

EVALUATION OF ANTI-DIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF *POUTERIA CAMPECHIANA* (Kunth)

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CHAPTER 1

INTRODUCTION

1.1. MEDICINAL PLANTS

India is the largest producer of medicinal plants and is rightly called the “Botanical garden of the World”. The medicinal plants, besides having natural therapeutic values against various diseases, also provide high quality of food and raw materials for livelihood.

India has a rich culture of medicinal herbs and species, which include about more than 2000 species and has a vast geographical area with high potential abilities for Ayurveda, Unani, Sidha traditional medicines but only very few have been studied chemically and pharmacologically for their potential medicinal value (Gupta et al., 2005; Sandhu and Heinrich, 2005).

Human beings have used plants for the treatment of diverse ailments for thousands of years. According to the World Health Organization, most populations still rely on traditional medicines for their psychological and physical health requirements, since they cannot afford the products of western pharmaceutical industries (Salie et al., 1996), together with their side effects and lack of healthcare facilities. Rural areas of many developing countries still rely on traditional medicines for their primary health care needs and have found a place in day-to-day life. These medicines are relatively safer and cheaper than synthetic or modern medicine (Iwu et al., 1996; Idu et al., 2007). People living in rural areas from their personal experience know that these traditional medicines are a valuable source of natural products to maintain human health, but they may not understand the science

behind these medicines, but knew that some medicinal plants are highly effective only when used at therapeutic doses (Maheswari et al., 1986).

Medicinal plants are assuming greater importance in the primary health care of individuals and communities in many developing countries. Indian medicinal plants and their derivatives have been an invaluable source of therapeutic agents to treat various disorders. Herbal products are often perceived as safe because they are "natural". In recent years herbal medicine is a major component in all traditional medicine system, and a common element in Siddha, Ayurvedic, Homeopathic, Naturopathic, Traditional Chinese medicine, and Native American medicine. Considerable efforts have been directed towards the development of natural products from various plant sources (Hazeena Begum V et al., 2011).

Today a substantial number of drugs are developed from plants which are active against a number of diseases. The majority of these involve the isolation of the active ingredient (chemical compound) found in a particular medicinal plant and its subsequent modification. In the developed countries 25 percent of the medical drugs are based on plants and their derivatives and the use of medicinal plants is well known among the indigenous people in rural areas of many developing countries (Ignacimuthu. S et al., 2009).

Plants used in traditional medicines have stood up to the test of time and contributed many novel compounds for preventive and curative medicine to modern science. India is sitting on a gold mine of well recorded and traditionally well practiced knowledge of herbal medicine. Specially, plants growing at high altitude in Himalayan pastures are time-honored sources of health and general well-being of local inhabitants. As of today, Himalayan plants are a major contributor to the herbal

pharmaceutical industry both of India and other countries. Plants growing at higher altitude are subjected to an assault of diverse testing situations including higher doses of mutagenic UV-radiation, physiological drought, desiccation and strong winds. Plants interact with stressful environment by physiological adaptation and altering the biochemical profile of plant tissues and producing a spectrum of secondary metabolites. Secondary metabolites are of special interest to scientists because of their unique pharmacophores and medicinal properties.

Secondary metabolites like polyphenols, terpenes and alkaloids have been reported to possess antimutagenic and anticancer properties in many studies. The fundamental aspiration of the current review is to divulge the antimutagenic/anticancer potential of five alpine plants used as food or medicines by the populations living at high altitudes. India is the largest producer of medicinal plants and is rightly called the “Botanical garden of the World”. The medicinal plants, besides having natural therapeutic values against various diseases, also provide high quality of food and raw materials for livelihood. Considerable works have been done on the traditional uses and scientific reports. These plants may promote host resistance against infection by re-stabilizing body equilibrium and conditioning the body tissues. Several reports describe that the anticancer activity of medicinal plants is due to the presence of antioxidants in them. In fact, the medicinal plants are available, cheaper and possess easily no toxicity as compared to the modern (allopathic) drugs. Hence, this review article contains 66 medicinal plants, which are the natural sources of anticancer agents.

