functions.

The fundamental agenda consists of theory of Hippocrates, which presupposes the presence of four humours in the body namely blood, phlegm, yellow bile and black bile. According to prehistoric percepts of Unani theory, there are four primary elements namely *Nar* or fire, *Hawah* or air, *Ma* or water and *Arz* or earth. This is a generally accepted theory among all schools of thoughts of Unani medicine. Air stands for gaseous state, water for liquid state, earth for solid state while fire stands for matters that has been transformed into heat. The properties of these four elements are: *Nar*- hot and dry, *Hawah*-hot and wet, *Ma*-cold and wet and *Arz*-cold and dry.

The prehistoric Unani scholars based their study on the hypothesis of humours which combined both physiology and pathology. According to them there are three types of matter in the human body namely solid, liquid and gas. Solid parts are known as organs or *A'da*, liquid parts are known as humours or *Akhlat* and the gaseous parts are known as pneuma or *Ruh*. The body fluids, which are humours, are further sub-divided into four types: *Dam* (Blood), *Balgham* (Phlegm), *Safra* (Yellow bile) and *Sauda* (Black bile). On the basis of different constitution, people can be categorized under four basic temperaments. The four are *damwi*, bilious, phlegmatic and melancholic temperament.

- **HOMEOPATHIC SYSTEM OF MEDICINE**

This system of medicine is a authority whose primary emphasis is on therapeutics. It is a low cost system employing entirely non toxic drugs. This system derives its name from two Greek words *Homoios* (like) and *Pathos* (treatment). Homoeopathic system of medicine was started by the chemist, physician and pharmacist Samuel Hahnemann of Germany. He promoted the law of similar which says that like cures like.

Homoeopathy is the system of healing based on scientific methods and demonstrable laws and principles, which are –Law of Similars, Law of Direction of Cure, Law of Single Remedy, Law of Minimum Dose. Homoeopathic medicines are used in the form of mother tinctures, small pills, powders and distilled water.

- **NATUROPATHY AND YOGA**

Until the recent past, Yoga was considered very exotic and secret, being a forte of the hermits and saints who trained it in aloofness to attain spiritual clarification. The word "Yoga " is derived from the root "Yujir Yoge ", which means "to unite" or "to bind" or "to yoke". According to Yajnavalkya, Yoga means "the Union" i.e. combination of the individual spirit (Jivatman) with the universal spirit (Parmatman). The word "Yoga" is derived from the root "Yuj Samadhau" meaning spiritual incorporation. According to Bhagwad Gita, the word Yoga means "Equanimity of Mind", which can only be acquired after getting recognized in discriminative wisdom (which is a consequent of strong meditation). While according to Maharishi Patanjali, Yoga is defined as the "cessation of modifications of Chitta, which results into individual soul enduring in itself and thereby attaining God Realization and Spiritual perfection." The system of Yoga is more than 5000 years old and Gita has rightly described it as ancient (Puratan) and eternal (Sanatan).

- **HERBAL MEDICINE**

Herbal remedy, or phytotherapy , is the science of using herbal drus to treat the sick. The term was introduced by the French physician Henri Leclerc (1870-1955). He had published several essays on the use of medicinal plants, most of them in *La Presse Medicale*, a leading French medical journal. These essays were exceptional for their style, and splendid examples of the art of presenting a subject. He summed up his life-time knowledge in *Pr&s de Phytothkrapie***,** a brief work that has since become a classic. Herbal medicine has move toward a long way since the days of the ancient 'herbalism'. The study of the use of medicinal plants is now a scientific subject, a branch of medicine in the same way as chemotherapy, hydrotherapy, electrotherapy and others. Information of medicinal plants and their uses has been recorded from antiquity - by Imhotep, the priest-physician of ancient Egypt who devised the Step Pyramid of Sakkara, by Galen, personal physician to the Roman emperor Marcus Aurelius, and later by Paracelsus, the

Abbess Hildegard of Bingen, and the authors of the great herbals of medieval times, exact to the present day.[45,46]

DIABETES MELLITUS

Pancreas has both endocrine and exocrine functions. The exocrine functions are largely the digestive enzymes. Speckled among the exocrine portion of the pancreas are millions of tiny clusters of endocrine tissue called pancreatic islets or islets of Langerhans, which contains four types of cells secreting different hormones.

- **Alpha cells:**

Constitutes about 20% and secrete glucagon, which raises blood glucose level.

- **Beta cells:**

Constitutes about 75% and insulin which lowers blood glucose level.

- **Delta cells:**

Constitutes about 3% and secrete growth hormone release inhibiting hormone or somatostatin.

- **Pp cells:**

Constitutes about 2% and secrete pancreatic polypeptide which regulates the release of pancreatic digestive enzymes.

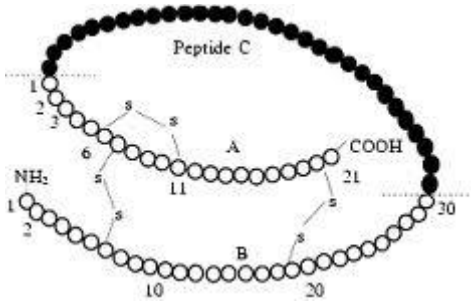


Fig 1: Structure of Insulin

It is composed of two chains (chain-A of 21 and chain-B of 30 amino acids) that are joined mutually by disulfide cross-bridges.

- **MECHANISM OF ACTION**

The insulin receptor in mammalian cells is a huge trans-membrane glycoprotein. It is composed of two α-subunits and two β-subunits linked by disulfide bonds to comprise a β-α-α-β heteroteramer. β-subunits contain tyrosine residues. When insulin binds to the α- subunit at the exterior of the cell surface, the tyrosine kinase activity, in β subunits is stimulated. Insulin in addition stimulates the glucose transport across cell membrane by ATP-dependent translocation of glucose transporter-4(GlUT_4) to the plasma membrane, an event that is vital for glucose uptake by skeletal muscle and fat.

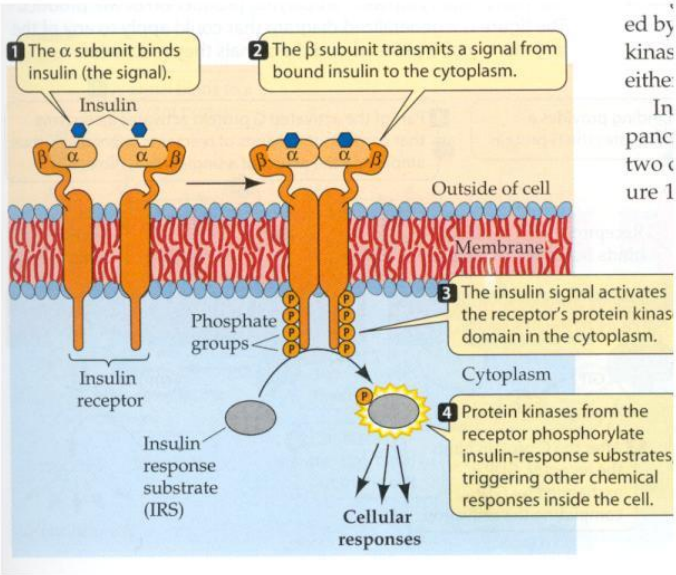


Fig 2: Mechanism of Insulin

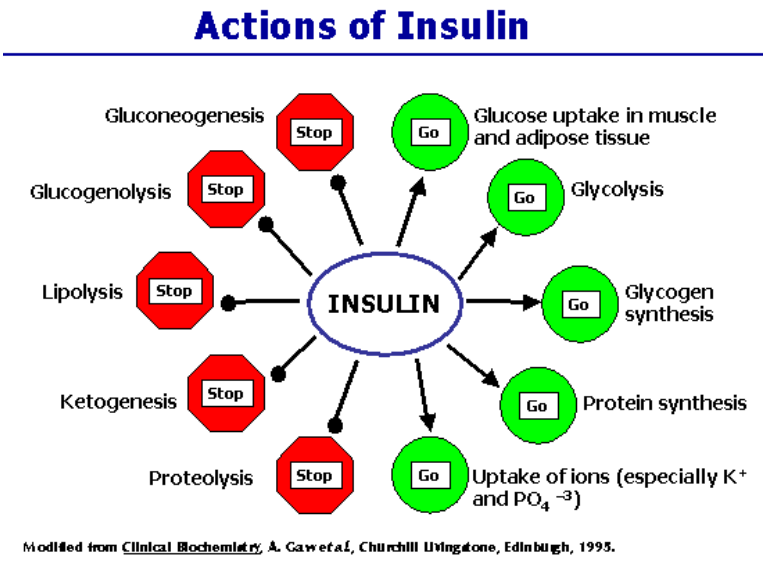


Fig 3: Actions of Insulin

The word diabetes mellitus describes a metabolic disorder of numerous etiology characterized by chronic hyperglycemia with instability of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The word "diabetes" stems from a Greek term for passing through, a location to increased urination (polyuria), a common symptom of the disease. "Mellitus" is the Latin word for honeyed, a indication to glucose noted in the urine of diabetic patients. Diabetes mellitus is sometimes referred to as sugar diabetes but as a rule is basically called diabetes. There is also a rare disease called diabetes insipidus (water diabetes) in which the kidneys release excessive water. Similar to diabetes mellitus, it has excessive urination as a symptom. The effects of diabetes mellitus include long–term damage, dysfunction and failure of various organs. Diabetes mellitus may present with typical symptoms such as thirst, polyuria, polydypsia, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non–ketotic hyperosmolar state may develop and direct to stupor, coma and, in absence of effective treatment, death. Often symptoms are not relentless, or may be absent, and consequently hyperglycemia sufficient to cause pathological and functional changes may be present for a extended time before the diagnosis is made. The lengthy–episode of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, Charcot joints, and features of autonomic

dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease.

- **ROOT CAUSES OF DIABETES MELLITUS (DM)**

The origin of diabetes is complex. Most cases begin with one of two processes:

- **METABOLIC**

Detrimental lifestyle factors such as overeating, physical inactivity and obesity can weaken the body's ability to use insulin. This is called insulin resistance. Unmanageable risk factors including genetics, family history and age can also be involved. Metabolic forms of diabetes include:

1. Type 2 diabetes

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

2. Gestational diabetes

Changes in hormone level contribute to this condition which can develop in any previously nondiabetic woman during pregnancy, especially those who are overweight.

- **FACTORS CONTRIBUTING DIABETES MELLITUS**

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

- **Vascular disease (diabetic angiopathy), atherosclerosis, heart conditions and stroke**

The cardiovascular disorders are the leading reason of death in people with diabetes.

- **Kidney disease (diabetic nephropathy)**

Diabetes is the chief cause of end-stage renal disease, which requires treatment with dialysis or a kidney transplant.

- **Eye diseases**

These comprise diabetic retinopathy, glaucoma and cataracts. Diabetes is a foremost cause of visual impairment and blindness.

- **Nerve damage (diabetic neuropathy)**

Includes peripheral neuropathy, which often causes pain or numbness in the limbs, and autonomic neuropathy, which can hinder digestion (gastroparesis) and contribute to sexual dysfunction and incontinence. Neuropathy may also damage hearing and other senses.

- **Impaired thinking**

Many studies have related diabetes to increased risk of memory loss, dementia, Alzheimer's disease and other cognitive deficits. Recently some researchers have suggested that Alzheimer's disease might be type 3 diabetes, involving insulin resistance in the brain.

- **Infections and wounds**

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

- **Cancer**

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

- **Musculoskeletal disorders**

Several conditions ranging from gout to osteoporosis to restless legs syndrome to myofascial pain syndrome are more common in diabetic patients than nondiabetics.

- **Pregnancy complications**

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

- **Emotional difficulties**

Several but not all of the studies exploring connections between diabetes and mental illness have found increased rates of depression, anxiety and other psychological disorders in diabetic patients. In addition to chronic hyperglycemia, diabetic patients can experience acute episodes of hyperglycemia (High glucose) as well as hypoglycemia (low glucose). Severe cases can cause seizures, brain damage and a potentially fatal diabetic coma. Acute glucose emergencies include:

- **Insulin shock:**

This complex stage of hypoglycemia is usually due to excessive amounts of insulin medication or certain antidiabetic agents.

- **Diabetic ketoacidosis:**

A lack of insulin can compel the body to burn fats instead of glucose for energy. The outcome is a toxic byproduct called ketones, along with severe hyperglycemia.

- **Hyperosmolar hyperglycemic non-ketotic state**

This involves severe hyperglycemia and dehydration.

- **TYPES AND DIFFERENCES OF DIABETES**

There are quite a lot of forms of diabetes.

- **Type 1 diabetes:**

Type-1 diabetes is an autoimmune disease in which the immune system erroneously destroys the insulin-making beta cells of the pancreas. It typically develops more quickly than other forms of diabetes. It is typically diagnosed in children and adolescents, and sometimes in young adults. Type 1 diabetes is also called as juvenile onset diabetes and insulin-dependent diabetes mellitus (IDDM).

A variant of type 1 that develops later in life, usually after age 30, called as latent autoimmune diabetes of adulthood (LADA). Sometimes patients with autoimmune diabetes develop insulin resistance because of weight gain or genetic factors. This condition is identified as double diabetes.

- **Type 2 diabetes:**

In the patients with type-2 diabetes , the pancreas makes insulin initially, but the body has difficulty using this glucose-controlling hormone. Ultimately the pancreas cannot produce enough insulin to react to the body's need for it. It accounts for 85 to 95% of the cases. Type 2 diabetes used to be called adult-onset diabetes and non-insulin-dependent diabetes mellitus (NIDDM).

- **Gestational diabetes:**

A temporary metabolic disorder that any formerly non-diabetic woman can develop during pregnancy, usually the third trimester. Hormonal changes contribute to this disease, beside with excess weight and family history of diabetes. About 4% of pregnant women develop

gestational diabetes, according to the American Diabetes Association. Gestational diabetes can cause harms for the mother and baby, together with preeclampsia, premature deli-very, macrosomia (oversized infant), and jaundice and breathing difficulties in the infant. This disease typically ends when the pregnancy does, but it increases the danger of type 2 diabetes later in life for the mother and the child.

- **Secondary diabetes:**

Diabetes caused through another condition. The many possible sources of secondary diabetes range from diseases such as pancreatitis, cystic fibrosis, Down syndrome and hemochromatosis to medical treatments including corticosteroids, other immune suppressives, diuretics and pancreatectomy.

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

There are also rare syndromes (clusters of conditions) that include diabetes, notably:

- **Wolfram syndrome**

Genetic disorder that involves insulin-dependent diabetes, vision problems, deafness and diabetes insipidus.

- **Autoimmune polyglandular syndrome (APS)**

Are group of autoimmune endocrine diseases. Two of the three forms of APS feature type 1 diabetes. Unsteady diabetes, also known as brittle or labile diabetes, is a term that may be used to describe any case of poorly controlled diabetes despite of the type. All of these conditions involve diabetes mellitus ("sugar diabetes"). Diabetes insipidus ("water diabetes") is an unrelated endocrine system disorder in which the kidneys release a large amount water.

- **RISK FACTORS AND CAUSES OF DIABETES**

The causes of diabetes are complex and only partially understood. This disease is generally well thought-out as multi-factorial, connecting to several predisposing conditions and risk factors. In many cases genetics, habits and environment may all contribute to a person's diabetes. Other diabetic risk factors and causes include:

- **Genetics and family history**

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

- **Family medical history is also significant to varying degrees**

For instance, a person whose parents both have type 1 diabetes has a 10 to 25% chance of developing that disease, according to the American Diabetes Association, and someone whose parents both have type 2 diabetes has a 50% chance of developing that disease.

- **Weight and body type**

Overweight and obesity are foremost factors in type 2 diabetes and gestational diabetes. Excess fat, especially around the stomach (central obesity), promotes insulin resistance and metabolic syndrome.

- **Sex**

Low testosterone levels (male hypogonadism), which scientists have associated with insulin resistance.

- **Height of physical activity**

Lack of standard exercise is responsible for much of the twin global epidemics of obesity and diabetes.

- **Diet**

The outcome of diet in the development of diabetes is controversial. Some studies have related heavy consumption of soft drinks and other simple carbohydrates to risk of metabolic diabetes, and foods with little glycemic index, such as whole grains, to reduced risk.

- **Other diseases**

Medical situations including high blood pressure, hyperlipidemia (unhealthy levels of cholesterol), polycystic ovarian syndrome, asthma and sleep apnea have been linked to type 2 diabetes . Celiac disease (gluten intolerance) and other autoimmune diseases have been linked to type 1. Some conditions that may cause secondary diabetes include pancreatitis, hemochromatosis, endocrine disorders including hyperthyroiddism, Cushing's disease and acromegaly, and genetic conditions including cystic fibrosis, Down syndrome and some forms of muscular dystrophy, Diabetic foot and urinary tract infection.

- **Hormones**

Hormones are the chemical messengers that can contribute to diabetes in various ways. For example, stress hormones such as cortisol have been associated with fluctuating glucose levels in type 2 diabetes, and stress hormones in women during pregnancy have been linked to risk of type 1 diabetes in the child. The release of growth and sex hormones during adolescence may make some teens more prone to diabetes. A broad range of hormonal treatments including anabolic steroids, growth hormone, estrogens, injected contraceptives, androgen deprivation therapy for prostate cancer and corticosteroids have been related with secondary diabetes.

- **Medical treatments**

In addition to hormonal therapies, drugs including diuretics, beta blockers (another class of antihypertensives), immunosuppressives, antiretrovirals (AIDS/HIV drugs) antipsychotics, lithium, and some antidepressants, anticonvulsants and chemotherapy drugs have been related to an increased threat of secondary diabetes. Pancreatectomy and radiation therapy may also result in secondary diabetes. Drugs including pentamidine (used to treat pneumonia) and L–asparaginase k(used to treat leukemia) have been linked to type 1 diabetes.

- **Other chemicals**

In addition to the pharmaceuticals, some studies have associated PCBs, other pollutants and certain pesticides including the defoliant Agent Orange and Dioxin (its active ingredient) to insulin resistance and type 2 diabetes. General consumer plastics and plastics ingredients including Phthalates and Bisphenol A have also been linked to insulin resistance in some cases. Exposure to agricultural pesticides during pregnancy has been tentatively connected to gestational diabetes. A rat poison called pyriminal has been linked to type 1 diabetes.

- **Other environmental factors**

Researchers hypothesize that free radicals may add to the development of type 1 and possibly other forms of diabetes. Free radicals are produced as a result of chemical reactions in the body. Smoke, air pollution and even genetics contribute to the formation of free radicals. Once these radicals build up, they can destroy cells, plus those concerned in the production of insulin. Cold weather is another likely environmental factor in type 1 diabetes. This disease occurs most commonly in cold climates and develops more repeatedly in the winter than the summer.

- **Viruses**

Some people are diagnosed with type 1 diabetes following a viral infection. Viruses thought to be related to type 1 diabetes consist of mumps, rubella and coxsackie virus (related to the virus family that causes polio and hepatitis).

- **Smoking**

Cigarette smoking is a risk factor for type 2 diabetes and possibly other forms of diabetes.

- **Alcohol**

Too much use of alcohol is a risk factor for diabetes. For instance, it can cause pancreatitis. But, some research has establish that light drinking may decrease the risk of becoming diabetic.

- **Signs and symptoms of diabetes**

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

Type 1 diabetes can build up rapidly and often occurs after a disease, but symptoms may be mistaken for the flu or other general conditions. Type 2 diabetes can take several years to develop and sometimes becomes obvious only after long-term complications transpire, such as sexual dysfunction or leg pain that is due to diabetic neuropathy or claudication (caused by peripheral artery disease).

Some people, particularly young people with type 1 diabetes, go undiagnosed until they are brought to a hospital with a crisis called diabetic ketoacidosis . Indicators of diabetic

ketoacidosis comprise sweet fruity-smelling or wine-smelling breath, confusion and heavy labored breathing (Kussmaul breathing). Sometimes patients are diagnosed with diabetes only after suffering other severe complications including insulin shock, hyperosmolar hyperglycemic non-ketotic syndrome or diabetic coma. To help prevent such complications, people are advised to undergo intermittent screening for diabetes with glucose tests, particularly if they have risk factors.

DIAGNOSIS METHODS FOR DIABETES:

Physicians employ glucose tests to diagnose diabetes. These blood tests determine the level of glucose (blood sugar) in a person's bloodstream. Often when people have a physical assessment they are screened for diabetes with a fasting plasma glucose test (FPG). An FPG is regularly performed in the morning since this makes it easier for the patient to fast for the required eight hours. Glucose is measured in milligrams per deciliter (mg/dl) of blood. FPG results below 100 mg/dl are normal. Glucose between 100 and 125 mg/dl is considered pre-diabetes. Glucose above 125 mg/dl indicates diabetes. To prove diagnosis, another glucose test must be performed on another day, according to the National Institute of Diabetes and Digestive and Kidney Disorders. If glucose testing determines that a patient has diabetes, further tests may be offered to ascertain the type.

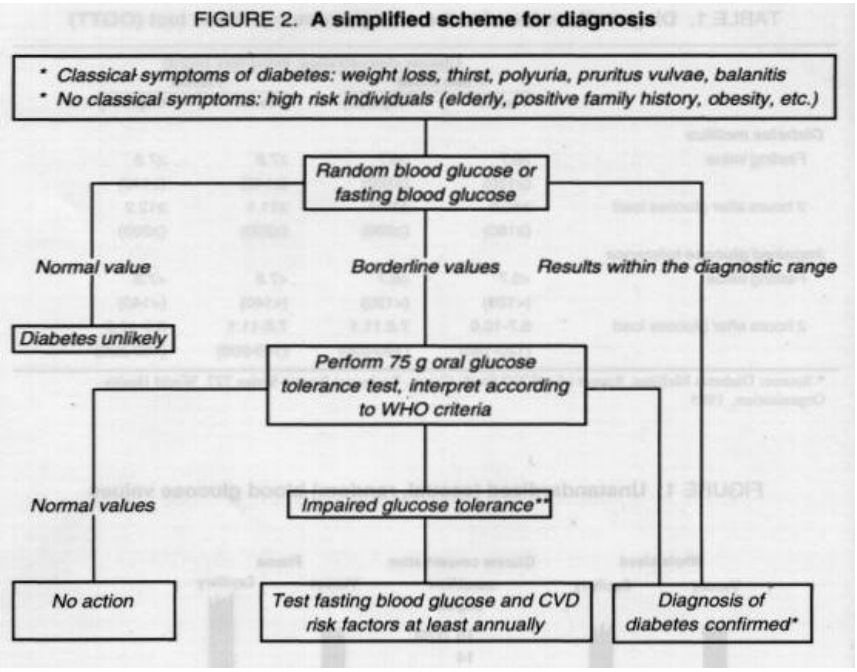


Fig 4: Schematic diagram for diagnosis

TREATMENT OPTIONS FOR DIABETES:

The various forms of insulin administration include syringe injections, insulin pumps, insulin pens, jet injectors and inhaled insulin. Oral diabetes medications include:

i) Alpha-glucosidase inhibitors

ii) Biguanides

iii) Meglitinides

iv) Sulfonylureas

v) Thiazolidinediones

vi) A new class called DPP-4 inhibitors

Other important aspects of treating and monitoring diabetes include:

i) Nutrition counseling

ii) Every day foot care and skin care

iii) Normal physical examinations and foot examinations

iv) Usual blood pressure readings and cholesterol tests

v) A pneumonia vaccination and yearly flu shots.

vi) A sick-day plan devised by a physician.

vii) Supplementary medical care before, during and after pregnancy.

viii) Cardiac and neurological testing as desired.

Following the care plan can help avoid overwhelming diabetic complications such as leg amputation, blindness, heart attack, stroke and chronic kidney failure.

PREVENTION METHODS FOR DIABETES

There is no recognized way of preventing autoimmune forms of diabetes (type 1 and latent autoimmune diabetes of middle age) or genetic conditions such as maturity-onset diabetes of the young and Wolfram disease. Genetic tests can tell who is in danger, and clinical trials are investigating probable methods of preventing type 1, including vaccines and pre-emptive use of insulin. People can take numerous steps to decrease their risk of developing metabolic forms of diabetes (type 2 and gestational diabetes). The principal focus is on managing weight during regular exercise and a reasonable diet. Such practices also help out people with other forms of diabetes circumvent insulin resistance and double diabetes. It is also advantageous to control blood pressure and cholesterol, avoid smoking and restrict alcohol. People with risk factors for

diabetes must be screened regularly with glucose tests. Early detection and treatment can ward off many diabetic complications.

ORAL HYPOGLYCAEMIC AGENTS:

Are the used to treat type-2 diabetes and they have the prospective to decrease blood glucose to subnormal levels and also help insulin release from pancreatic β-cells. There are two classes of oral hypoglycaemic agents: sulfonylureas and meglitinides.

1. **Sulfonylureas:**

1.1.First generation: Tolbutamide, chlorpropamide

1.2.Second generation: Glibenclamide, Glipizide, Gliclazide, Glimepiride

The Sulfonylureas largely exert their action on β-cells, stimulating insulin secretion and thus dropping blood glucose levels. These drugs diminish the permeability of k^+ by competitively blocking the sulfonylurea receptors present on ATP-sensitive k^+ channels, causing depolarization, ca^{2+} entry and insulin secretion.

2. **Biguanides**: Metformin:

Their major action is to inhibit the neoglucogenesis and decrease the hepatic glucose output. They in addition increase the peripheral glucose utilization by improving anerobic glycolysis. They raise the sensitivity of peripheral tissues to insulin.

3. **Meglitinide Analogues:** Repaglinide, Nateglinide.

They act by subsequent mechanisms:1, Improved uptake and utilization of glucose by skeletal muscles, which reduces insulin resistance 2, Inhibition of hepatic as well as renal gluconeogenesis 3, Slows down glucose absorption from GIT which increases availability of glucose for its alteration to lactate by enterocytes 4, Promotion of insulin binding to its receptors and reduction in plasma glucagon levels.

4. **α-Glucosidase inhibitors:** acarbose, miglitol

These are competitive inhibitors of intestinal α-glucosidases. Acarbose also inhibits α-amylase. Thus, these drugs diminish post-prandial digestion and absorption of carbohydrates and thus lower postmeal hyperglycaemia.

5. **Thiazolidinediones(Glitazones):** rosiglitazone, pioglitazone.

The Glitazones work as agonist to a nuclear receptor called Peroxisome Proliferator-Activated Receptor-gamma (PPAR-γ), which makes a complex with retinoid-X Receptor (RXR). This activation mediate differentiation of adipocytes, increases lipogenesis and promotes uptake of fatty acids and glucose.[5, 7 ,53]

REVIEW OF LITERATURE

LITERATURE REVIEW

1. **Guang-Zhi Zeng *et al.,*** *(*2009). Apoptosis inducement of bigelovin from extracts of *Inula helianthus-aquatica* on human leukemia U937 cells. Phytotherapy Research, volume 23,issue 6, pages 885–891, June 2009. Anti-leukemic activity of *Inula helianthus-aquatica* is due to the presence of a sesquiterpene lactone called Bigelovine. Bigelovin, a sesquiterpene lactone isolated from *Inula helianthus aquatica* potentially inhibits the growth of a panel of eight cancer cell lines, especially in human monoblastic leukemia U937 cells with an IC(50) value of 0.47 microM.