The rediscovery of Ayurveda is a sense of redefining it is modern medicines. Emerging concept of combining Ayurveda with advanced drug discovery

programme is globally acceptable. Traditional medicine has a long history of serving peoples all over the world. The ethnobotany provides a rich resource for natural drug research and development. In recent years, the use of traditional medicine information on plant research has again received considerable interest. The Western use of such information has also come under increasing scrutiny and the national and indigenous rights on these resources have become acknowledge by most academic and industrial researchers. According to the World Health Organization(WHO), about three quarters of the world's population currently use the herbs and other forms of traditional medicines to treat diseases. Traditional medicines are widely use d in India. (Debjit Bhowmik et al.,2013)

A survey of current pharmaceutical use revealed that, of the total ptescription drugs dispensed, 25% are plant derived (Farnsworth and Morris, 1976). Plant comopounds are highly varied in structure ; many are aromatic substances, most of which are phenols or their oxygen substituted derivatives. However, there is an increased attention on extracts and biologically active compounds isolated from plant species used in herbal medicine, due to the side effects and the resistance the pathogenic micro organism build against the antibiotics(Essawi and Srour, 1999). New compounds inhibiting micro organisms such as benzoin and emetine have been isolated from plants. Of the various pharmaceuticals used in modern medicine, atropine, ephedrine, digoxin, morphine, quinine, reserpine and tubocurarine serve as examples of drugs discovered through observations of indigenous medical practice (Gilani and Rahman,2005).

Plant constituents may be isolated andused directly as therapeutic agents or as starting materials for drug synthesis or they may serve as model for

pharmacologically active compounds in drug synthesis. The general research methods includes proper selection of medicinal plants, preparation of crude extracts, biological screening, detailed chemo pharmacological investigations, toxicological and clinical studies, standardization and use of active moiety as the lead molecule for drug design(Wink et al.,2005).

1.2. HERBAL PLANTS

Herbal drugs referred as plants materials or herbalism, involves the use of whole plants or parts of plants, to treat injuries or illness. Herbal drugs are use of therapeutic herbs to prevent and treat diseases and ailments or to support health and healing. These are drugs or preparations made from a plant and used for any of such purposes. Herbal drugs are the oldest form of health care known to mankind. They are many herbal products offered that assert to treat the symptoms of a broad range of problems, from depression to cold and flu. World Health Organization (WHO) has distinct herbal drugs as complete, labeled medicinal products that have various ingredients, aerial or secretive parts of the plants or other plant material or combinations. World Health Organization has set precise guidelines for the evaluation of the safety, efficacy, and quality of herbal medicines.

WHO estimates that 80% of the world populations currently use herbal drugs for major health care. Exceptionally, in some countries herbal drugs may also enclose by tradition, natural organic or inorganic active constituents which are not of plant source. Herbal drug is a chief constituents in traditional medicine and common constituents in Ayurvedic, Homeopathic, Naturopathic, and other medicine systems. Herbs are usually considered as safe since they belongs to natural sources. The use of herbal drugs due to toxicity and side effects of Allopathic medicines, has led to

rapid increase in the number of herbal drug manufacturers. For the past few decades, herbal drugs have been more and more consumed by the people with no prescription.

Seeds, leaves, stem, bark, roots, flowers, and extracts of all of these have been used in herbal drugs over the millennia of their use. Herbal products have reached extensive adequacy as beneficial agents like anti microbial, anti diabetic, anti fertility, anti aging, anti arthritic, sedative, anti depressant, anti anxiety, anti spasmodic, analgesic, anti inflammatory, anti-HIV, vasodilatory, hepatoprotective, treatment of cirrhosis, asthma, acne, impotence, menopause, migraine, gall stones, chronic fatigue, Alzheimer's disease and memory enhancing activities. Herbal drugs have been recognized for approximately 4000 years. These drugs have survived real world testing and thousands of years of human testing. Some drugs have been discontinued due to their toxicity, while other have been modified or combined with additional herbs to side effects.

1.2.1 Advantages of Herbal Drugs

- Low/Minimum cost
- Potency and efficiency
- Enhanced tolerance
- More protection
- Fewer side-effects
- Complete accessibility
- Recyclable

1.2.2. Disadvantage of Herbal Drug

- Not able to cure rapid sickness and accidents
- Risk with self dosing
- Complexity in standardizations.