2. **Fenglin Li *et al.,*** *(2009).* Preparation and antidiabetic activity of polysaccaharide from *Portulaca oleracea L*. African J Biotech, volume 8, issue 4, pages 569-573, Feb 2009. The study was conducted to find out the extraction parameters of polysaccharide from *Portulaca oleracea L.* (POP) and the antidiabetic activity of POP on alloxan induced diabetic mice using single factor test. The study revealed that the polysaccharide extracted from *Portulaca oleracea L*. can control blood glucose and modulate the metabolism of glucose and blood lipid in diabetes mellitus mice.

3. **Hee Yun *et al.*,** *(2009).* Insulin increase glucose transport in C2C12 Myotubes and HepG2 cells via activation of AMP-Activated Protein Kinase and Phosphatidylinositol 3-Kinase Pathways. J Med Food, volume 12, issue 5, pages 1023-1028, Oct 2009. The study was performed to associate the potential health benefits of inulin via AMPK and PI3-K activation. Inulin increased the uptake of glucose in C2C12 myotubes, both through AMP kinase and Phosphatidylinositol kinase signalling pathways. The results concluded the anti-diabetic activity of inulin through AMPK and PI3-K activation.

4. **Han Hong Jin *et al.,*** *(2009)* New Cytotoxic thymol derivatives from *Inula helianthus aquatica* (Compositae family). Acta Botanica Yunnanica, volume 31, issue 2, pages 190-192 (2009). The study was conducted to show the cytotoxicity on cancer cell lines. The results indicated that four thymol derivatives were produced from 95% ethanolic extract of *Inula helianthus* flowers that produced cytotoxicity.

5. **Huang Huo Qiang *et al.,*** *(2007)* Seven sesquiterpene lactones from flower of *Inula helianthus aquatica*. Chinese J Experimental Traditional Medical Formulae, volume 83, issue

6, pages 2011-2017, 2007. The study was elucidated to investigate the chemical constituents of *Inula helianthus aquatica* flower using various chromatographic techniques subjected to spectral analysis. The results indicated that aromaticin, 8-epihelenalin, bigelovin, 6-epi-desacetylisotenulin, carpesiolin, 2,3-dihydroaromaticin and ergolide were produced for the first time from the say plant.

6. **Zeggwagh NA *et al.*, *(2006).*** Hypoglycaemic and hypolipidemic effects of *Inulia viscosa* L.aqueous exracts in normal and diabetic rats .J Ethnopharmacol, volume108,issue 2, pages 223-227 May 2006.The study was designed to examine the hypoglycaemic and hypolipidemic activity of *Inula viscosa* aqueous extract on normal and diabetic rats. In normal rats, a significant reduction in blood glucose levels 2 h was observed after a single oral administration ($p<0.001$). Repeated daily oral administration significantly reduced blood glucose levels after 4 days of treatment ($p<0.01$). In diabetic rats, a significant reduction in blood glucose levels was observed 1 h after a single oral administration ($p<0.001$). Repeated oral administration reduced blood glucose levels at the 4th day ($p<0.001$). No change in total plasma cholesterol and triglyceride levels was observed after both a single and repeated oral administration in both normal and diabetic rats. We conclude that *Inula viscosa* possess a hypoglycaemic but not hypolipidemic activity in normal and diabetic rats. The observed hypoglycaemic activity seems to be independent of insulin secretion.

7. **Shan JJ *et al.*, *(2006).*** Effect of an antidiabetic polysaccharide from *Inula japonica* on constipation in normal and two models of experimental constipated mice. After administration of IJP, the gastrointestinal propulsive rate was increased by 9.79% and 10.42%, the start time of defecation was shortened by 37.27% and 44.06%, the number of faeces increased by 115.4% and 130.8% in normal mice. In fasting-water constipated mice, the start time of defecation was shortened by 9.69% and 30.52% by IJP, defecation granules raised by 22.09% and 39.53%, wet faeces weights were increased by 23.50% and 39.14% compared with the untreated constipated mice. In diphenoxylate-induced mice, the start time of defecation was shortened by 25.48% and 28.13%, defecation granules raised by 100.0% and 118.0%.

8. **Mahmood Mosaddegh *et al.,*** *(2006).* Cytotoxic effects of five species of Inula against some tumor cell lines. Iranian J Pharm Sciences, volume 2, issue 4, pages 203-208, August 2006. Fractions of five Inula extracts belonging to compositae family were evaluated using MTT assay for cytotoxicity against various cell lines including CACO 2 (human colon adenocarcinoma), MCF 7 (human breast adenocarcinoma), HEPG2 (human hepatocellular carcinoma), VERO (green African monkey kidney), and WEHI 164 (balb C mouse fibrosarcoma). The results proved that the species, *Inula oculus christi* showed better cytotoxicity.

9. **Jun-Jie Shan *et al.,*** *(2005)* Anti-diabetic and hypolipidemic effects of aqueous extract from the flower of *Inula japonica* in Alloxan-Induced Diabetic Mice. The antidiabetic and hypolipidemic effects of aqueous-extract from the flower of *Inula japonica* and its two fractions (IJR and IJP) were investigated in alloxan-induced diabetic mice. An oral glucose tolerance test (OGTT) of IJ was also performed in normal and diabetic mice. The results showed that IJ (1000 mg/kg), IJR (500 mg/kg) and IJP (250 mg/kg) significantly reduced blood glucose levels in diabetic mice by oral administration (*p*_0.01). IJ and IJP markedly decreased serum triglyceride concentrations (*p*_0.05) in diabetic mice. Their hypoglycemic activities were better than gliclazide (40 mg/kg) and compared with metformin (250 mg/kg). IJ raised plasma insulin levels in alloxan-induced diabetic mice.

10. **Gholap S *et al.,*** *(2004) Inula racemosa, Boerhaavia diffusa* and *Ocimum sanctum* (Holy Basil) exhibited anti-peroxidative , hypoglycemic and cortisol lowering activities- Green Medinfo Summary. Pharmazie, volume 59, issue 11, pages 876-878 (2004). The study performed proved that the hypoglycaemic effects of certain plant extracts are mediated through inhibition in corticosteroid concentration.

11. **Liqun Rao *et al.,*** *(2011)* Optimization of the technology of extracting water soluble polysaccharides from *Morus alba L.* leaves. African Journal of Biotechnology, volume 10, issue 59, pages 12714-20, October 2011. The study was carried out to optimize the parameters for water soluble polysaccharides from mulberry leaves using hot water extraction by orthogonal test through single factor experiment. The results indicated the following extraction conditions such as material concentration equivalent to 1:24, an extraction

temperature of 70° C, an duration of 90 minutes and a concentration of ethanol equivalent to 80% which gave a maximum polysaccharide yield.

12. **Nathalie M Delzenne *et al.*, *(2007)*** Modulation of Glucagon like Peptide 1 and Energy metabolism by Inulin and Oligofructose Experimental data. Journal of Nutrition, volume 137, issue 11, pages 2547-51, 2007. The present work depicted the modulation of oligo fructose in animal models. The results proved that glucagon like peptide 1 can exert antidiabetic properties. It could also modulate other gastro intestinal peptides involved in the control of food intake.

13. **Jia xian Zhu *et al.*, *(February 2012)*** Chemical Constituents from the Aerial parts of *Inula japonica Thunb*. Natural Product Research and development, volume 23, issue 6, pages 999-1001, 2012. This was carried out to demonstrate the isolation and purification of ten compounds from *Inula japonica* for the first time using column chromatography. The compounds elucidated were ayapin, 7-hydroxycoumarin, daphnetin, citrusin, wedelolactone, scopoletin, medioresinol, syringic acid, vanillic acid and isovanillic acid.

14. **Yang Zhenmin *et al.*, *(2011)*** Isolation and quantitative determination of inulin type oligosaccharide in roots of *Morinda officinalis.* Carbohydrate Polymers: Elsevier, volume 8, issue 4, pages 1997-2004, 2011. Based on size exclusion chromatography, inulin type oligosaccharides with different degrees of polymerization were isolated followed by determination of purity using HPLC- ELSD. The study was performed to analyse monosaccharide and oligosaccharide in three types of roots of M. officinalis and provides a new basis of assessment on quality of M. officinalis.

15. **Hokputsa Sanya *et al.*, *(2004)*** Water soluble polysaccharides with pharmaceutical importance from *Durio zibethinus*: isolation, fractionation, characterization and bioactivity. Carbohydtrate Polymers: Elsevier, volume 56, issue 4, pages 471-81, 2004. The activity was performed to study the relationship of chemical features of various polysaccharide fractions that had been isolated from *Durio zibethinus* by hot water extraction and alcohol precipitation. The physical features of fractionated segments were analysed by size exclusion chromatography coupled to multi angle laser light scattering.

16. **Sylvia Czapla *et al.*,** *(2000)* Transgenic potato (*Solanum tuberosum*) tubers synthesize the full spectrum of inulin molecules naturally occurring in globe artichoke (*Cynara scolymus*) roots. Proc.Natl Acad Sci, volume 97, issue 15, pages 8699-8704, 2000. The work was performed to show that the synthesis of inulin molecules of all chain lengths naturally occurring in a given plant species can be produced in planta by means of various enzymes.

17. **Gholap S *et al.,*** *(2003)* Effects of *Inula racemosa* root and *Gymnema sylvestre* leaf extracts in the regulation of corticosteroid induced diabetes mellitus: involvement of thyroid hormones. Pharmazie, volume 58, issue 6, pages 413-5, 2003. The above study evaluated the individual and combined efficacy of *Inula racemosa* root and *Gymnema sylvestre* leaf extract in corticosteroid induced hyperglycaemia. The results indicated that the combination was more effective than the individual extracts but no changes were seen in the thyroid hormone concentrations and therefore not effective for the same, but for steroid induced diabetes mellitus.

18. **Gaafar A M *et al.,*** *(2010)* Extraction conditions of Inulin from *Jerusalem Artichoke* tubers and its effects on Blood glucose and Lipid Profile in diabetic rats. Journal of American Science, volume 6, issue 5, pages 36-43, 2010. The analysis for the contents of tubers of *Jerusalem artichoke* was aimed and optimising the conventional extraction of inulin using various factors like time extract, temperature, solvent ratio. Accordingly the maximum yield was obtained by using a sample to solvent ratio of 1:5 w/v at 80° C for about 90 minutes. Also a high level of inulin 15% led to amore reduction of blood glucose level.

19. **Thomas Barclay *et al.,*** *(2010)* Inulin – a versatile polysaccharide with multiple pharmaceutical and food chemical uses. J Excipients and Food Chem, volume 1, issue 3, pages 27-50, 2010. The authors proclaim about inulin, a natural plant derived polysaccharide with intense range of food and pharmaceutical applications. It also shows about inulin's physicochemical properties that bestow it with many pharmaceutical applications.

20. **Greg Kelly *et al.,*.** *(2008)* Inulin type Prebiotics-A Review Part I. Alternative Medicine Review, volume 13, issue 4, pages 315-30, 2008. The article is a review part of inulin type

prebiotics which are a group of compounds joined together by the ability to promote the growth of specific beneficial gut bacteria.

21. **Holownia Piotr *et al.,*** *(2010)* The benefits and potential health hazards posted by the prebiotic Inulin- a Review. Pol J.Food Nutr. Sci, vol 60(3):201-11 (2010). The work demonstrated that inulin prebiotic is a non digestible carbohydrate normally occurring through out the normal human diet. The most beneficiary effects to human health are maintenance of microbial gut homeostasis, reduction in gut inflammation, prevention of colonic cancer, increase in mineral absorption, and decrease in cholesterol and bowel level improvements. The ultimate aim was to discuss the scientific evidence and to address the general concerns of consumers.

22. **Hariono Maywan *et al.,*** *(2009)* Extraction, identification and acetylation of inulin from *Dahlia tubers*. The 9th national Symposium on Polymeric materials: Pages 572-9, 2009. The study depicts the acetylation of inulin in polar aprotic organic solvents such as pyridine and dimethyl formamide. The results showed that the yield produced using pyridine presented 40% and this method was a preferable way to graft the inulin backbone with acetylation.

23. **Ll He *et al.,*** *(2012)* Research Progress on polysaccharides from Ginkgo biloba. Journal of Medicinal plants and Research, volume 6, issue 2, pages 171-6, 2012. The present activity was conducted to prove that *Ginkgo biloba* polysaccharides reduce blood sugar, immune regulation, anti oxidation, anti inflammatory effects. The extraction was carried out using ht water extraction and alcohol precipitation. The extracted methods were then purified using step by step precipitation, salting out and column chromatography.

24. **Narindoe Kaur *et al.,*** *(2002)* Applications of Inulin and oligo fructose in health and nutrition. J Biosci, volume 27, issue 7, pages 703-14, 2002. The work was extended to prove the extensive health promoting applications of inulin and oligo fructose in various lines, both pharmaceutically and biologically.

PLANT PROFILE

PLANT PROFILE

FIGURE 3.1

***INULA HELIANTHUS AQUATICA* PLANT**

3.1 PLANT PROFILE

SCIENTIFIC NAME: ***Inula helianthus aquatica***

SYNONYMS **:** ***Aquatic Sunflower***

FAMILY **:** ***Asteraceae***

COMMON NAME : 1) Chinese : Shui chao yang cao

2) Tamil : Kaattu sooryakanthi

3) Malayalam : Kaattu sooryakanthi

Inula is a large genus of about 90 species of flowering plants in the family Asteraceae, native to Europe, Asia and Africa.

They are mostly perennial herbs that vary greatly in size, from small species a few centi meters tall to enormous perennials over 3 meters tall. Some common characteristics include pappus with bristles, flat capitalum, and lack of chaff. Several species are popular flowers for the garden, with cultivation going back to antiquity. The name *Inula* was already used by the Romans and derived from Helen of Troy fame.

Inula helianthus aquatic is found in parts of south west of China , in India ita found in Kerala, Maharashtra, Gujarat, it is a wild plant, but it cultivated also as ornamental plant.

3.2 PARTS USED:

Flowers and Leaves

3.3 DESCRIPTION

Inula helianthus aquatica belonging to the Asteraceae family is characterised by many small flowers, arranged in a head looking like a single flower and subtended by an involucres of bracts .The head may consist of both ray flowers and disk like flowers as in sunflower.

It grows about2 meters long. it is a annual plants stem is leafy smooth and greenish and middle leaf densely, 2.5-4.5*2-3cm, with ovate base chordate and amplexicaul. Coloured leaves arranged into alternate phyllotaxy and reticulate venation. Margin coarsely serrate and apex rounded.

3.4 PROPERTIES AND MEDICINAL USES

Inula helianthus aquatica was traditionally used for different ailments in Chinese and in Ayurveda. The entire plant is used and is considered acrid, sweet, and warm. It expels wind-dampness, mend sinew and bone, stop bleeding, and resolve toxin. It is used for wind-dampness pain, broken bones, external injury with bleeding, and clove sores with toxic swelling. Seeds used for optic disorder and pitha and kapha treatment in ayurveda, it is denoted in the book of bavaprakeshnighudu. It possess anti-tumour, anti-diabetic, anti-inflammatory, anti- ulcer, anti-oxidant properties**.**[2,54]

SCOPE OF WORK

SCOPE OF WORK

The world has an approximate estimation of 300-350 millions of people with type II diabetes. Diabetes mellitus is becoming more prominent and wide spread due to the changes in life style and food habits and due to genetic variation. It causes defects in protein, lipid and carbohydrate metabolism, resulting in an increase in the incidence of related diseases such as cardio vascular disorders, renal disorders, even affects brain and the central nervous system, finally impairing functions of almost all organs in the body. About 400- 500 species of plants were identified as anti-diabetic and many have hypoglycaemic effects exerted by different mechanisms of action. Very few have been introduced into the modern system, despite their roles in traditional systems such as Ayurvedic and Chinese systems.

Inula helianthus aquatica belonging to the *Asteraceae* family is widely used in the Chinese medicine and also in Ayurveda. It possess anti-tumour, anti-diabetic, anti-inflammatory, anti- ulcer, anti-oxidant properties. The entire plant is used and is considered acrid, sweet, and warm. It expels wind-dampness, mend sinew and bone, stop bleeding, and resolve toxin. It is used for wind-dampness pain, broken bones, external injury with bleeding, and clove sores with toxic swelling. It also possess anti-ulcer, anti-inflammatory, anti-tumour, anti-epileptic, anti-helminthic, anti-septic, anti-tussive, cardiotonic, diuretic, diaphoretic and analgesic properties.

The present study was done to standardise *Inula helianthus aquatica* and to evaluate the anti-diabetic potential with its mechanism of action. The anti-diabetic property was validated by correlating various biochemical parameters. [4, 25, 40]

PLAN OF WORK

PLAN OF WORK

The study was carried into five various sections as follows.

A. PHYTOCHEMICAL ANALYSIS:

a) Collection and authentication of the plant Inula *helianthus aquatica.*

b) Extraction of active constituents from *leaves of Inula helianthus aquatica* by hot water extraction using reflux and continuous hot percolation by using Hexane, Ethanol and Water.

c) Determination of phyto constituents present in the 3 extracts.

B. ISOLATION OF ACTIVE CONSTITUENTS FROM LEAVES OF *INULA HELIANTHUS AQUATICA*:

The extraction process was carried out using Soxhlet extraction and reflux condensation at a pre-determined temperature setting. Appropriate solvent usage contributed to a maximum yield of the product. Precipitation technique using alcohol was used to bring about the separation of the final product followed by the quantitative calculation of the collected net content.

C. PHARMACOLOGICAL EVALUATION OF LEAVES OF *INULA HELIANTHUS AQUATICA*:

a) Anti-diabetic evaluation

1. Alloxan induced diabetes on rats

D. **BIOCHEMICAL PARAMETER FOR ANTI DIABETIC ACTIVITY:**

a) Serum glucose

b) Liver function test

1. Serum Glutamate Oxaloacetate Transaminases (SGOT)
2. Serum Glutamate Pyruvate Transaminases (SGPT)
3. Alkaline Phosphatase (ALP)

E. STATISTICAL ANALYSIS:

The analysis was carried out using graph pad prism software. The collected data was subjected to appropriate statistical test including one way ANNOVA, followed by an appropriate Dunnett's t-test.

PRELIMINARY PHYTOCHEMICAL STUDIES

PRELIMINARY PHYTOCHEMICAL STUDIES

6.1 MATERIALS AND METHODS

6.1.1 Collection and authentication of plant material

The plant *Inula helianthus aquatica* belonging to the family Asteraceae (Compositae), is found throughout the parts of India, south west China, and other south east Asian countries, Africa and some parts of Europe. The plants were collected from parts of Malappuram District of Kerala. It was identified and confirmed by botanist.

6.1.2. Animal approval

The study was conducted after obtaining the approval from Institutional Animal Ethics Committee (IAEC), and the experimental procedure were in accordance to the guidelines of IAEC (No:688/02/c/CPCSEA).

6.2 PREPARATION OF EXTRACTS

Preparation of the different extracts of the powdered leaves of *Inula helianthus aquatica* was carried out successively by hot water reflux and by continuous hot percolation method by using the following reagents. The defatted leaves with petroleum ether and were extracted by using the following reagents

6.2.1 HEXANE EXTRACT

Hexane extraction was carried out by extracting powdered leaves of *Inula helianthus aquatica* with hexane. The powdered leaves about 250 gm is packed well in a soxhlet apparatus and extracted with hexane (60-75°C), until colour of solvent is retained in it's original form in the siphon tube. The extract was filtered while hot and vacuum distilled and dried in desiccators. It was dried and percentage was calculated.

6.2.2 ETHANOL EXTRACT

After the Hexane extraction, the marc obtained was taken out and air dried, again packed in soxhlet apparatus and extracted with Ethanol, until extraction was complete. It was

then filtered and distilled , in reduced pressure and dried in desiccators . The extract was weighed and percentage yield was calculated.

6.2.3. AQUEOUS EXTRACT [30, 32, 33, 43, 44, 49]

Polysaccharides are polar macromolecular compounds and the method of hot water extraction and alcohol precipitation using reflux condensation was used for the extraction of polysaccharides. In the above method, often a portion of the residual protein would be removed and a suitable reagent that brings about polysaccharide precipitation was complemented towards the finalising stage. The temperature and the duration of the extraction procedure were determined using investigational studies. An aliquot portion of the collected sample was transferred to a high grade round bottom flask and continuously refluxed for a calculated time period of three hours at a pre-determined extraction temperature of 60-70°C. Subsequently, alcohol precipitation was followed (using various concentration) and the precipitate obtained was redissolved in water and the polysaccharide content was determined.

TABLE 6.1

Data for successive extraction values of various extracts of *leaves of Inula helianthus aquatic*.

SI NO.	EXTRACTS	%YEILD(W/W)
1	Hexane extract	3.7 %
2.	Ethanol extract	4.5 %
3	Aqueous extract	3.4 %

FIGURE 6.1

AQUEOUS EXTRACTION

6.3 QUALITATIVE PHYTOCHEMICAL ANALYSIS

The Hexane, Ethanol, and Aqueous extracts of *Inula helianthus aquatica* were subjected to various phyto chemical tests, for detecting the presence of various chemical constituents that might be present in that plant.

6.3.1 TEST FOR ALKALOIDS

Aliquot portion of the plant extract was acidified with few ml of Hydrochloric acid and the resulting solution was used for performing the test for Alkaloids.

a. **Dragendroff's Test:** The extract was treated with few drops of Dragendroff's reagent. (Potassium Bismuth Iodide solution). Orange coloured spots indicated the presence of alkaloids).
b. **Hager's Test:** The extract was treated with a few ml of Hager's reagent (Saturated Solution of Picric Acid) and a yellow precipitate was produced showing the presence of alkaloids.
c. **Wagner's Test:** The extracts were treated with aliquot quantities Wagner's reagent (Iodine-Potassium Iodide). Reddish brown precipitate indicated the presence of alkaloid.
d. **Mayer's Test:** Extract gives cream colour precipitate with Mayers reagent (Potassium Mercuric Iodide solution).

6.3.2 TEST FOR SAPONINS

The following tests of saponins were carried out:

a. **Foam Test:** 1 gm of extract was with diluted with 20 ml distilled water in graduated cylinder and shaken for 15 minutes. A foam layer of 1cm indicated the presence of saponins.
b. **Hemolysis Test:** Saponins produces hemolysis of red blood cells

6.3.3 TEST FOR GLYCOSIDES

a. **Legal's Test:** About 0.1gm of the extract was treated with pyridine (2ml) and added sodium nitro prusside solution (2 ml) and made alkaline with sodium hydroxide solution (pink to red colour solution indicated the presence of glycosides).
b. **Keller-Killiani Test:** Extract with chloroform mix well and evaporated it to dryness. Add 0.4 ml of glacial acetic acid containing trace amount of ferric chloride. Transfer to a small test tube, added carefully 0.5 ml of concentrated sulphuric acid by the side of the test tube. Acetic acid layer shows blue colour.
c. **Raymond Test:** When the extract was treated with hot methanolic alkali, a violet colour is produced.
d. **Baljets Test:** Extract with picric acid or sodium picrate, orange colour is produced.

6.3.4 TEST FOR CARBOHYDRATES

a. **Molisch's Test**: A small quantity (300 mg) of the extract was dissolved in 4 ml distilled water and filtered. The filtrate was subjected to Molisch's test (Formation of reddish brown ring indicated presence of carbohydrate).
b. **Fehling's Test:** Dissolved a small portion of extract in water and treated with Fehling's solution. (Brown colour indicated the presence of carbohydrate).
c. **Benedict's Test:** Extract solution about 1ml is added to 5ml of benedict's reagent and boiled for 2 minutes and cooled. A red precipitate is produced.
d. **Selivnoff's Test:** To a few ml of extract solution, add about 2ml of Selivanoff's reagent ketohexose which forms a deep red colour when reacted with Seliwanoff's reagent. An aldo hexose, will show a light pink colour that takes a longer time to develop when reacted with Seliwanoff's reagent.
e. **Iodine test for Polysaccharides:** Polysaccharides react to a lesser with iodine to form a red-brown or reddish-purple colour.

6.3.5 TEST FOR PHENOLIC COMPOUNDS AND TANNINS

a. **Braemer's Test:** To a 2-3 ml of extracts, 10% alcoholic ferric chloride solution was added. (Dark blue or greenish grey coloration of the solution indicated the presence of tannins).

6.3.6 TEST FOR PROTEINS AND AMINO ACIDS

a. **Ninhydrin Test:** Dissolved a small quantity of extract in a few ml of water and subjected to ninhydrin. (Blue coloration indicated the presence of amino acids).
b. **Biuret Test:** Extract added to Biuret reagent and a violet colour indicates the presence of proteins.

6.3.7 TESTS FOR FLAVONOIDS

a. **Shinoda Test:** To 2-3 ml of extract, a piece of magnesium ribbon and l ml of concentrated hydrochloric acid was added. (Pink red or red coloration of the solution indicated the presence of flavanoids).
b. **Lead Acetate Test:** To 5 ml of extract solution added l ml of lead acetate solution. (Flocculent white precipitate indicated the presence of flavanoids).

6.3.8 TEST FOR STEROIDS / TERPENOIDS

a. **Libermann - Burchard Test:** To l ml of extract, l ml of chloroform followed by 2-3 ml of acetic anhydride, and 1 to 2 drops of concentrated sulphuric acid were added. (Dark green coloration of the solution indicated the presence of steroids and dark pink or red coloration of the solution indicated the presence of terpenoids).
b. **Salkowski Test:** Extract was treated with few drops of concentrated sulphuric acid. Red colour at lower layer indicates that the presence of Steroids and formation of yellow colour at lower layer indicates presence of triterpenoids.

6.3.9 TEST FOR FIXED OILS AND FATS

Press small quantities of latex extract between two filter papers. Oil stains on the paper indicated that the presence of fixed oils.

6.4 RESULT AND DISCUSSION

The leaves of Inula helianthus aquatica was extracted with various solvents like Hexane, Ethanol, Water and subjected to preliminary phyto chemical screening for the presence of different chemical groups.

TABLE 6.2

Data for qualitative Phytochemical analysis of various extract of *Inula helianthus aquatica* leaves.

PHYTOCONSTITUENTS	HEXANE EXTRACT	ETHANOL EXTRACT	AQUEOUS EXTRACT
ALKALOIDS	-	+	-
CARBOHYDRATES	-	-	+
FIXED OILS AND FATS	+	-	-
FLAVONOIDS	-	+	+
GUMS AND MUCILAGE	-	+	+
GLYCOSIDES	-	+	+
PROTEINS AND AMINO ACIDS	-	-	-
SAPONINS	-	-	-
STEROIDS	+	+	-
TERPENOIDS	+	+	-

Present (+) Absent (-)

ISOLATION AND CHARACTERIZATION

ISOLATION AND CHARACTERIZATION

Inula helianthus aquatica (Asteraceae) is an important member of the species *Inula.* The species is rich in polysaccharides such as Inulin, which constitute majority of the oligofructoses, mainly responsible for the hypoglycemic effect. Inulin and inulin like Oligo fructoses, functional food ingredients found in various edible plants, has been reported to exert potential health benefits, including decreased risk of colonic diseases, non–insulin-dependent diabetes, obesity, osteoporosis, and cancer etc. Several methods such as TLC, HPTLC, GSC, HPLC, etc can be used for the isolation of oligofructoses, such as Inulin.

7.1. CHROMATOGRAPHIC STUDIES

These are some of the preliminary and common analytical techniques, used for the purification and separation of organic and inorganic substances.

7.1.1 THIN LAYER CHROMATOGRAPHY[50]

Thin layer chromatography is described as a method for chromatographic analysis on thin layers of adsorbents. The technique is rapid and helps in the separation of micrograms of the substances.