1.2.3. Usage and Preparation of Herbal Drugs

The usage of herbal drugs in the correct Way provides effectual and safe treatment for many ailments. The efficiency of the herbal drugs is typically subjective to the patient. The strength of the herbal drugs varies based on the genetic distinction, growing conditions, timing and method of harvesting, revelation of the herbs to air, light and dampness, and type of conservation of the herbs. Some of the plants that make up herbal drugs are cultured and processed in the country and others are imported from around the world. Raw materials for herbal drugs may be derived from carefully cultivated plants or collected in the wild. Herbal drugs are accessible in several forms and often require preparation before their use. They can be normally purchased in mass form as dried plants, plant parts or insecurely packed for herbal teas and decoctions. Decoctions are made by boiling the herb in water, then straining out of the plant material. More intense form of herbal drugs are available in the form of hydro alcoholic tinctures and fluid extracts. Methods of preparation may differ because of the nature of the plants active chemical constituents.

1.2.4. Pharmacological Actions of Herbal Drugs

1.2.4.1. Anti-inflammatory activity

The extracts of *Achillea millefolium*, *Artemisia vulgaris*, *Bauhinia tarapotensis*, *Curcuma longa*, *Forsythia suspense*, *Houttuynia cordata*, *Glycyrrhiza uralensis*, *Lonicera japonica*, *Ruta graveolens*, *Securidaca longipedunculata* and *Valeriana wallichii* have shown anti-inflammatory activity.

1.2.4.2. Anti diabetic activity

From earliest period, people are using herbal plants as home remedies for the treatment of diabetics. A variety of herbal plants with anti diabetic activity are *Abroma augusta*, *Acacia melanoxylon*, *Acacia modesta*, *Acacia nilotica*, *Aconitum ferox*, *Adatoda vasika*, *Adiantum capillus*, *Adiantum incisum*, *Agrimonia eupatoria*, *Allium sativum*, *Aloe barbadensis*, *Althae officinalis*, *Apium graveolens*, *Arcitium lappa*, *Commiphora abyssinca*, *Embilica officinalis*, *Eucalyptus globules*, *Ginseng panax*, *Gymnema sylvestre*, *Inula helenium*, *Juniperus communis*, *Medicago sativa*, *Orthosiphon stamineus*, *Panax quinquefolius*, *Polygala senega*, *Plantago ovata*, *Punica granatum*, *Salvia officinalis*, *Scoparia dulcis*, *Tanacetum vulgare*, *Taraxacum officinale*, *Tecoma stans*, *Trifolium alexandrium*, *Trigonella foenum*, *Turnera diffusa*,.

1.2.4.3. Analgesic activity

The extracts of *Bougainvilla spectabilis*, *Chelidonium majus*, *Ficus glomerata*, *Dalbergia lanceolaria*, *Glacium paucilobum*, *Nepeta italic*, *Polyalthia longifolia*, *Sida acuta*, *Stylosanthes fruticosa*, *Toona ciliate*, *Zataria multiflora*, and *Zingiber zerumbet* are used as analgesic agents.

1.2.4.4. Anticancer activity

Medicinal plant products exhibiting anticancer activity continue to be the subject of extensive research aimed at the development of drugs for the treatment of different human tumors. The medicinal plants used for the treatment of cancer are, *Acalypha fruticosa*, *Alangium lamarki*, *Catharanthus roseus*, *Celastrus paniculatus*, *Embelica ribes*, *Ficus glomerata*, *Terminalia chebula*, *Tylophora indica*, The extracts used for the treatment of breast cancer are *Buthus martensi*, *Colla cornu*, *Herba epimedi*, *Radix glycyrrhizae*, *Squama manitis*, *Tuber curcumae*, The herbal drugs used for the treatment of pancreatic cancer are *Embilica officinalis*, *Nigella sativa*, and *Terminalia belleric*.

1.2.4.5. Anti- ageing activity

Cell membranes are particularly susceptible to the hostility of free radicals. When the nucleus is injured, the cell loses its ability to replicate itself. The impaired cell replication results in the destabilized immune system, skin ageing and may age related disorders. Various antioxidants neutralize the free radicals and prevent oxidation on a cellular level. The most effectual antioxidants include pine bark extract, grape seed extracts, and blue berries were effectual against the hostility of free radicals. Some commonly used herbs as anti-ageing agents are *Allium sativum*, *Arnica Montana*, *Cucumis sativum*, *Curcuma longa*, *Ocimum sanctum*, *Panax ginseng*, *Rosa damascence* and *Withania somnifera*.