Some workers refer this technique as open column chromatography , spread layer chromatography and surface chromatography.

Though thin layer chromatography is similar to paper and column chromatography, but it is considered to be far superior to both of them because of the certain specific reasons.

a. Separation is sharpest as compared to paper and column chromatography.
b. It requires less time and less amount of substance.
c. Acid is directly sprayed on TLC plates as identifier.
d. Individual spots are less diffused as compared to paper chromatography.
e. Plates can be heated to high temperature without causing damage.
f. Several coating substances can be used, such as alumina, silica gel, and cellulose.
g. The capacity of thin layer of an adsorbent is higher than that of paper.

h. It is easy to coat the paper with variety of corrosive reagents that would destroy the paper.

7.1.2 BASIC OPERATIONS INVOLVED

a. Preparation of chromatographic plates.
b. Application of samples to chromatographic plates.
c. Choice of adsorbent.
d. Selection of solvent.
e. Proper developing system.
f. Location of compounds on chromatographic plates.
g. Detection and identification.

The aqueous extract of leaves of *Inula helianthus aquatica* leaves shown a clear spot on thin layer chromatographic plate with the solvent system containing, glacial acetic acid :chloroform: water(7:6:1). Detected by spraying with Aniline-Diphenylamine reagent, a deep browinsh colored spot was produced.[3]

FIGURE 7.1
Photo of TLC plate showing spot of aqueous extract.

7.2.1 COLUMN CHROMATOGRAPHY [51]

Column chromatography is defined as the uniform percolation of a liquid through a column of finely divided substances. The selected substances selectively retard certain components of the liquid.

The interaction of a substance with the stationary phase may occur in several ways.

- There may be direct interaction between the substance and the surface of the phase.
- The stationary phase may merely hold a second fluid phase so that the distribution involves a partition between two liquid phases.

This method been used successfully in many complicated systems and is not limited to colored solutions only. For each type there are definite disciplines which are taken into considerations.

The important things to be considered are types of solvents, adsorbents, design of the apparatus and the nature of the substance to be identified. The rate of flow of solvent is increased or decreased depending on the viscosity and other factors.

7.2.1.1 REQUIREMENTS OF COLUMN CHROMATOGRAPHY

Adsorbent used	:Silica gel (column grade 60-120)
Eluent	:Chloroform to Water in gradation
Length of column	:60cm
Diameter of column	: 3.5cm
Quantity of aqueous extract	: 5gm
Length of column packed	:40cm

7.2.1.2 PROCEDURE

- **Column preparation**

Column was dried and a glass wool was plugged to its bottom. Then a layer of sand bed was placed over the glass wool, to give a flat level. After that the column grade silica gel was poured slowly into the column. It was allowed to stand for some time.

Aqueous extract of *Inula helianthus aquatica* was kept over the layer of adsorbent, with a layer of paper disc and sand bed over the adsorbent that prevents any disturbance of the adsorbent layer.

- **Development of chromatograph**

Column work was done by adding the aqueous extract of *Inula helianthus aquatica* over the column grade silicagel 60-120 mesh.The process was carried out using solvents in the increasing order of polarity, including Benzene,Chloroform, Water.

About 25 ml of the fractions were collected and evaporated to get the residue, and tested for the presence of different constituents, by thin layer chromatography. Chloroform :water (85:15) gave single spot on TLC . It was confirmed by characterization using IR, NMR and MASS. [9, 17, 30, 50]

TABLE 7.1

Data for column chromatography

Fraction	Solvent	Ratio	Nature of residue	Analysis by TLC
1-5	Benzene	100	No residue	No spot
16-25	Benzene: Chloroform	85:15	Yellow	Merged spot
26-35	Benzene: Chloroform	50:50	Greenish yellow	Merged spot
47-60	Chloroform: Water	85:15	Brown	Single spot
60-72	Chloroform: Water	50:50	Brownish yellow	Two distinct spot

- **Chemical characterization of the isolated compound**

47-60th fractions of Chloroform: Water solvent system was treated with 0.5ml of Hydrochloric acid and heated for 2 minutes, cooled and neutralized to litmus paper with 5M Sodium hydroxide. Added 5ml of cupric-tartaric acid solution and heated. A red precipitate was produced which conforms the presence of Oligo Fructose.[3]

FIGURE NO. 7.2

IR Spectrum of isolated fraction

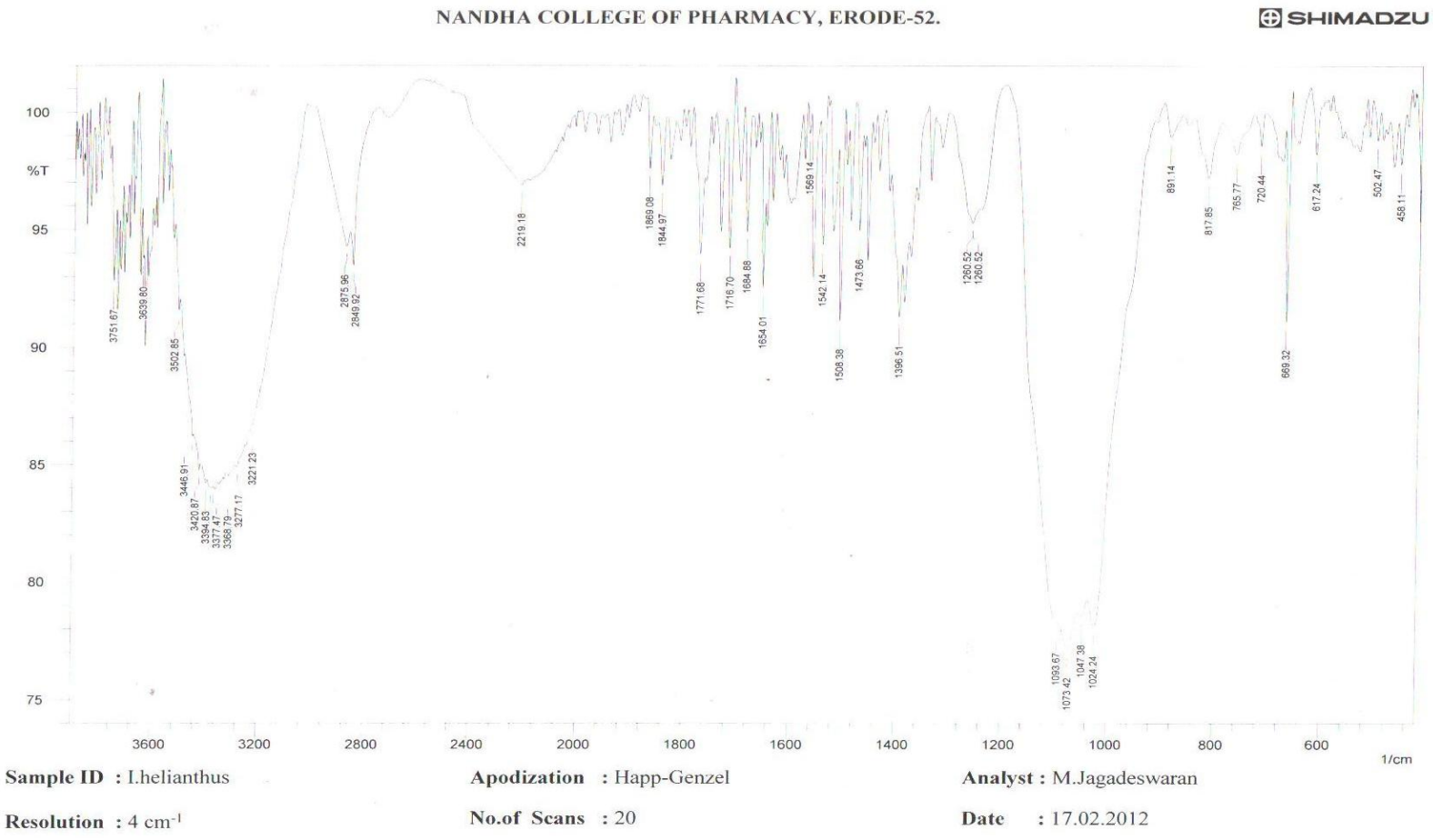


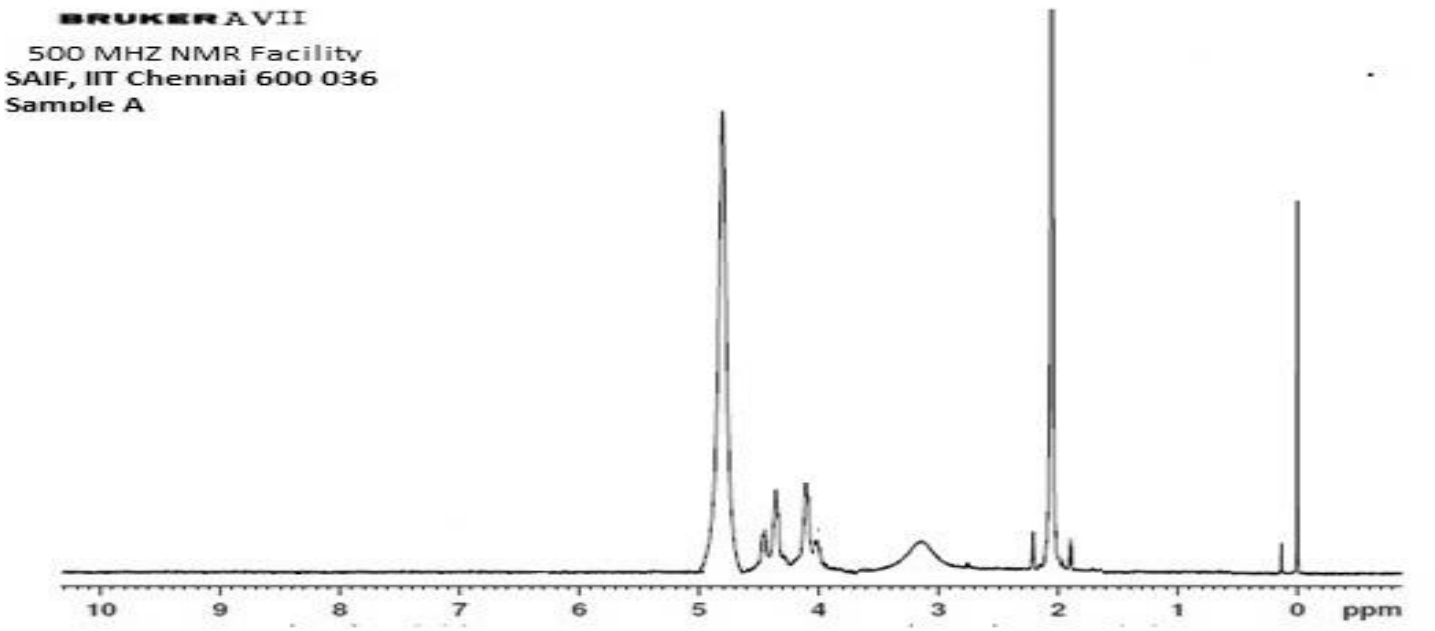
TABLE NO. 7.2

IR Interpretation of the isolated fraction:

Sr. No:	Functional groups	Wave number (cm^{-1})
1.	Aliphatic OH stretching	3639.80
2.	Aliphatic C-H stretching	2875.96
3.	Aliphatic C-H stretching	2849.92
4.	Ether C-O-C stretching	1260.52

FIGURE NO. 7.3

NMR Spectrum of isolated fraction

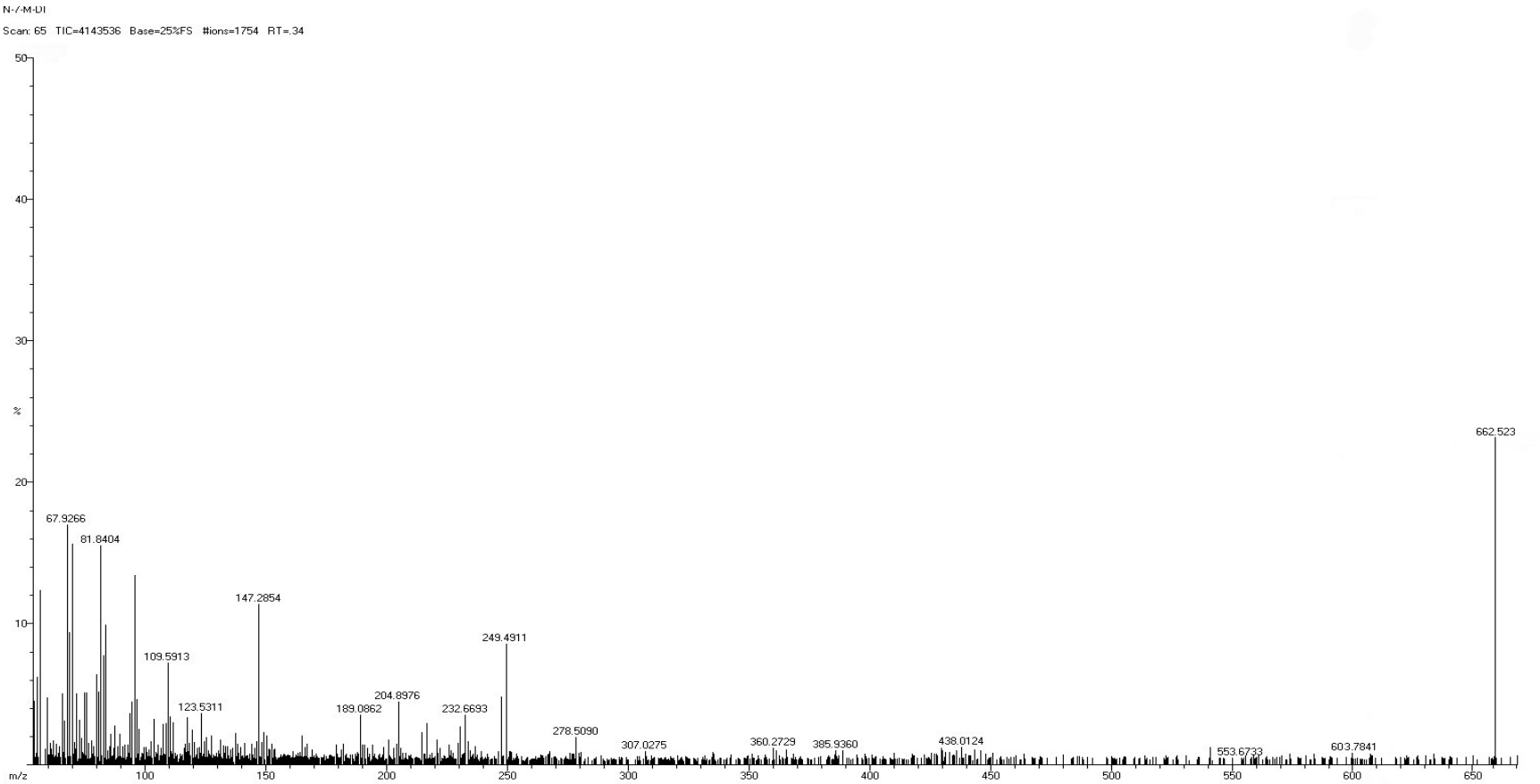


NMR Interpretation of the isolated fraction:

2.1 (2H of CH_2), 4.8 (4H of tetrahydro furan, m), 4.2 (1H of OH), 3.2 (2H of OCH_2)

FIGURE NO. 7.4

MASS Spectrum of isolated fraction



MASS Interpretation of the isolated fraction:

Mass: 662.523(M+)

PHARMACOLOGICAL SCREENING

EVALUATION OF ANTIDIABETIC ACTIVITY

8.1 INTRODUCTION

Diabetes mellitus is a metabolic disorder in the endocrine system. This dreadful disease is found in all parts of the world and is becoming a serious threat to mankind health. There are lots of chemical agents available to control and to treat diabetic patients, but total recovery from diabetes has not been reported up to this date. Alternative to these synthetic agents, plants provide a potential source of hypoglycemic drugs and are widely used in traditional systems of medicine to prevent diabetes. Several medicinal plants have been investigated for their beneficial uses in different types of diabetes. The effects of these plants may delay the effect of diabetic complications and correct the metabolic abnormalities using variety of mechanisms.

8.2 MATERIALS AND METHODS

8.2.1 Selection of animals

Male albino rats of Wistar strain and weighing about 100-200 gm were used for the study. The animals were got from Nandha College of pharmacy and research institute, Erode, and were approved by Ethical committee (NCP/IAEC/PG-2010/20).

8.2.2 Maintenance of animals

The animal house was well ventilated and animals had 15-20 $\pm 2^{O}$C. The animals were housed in large spacious hygienic cages during the course of the experimental period.

The animals were fed with rat pellets feed supplied by M/s Hindustan Lever Limited, Bangalore, India and filtered water and lithium. Animals described as fasted were deprived of food for $\geq$16 hr but allowed free access to water. The place where the experiments were conducted was kept very hygienic by cleaning with antiseptic solution, as the diabetic animals are susceptible to infections.

8.2.3 Chemicals

1. Alloxan monohydrate(Sigma chemicals, Bangalore)
2. Diethyl ether (Nice chemicals, cochin)
3. Carboxy methyl cellulose
4. Carbon tetra chloride (Nice chemicals, cochin)

8.2.4 Drugs

1. Leaf extract of Inula helianthus aquatica
2. Glibenclamide 5mg/kg

8.3 PROCEDURE:

8.3.1 Aqueous Extraction:

Aqueous extract was prepared from a powder of the leaf extract of *Inula helianthus aquatica* was prepared in an electric grinder. The 200 gm powder was extracted with water. The extract was evaporated to dryness under vacuum and dried in vacuum desiccators (5%w/w).

8.4 EXPERIMENTAL DESIGN:

Different groups of rats were used to study the effect of aqueous extract of Inula helianthus aquatica. The rats were divided into five groups each consisting of six rats:

Group 1: The rats received 2 ml CMC. These animals serve as normal controls.

Group 2: Received a single dose of (150mg/kg body weight), Alloxan monohydrate in CMC through intraperitoneal route and served as negative control.

Group 3: Received the aqueous leaf extract 200mg/kg for 21days and served as test 1.

Group 4: Received the leaf extract 400mg/kg for 21 days and served as test 2.

Group 5: Received Glibenclamide 5mg/kg for 21days and served as positive control.

8.5 INDUCTION OF DIABETES:

Animals were allowed to fast 24 hr and were injected with freshly prepared aqueous solution of alloxan monohydrate (150 mg/kg i.p.) as reported previously (Kameswara Rao et al., 1999). After a week rats with marked hyperglycemia (fasting blood glucose>200mg/dl) were used for the study.

8.6 DETERMINATION OF BLOOD GLUCOSE:

Blood glucose was collected for the measurement of blood glucose from the tail vein at 0, 1, 2 and 3 hr after feeding the plant extracts. The blood glucose levels were determined by using one touch glucometer.

8.7 BIOLOGICAL ESTIMATION:

After the experimental regimen, the blood is collected through the retro-orbital puncture of eye of animals under mild diethyl ether anesthesia in Eppendorff's tube (1ml) containing 50µl of anticoagulant (10% trisodium citrate) and the serum was separated by centrifuging at 6000rpm for 15min. the biochemical parameters, cholesterol, triglycerides, total protein, SALP, SGOT and SGPT are determined by using the commercial kit available (Ecoline, manufactured by Merck Specialities private limited, Ambernath.)

8.8 STATISTICAL ANALYSIS:

The collected data were subjected to appropriate statistical test including one way ANOVA, followed by an appropriate Dunnett's t-test, P-value of less than 0.05, 0.01 and 0.001 were considered as less significant, significant and more significant respectively. The analysis was carried out using graph pad prism software.

8.9 RESULT AND DISCUSSION:

Screening of anti hyperglycemic activity of extracts in Alloxan induced diabetic rats.

The effect of *Inula helianthus aquatica*, leaf extract on fasting blood glucose level in alloxan induced diabetic rats were give in Table8.1 and Graph 8.2. The lower dose of root extract 200gm/kg produced a slight decrease in fasting blood glucose level on the 28^{th} day, when compared with diabetic controlled animal. The higher dose of leaf extract, 400mg/kg produced a less significant reduction ($P<0.05$) from the 14^{th} day and a significant reduction ($p<0.01$) from 21^{th} days treatment the leaf extract was found to be more potent at the higher dose, 400mg/kg and it brought down the elevated blood glucose level in alloxan induced diabetic rats nearer to the normal range. [29,31,34,37,41,42]

TABLE 8.1

The effect of aqueous leaf extract of *Inula helianthus aquatica* on fasting blood glucose level in Alloxan induced diabetic rats.

GROUPS	1st Day	1st week	2nd week	3rd Week
CMC (0.5 %)	88.5±3.81	98.33±6.41	105.33±5.28	101.33±2.82
Diabetic control (150 mg/kg ALLOXAN)	135±7.41	255±6.85	274.16±6.11	289±6.53
Test-I(Aq. extract -200mg/kg)	105.83±6.93**	161.83±8.75**	158±8.87**	135.5±3.21**
Test-II (Aq. extract - 400mg/kg)	102.83±6.93**	154.66±5.06**	143.16±4.36**	123.66±5.73**
Standard (Glibenclamide) (5mg/kg)	104.17±4.57**	143.16±7.09**	135.33±5.92**	131±3.48**

Data represents mean ± S.D(n=5)

*p<0.05 significant as compared to normal control.

**p<0.01significant as compared to Alloxan control.

***p<0.01 significant as compared to Alloxan control.

ns is non significant as compared to normal control.

FIGURE 8.1

Effect of aqueous leaf extract of *Inula helianthus aquatica* on fasting blood glucose level in Alloxan induced diabetic rats

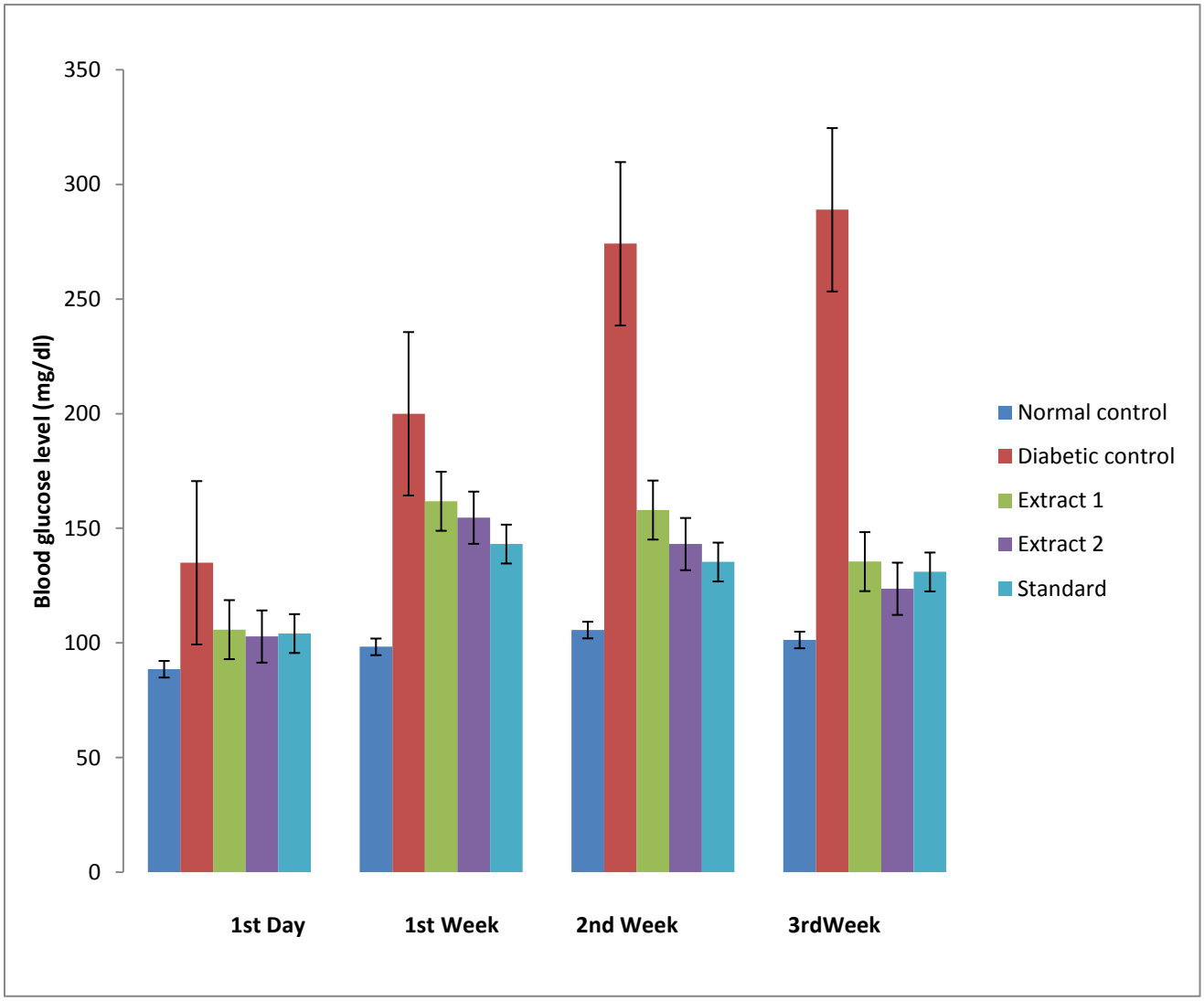


TABLE 8.2

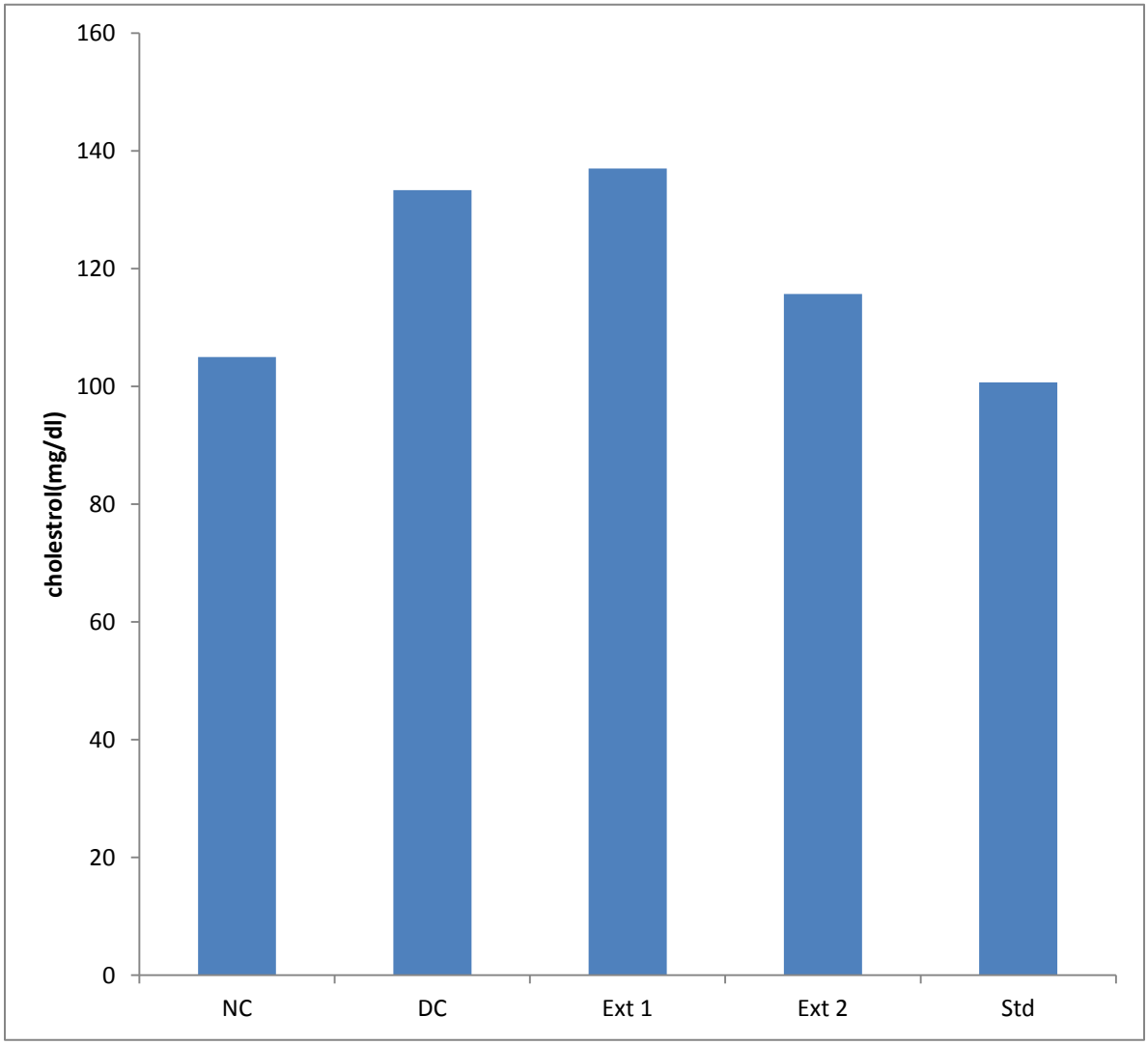
Effect of aqueous extract of leaves of *Inula helianthus aquatica* on cholesterol

GROUPS	CHOLESTEROL (mg/dl)
Normal control (0.5% CMC)	105±1.62
Diabetic control (150mg/kg ALLOXAN)	133.33±1.49
Test-I(Aq. extract -200mg/kg)	127±1.29**
Test-II Aq. extract - 400mg/kg)	115.66±2.31**
STANDARD DRUG(5mg/kg)	100.66±1.33**

**p<0.01significant as compared to Alloxan control.

FIGURE 8.2

Effect of aqueous extract of leaves of *Inula helianthus aquatica* on cholesterol.



TABL 8.3

Effect of Aqueous extract of leaves of *Inula helianthus aquatica* on Triglycerides

GROUPS	TRIGLYCERIDES (mg/dl)
Normal control (0.5% CMC)	75.166±1.16
Diabetic control (150mg/kg ALLOXAN)	104.5±1.54
Test-I(Aq. extract -200mg/kg)	96.16±1.01**
Test-II (Aq. extract- 400mg/kg)	82.5±0.76**
STANDARD DRUG(5mg/kg)	82.16±1.19**

**p<0.01significant as compared to Alloxan control.

FIGURE 8.3

Effect of Aqueous extract of leaves of *Inula helianthus aquatica* on Triglycerides

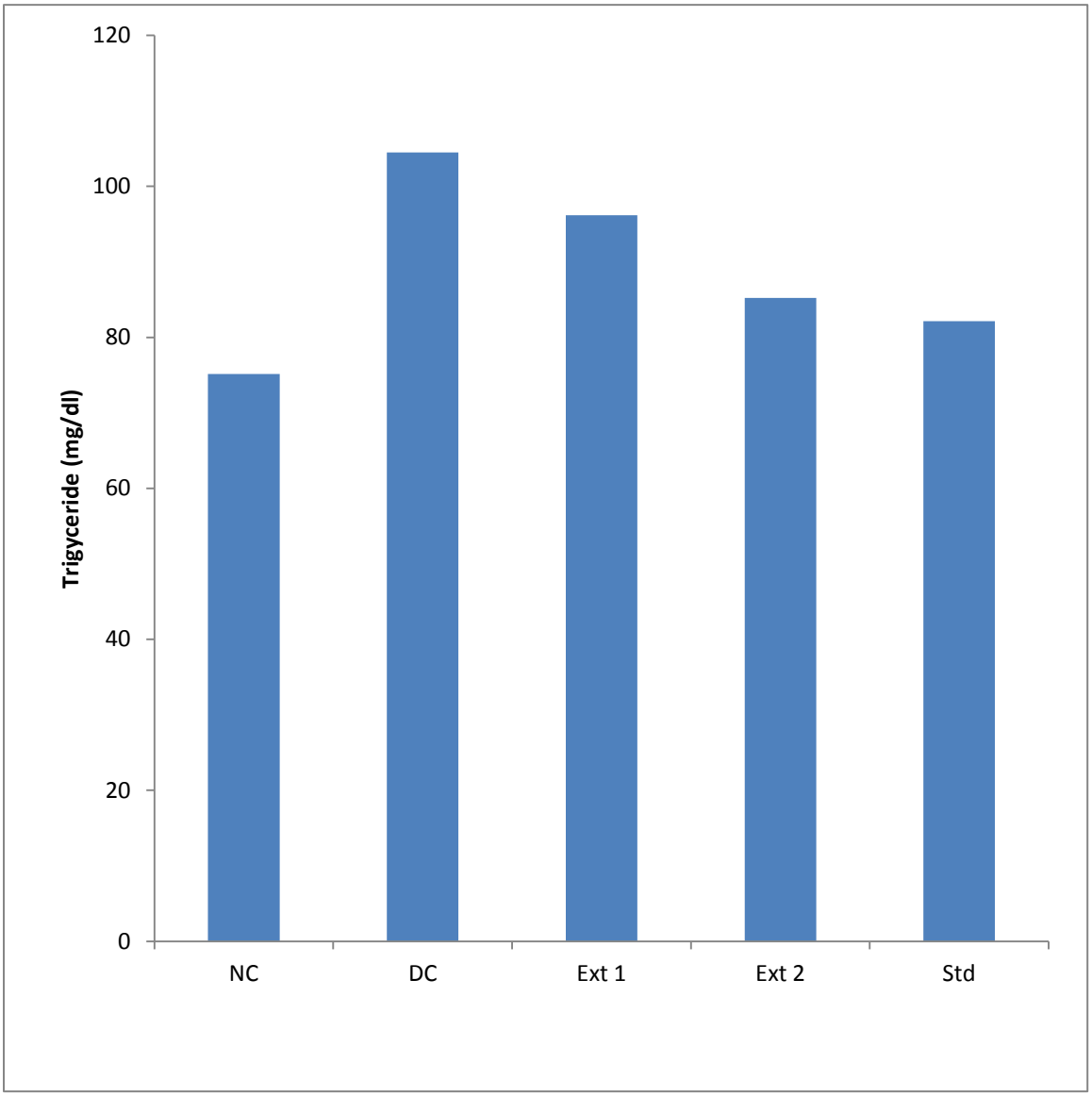


TABLE 8.4

Effect of Aqueous extract of leaves of *Inula helianthus aquatica* on total proteins

GROUPS	TOTAL PROTEIN (g/dl)
Normal control (0.5% CMC)	7.5±0.42
Diabetic control (150mg/kg ALLOXAN)	4.33±0.49
Test-I(Aq. extract -200mg/kg)	5.83±0.30*
Test-II(Aq. extract - 400mg/kg)	6.16±0.30*
STANDARD DRUG(5mg/kg)	6.83±0.40**

*p<0.05 significant as compared to normal control.

**p<0.01significant as compared to Alloxan control.

FIGURE 8.4

Effect of Aqueous extract of leaves of *Inula helianthus aquatica* on total proteins.

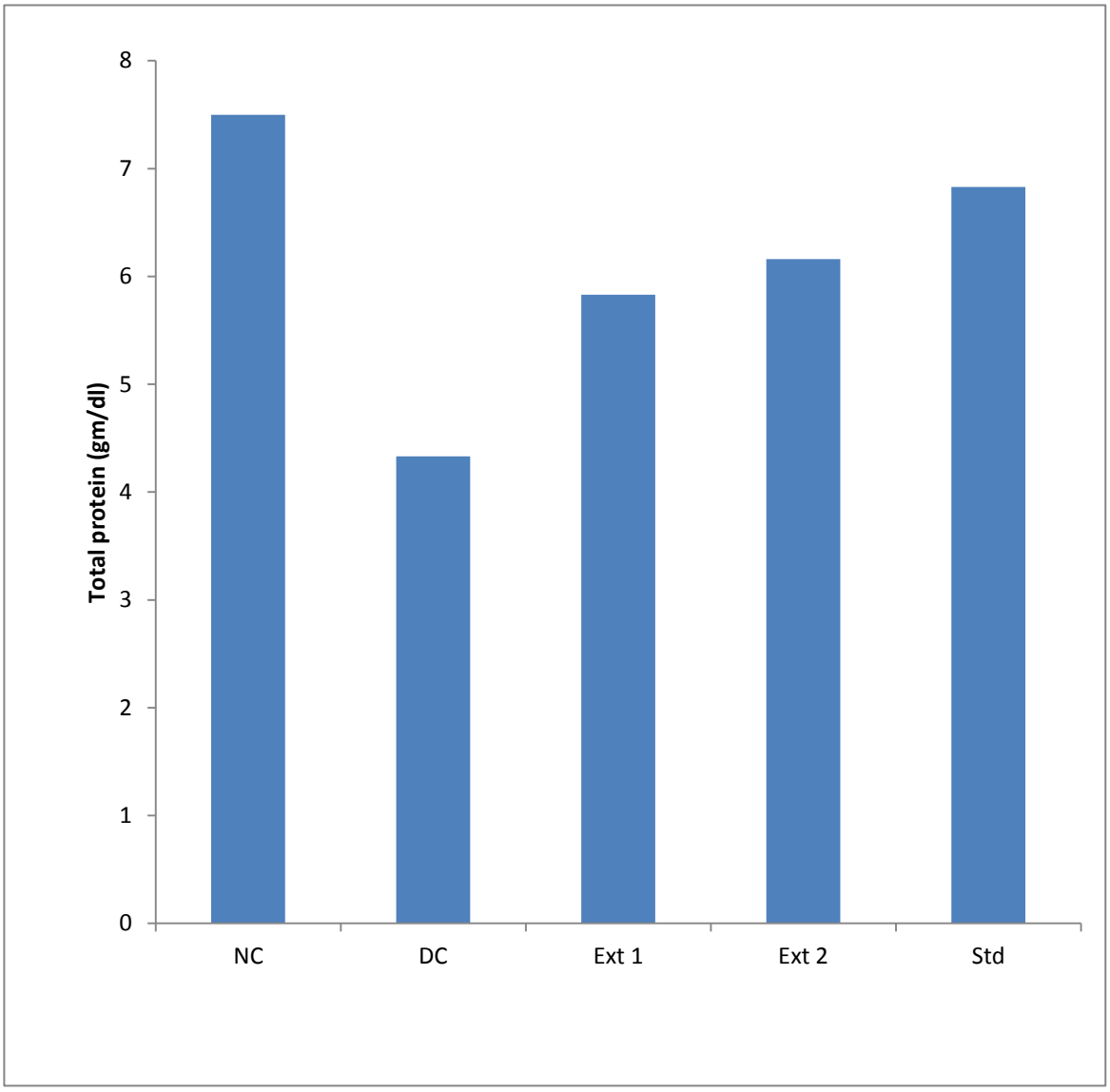


TABLE 8.5

Effect of Aqueous extract of leaves of *Inula helianthus aquatica* on SALP

GROUPS	SALP (IU/L)
Normal control (0.5% CMC)	265.50±3.69
Diabetic control (150mg/kg ALLOXAN)	431.16±0.98
Test-I(Aq. extract -200mg/kg)	320.33±1.35**
Test-II(Aq. extract - 400mg/kg)	261±1.46**
STANDARD DRUG(5mg/kg)	309.16±1.22**

**p<0.01significant as compared to Alloxan control.

FIGURE 8.5
Effect of Aqueous extract of leaves of *Inula helianthus aquatica* on SALP

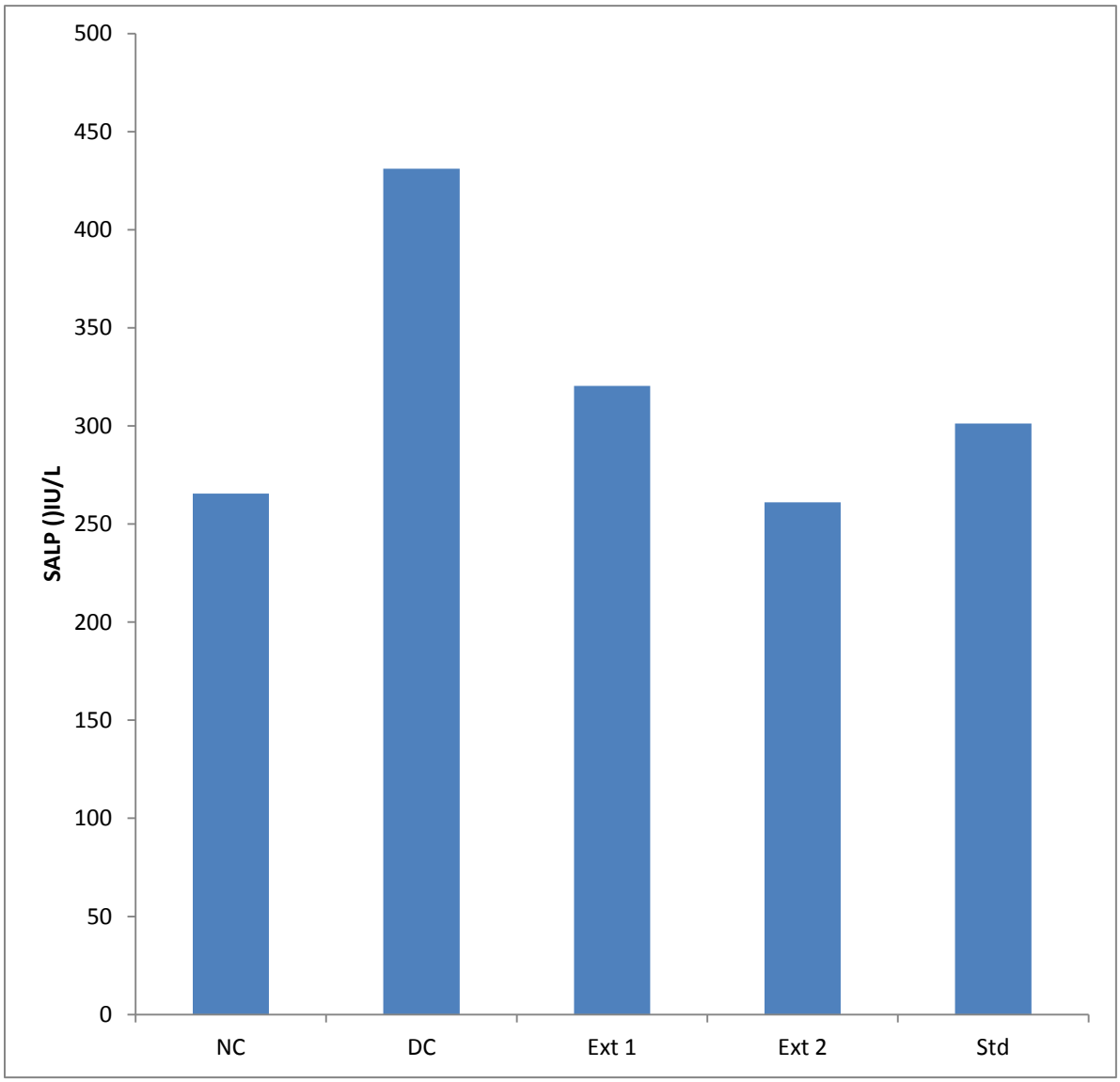


TABLE 8.6

Effect of aqueous extract of leaves of *Inula helianthus aquatica* on SGOT

GROUPS	SGOT(IU/L)
Normal control (0.5% CMC)	35.17±2.80
Diabetic control (150mg/kg ALLOXAN)	89.83±2.81
Test-I(Aq. extract -200mg/kg)	58±2.16**
Test-II(Aq. extract - 400mg/kg)	49.83±1.87**
STANDARD DRUG(5mg/kg)	40±1.77**

**p<0.01significant as compared to Alloxan control.

FIGURE 8.6
Effect of aqueous extract of leaves of *Inula helianthus aquatica* on SGOT

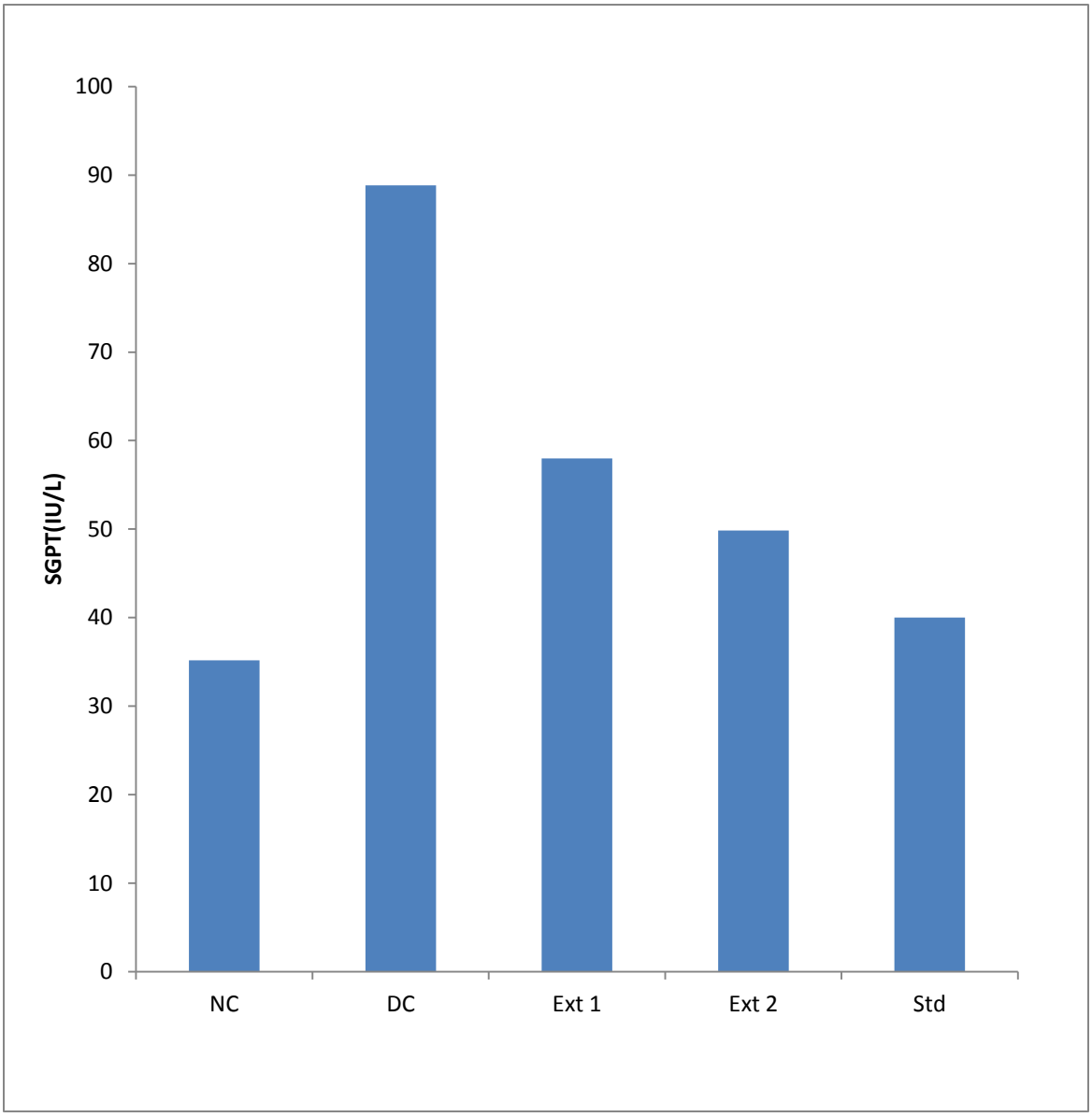


TABLE 8.7

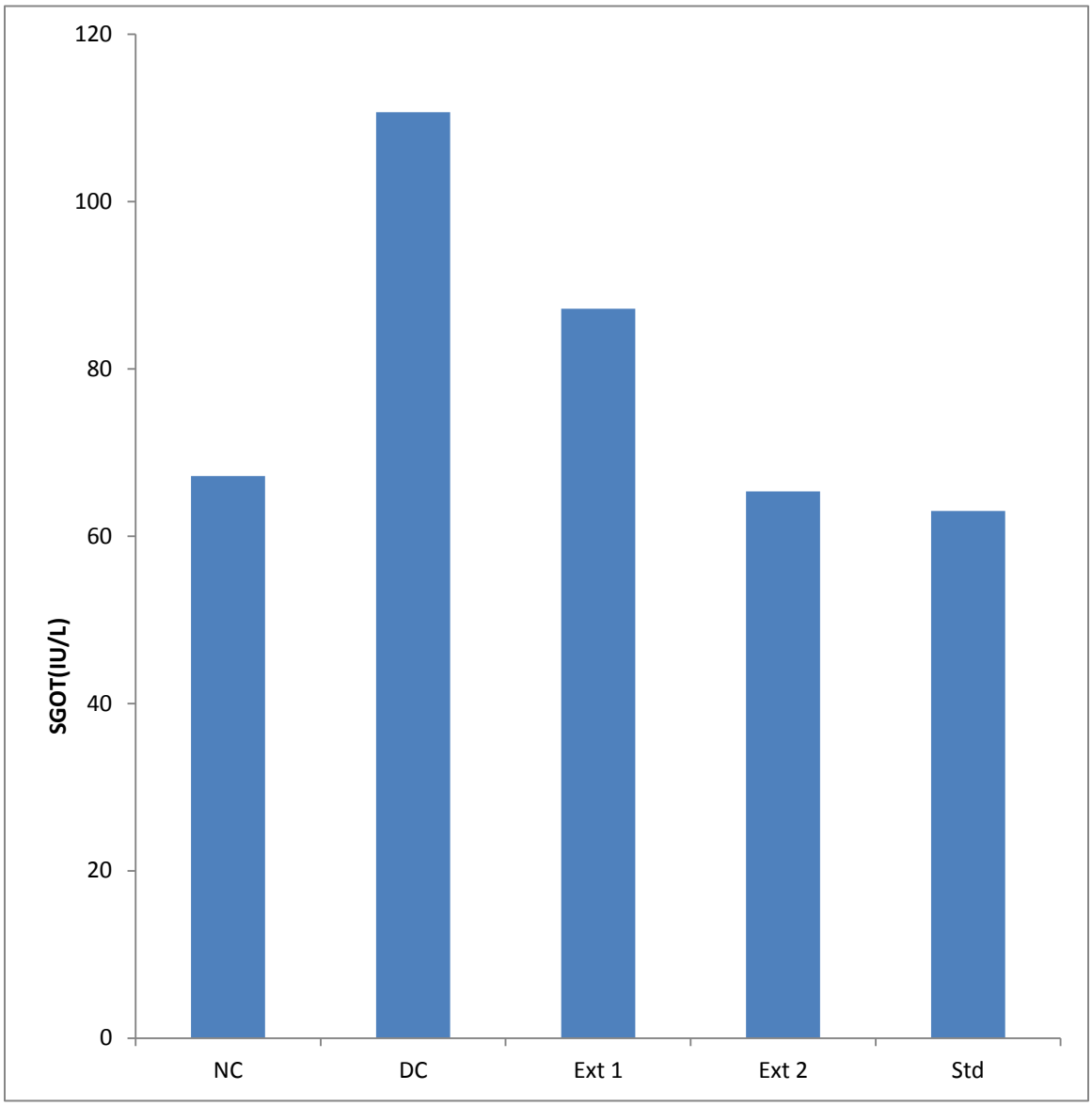
Effect of aqueous extract of leaves of *Inula helianthus aquatica* on SGPT

GROUPS	SGPT(IU/L)
Normal control (0.5% CMC)	67.17±2.44
Diabetic control (150mg/kg ALLOXAN)	110.67±2.57
Test-I(Aq. extract -200mg/kg)	87.17±2.17**
Test-II(Aq. extract - 400mg/kg)	65.33±3.67**
STANDARD DRUG(5mg/kg)	63±2.14**

**p<0.01significant as compared to Alloxan control.

FIGURE 8.7

Effect of aqueous extract of leaves of *Inula helianthus aquatica* on SGPT



RESULT

AND

DISCUSSION

RESULTS AND DISCUSSION

The plant *Inula helianthus aquatica* belonging to the family *Asteraceae* was selected and various studies such as phytochemical and pharmacological activities were carried out. The plant was collected from the Malappuram district of Kerala, and was authentified by the botanist for confirmation.

Plant leaves were collected and was shade dried for 20 days, until it was dry enough for extraction. It was then powdered in a grinder and extracted with solvents such as hexane, ethanol, and water.

The extracts were subjected to various preliminary phytochemical tests and the results indicated the presence of Oligo fructoses, in the aqueous extract as shown in Table No 6.2.

The compounds were identified by TLC and isolated by column chromatography.

The spectral studies were carried out from the aqueous extract of *Inula helianthus aquatica* leaves and subjected for purity testing. Then the purified fractions were subjected to IR, 1- H NMR, MASS, studies for the confirmation of the functional groups, number of protons and molecular weight.

The results demonstrates that the lower dose of leaf extract 200mg/kg produced a slight decrease in fasting blood glucose level on the 28^{th} day, when compared with diabetic controlled animal whereas the higher dose of leaf extract, 400mg/kg produced a less significant reduction ($P<0.05$) from the 14^{th} day and a significant reduction ($p<0.01$) from 21^{th} days treatment the leaf extract was found to be more potent at the higher dose, 400mg/kg and it brought down the elevated blood glucose level in alloxan induced diabetic rats nearer to the normal range.

The results obtained from the chemical tests, IR, NMR, MASS spectroscopy gave the conclusion that Oligo fructoses were present in the extract.

The anti-diabetic activity of the aqueous extract of *Inula helianthus aquatica* leaves were compared with that of standard drugs. The aqueous extract of *Inula helianthus aquatica* leaves produced significant anti diabetic activity in dose dependent manner as shown in Table No 8.1.

CONCLUSION

CONCLUSION

The selected plant, *Inula helianthus aquatica* belonging to the family Asteraceae gave a detailed report on preliminary phytochemical, isolation of active constituents and pharmacological studies.

The plant was selected for the work on the basis of its traditional use. Some related species have already proven for their anti diabetic activity. The *Asteraceae* family in general has anti-diabetic potential along with anti-ulcer, anti-inflammatory, anti- tumour, anti-epileptic, anti-helminthic, anti-septic, anti-tussive, cardiotonic, diuretic, diaphoretic and analgesic properties.

The phyto chemical studies, isolation, and spectral studies were carried out. In the present study the aqueous extract of leaves has proven anti-diabetic potential and helps in prevention and management of diabetes.

The anti-diabetic work was done on alloxan induced diabetic rats, and the results obtained has proven the potential of anti diabetic properties in aqueous extract of *Inula helianthus aquatica* leaves.

Detailed studies on the phyto constituents of the plants are yet to be established regarding the antidiabetic potential of this plant.

REFERENCE

REFERENCES

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