1.2.4.6. Antifertility activity

of naturally occurring fertility regulating agent because of their little or no side effects. The plants that have been reported to have anti fertility activity are

Amaranthus retroflexus, *Artabotrys odoratissimus*, *Barberis vulgaris*, *Carica papaya*, *Dieffenbachia seguine*, *Evodia rutacapra*, *Fatsia horrid*, *Ferula assafoetida*, *Hibiscus rosasinensis*, *Lonicera ciliosa*, *Magnolia virginiana*, *Mardenia cundurango*, *Pisum sativum*, *Podophyllum peltatum*, *Punica granatum*, *Raphanus sativus*, *Rehmannia glutinosa*, *Semecarpus anacardium*, *Sesbania sesban*, *Thuja occidentalis*, *Taxus baccata*, and *Verbena officinalis*.

1.2.4.7. Anti-psoriasis activity

A variety of natural proprietary formulas and preparations containing plant materials have been used to provide symptomatic relief in psoriasis. The different herbal remedies for psoriasis are, turmeric, curcumin, shark cartilage extract, oregano oil, milk thistle. Various antimicrobial agents *Azadirachta indica*, *Calendula officinalis*, *Cassia tora*, *Wrightia tinctoria* have been used in the management of psoriasis.

1.2.4.8. Anti depressant activity

A number of nutritional and herbal supplements have shown promise as alternative treatment for depression. A large number of plants have potential functions to treat depression which are described as, *Bacopa monniera*, *Panax quinquefolius*, *Piper methysticum*, *Rhodiola rosea*, *Valerina officianalis* and *Hypericum perforatum*.

1.2.4.9. Anti vitiligo activity

Anti vitiligo oil is a herbal remedy manufactured with potent herbs and is produced with traditional methods and is also a complete traditional herbal formulation. The plants which can be used in the treatment of vitiligo are *Acorus*

calamus, Adiantum capillus, Bowellia serrate, Cassia angustifolia, Cassia tora, Cinnamom cassia, Fumaria officinalis, Glycyrhiza glabra, Lavandula stoechas, Rosa damascene, Vitis vinifera, Zingiber officinale and Zizyphus sativa.

1.2.4.10. Treatment of dental diseases

The plant having the dental care properties are *Acacia catechu, Acacia arabica, Althea officinalis, Anacylus pyrethrum, Azardirachta indica, Barleria prionits, Cinnamomum camphora, Cuminum cyminum, Eucalyptus globules, Gardenia gummifera, Holarrhenia antidysentrica, Jasmimum grandiflorum, Juglans regia, Myrica sapida, Ocimum sanctum, Origanum vulgare, piper longum, piper nigrum, Salvadora persica, Saliva officinalis, Thalictrum foliolosum* and *Zanthoxylum alatum*. All these regimens play an important role in suppressing the dental problems.

1.2.5. Standardization of Herbal Drugs

Herbal drugs imply knowledge and practice of herbal healing for the prevention, diagnosis, and elimination of physical, mental, or social imbalance. The costs for healthcare are rising at an alarming rate throughout the world. At the same time, the world market for phytopharmaceuticals is growing progressively. The world Bank estimates that trade in medicinal plants, botanical drug products, and raw materials are growing at an annual rate of between 5 and 15 %. It is a common observation that people diagnosed with incurable chronic disease states such as diabetes, arthritis, and AIDS turned to herbal therapies for a sense of control and mental comfort from taking action. Herbal product studies cannot be considered scientifically valid if the product tested has not been authenticated and characterized

in order to ensure reproducibility in the manufacturing of the product in question. Several studies have indicated quantitative variations in marker constituents in herbal preparations.

Moreover, many dangerous and lethal side effects have recently been reported, including direct toxic effects, allergic reactions, effects from contaminants, and interactions with drugs and other herbs. The 10 most commonly used herbs in the United States, systematic reviews have concluded that only 4 are likely to be effective and there is very limited evidence to evaluate the efficacy of the approximately 20,000 other available herbal products. Standardized herbal products of consistent quality and containing well-defined constituents are required for reliable clinical trials and to provide consistent beneficial therapeutic effects. Pharmacological properties of an herbal formulation depend on phytochemical constituents present therein. Development of authentic analytical methods which can reliably profile the phytochemical composition, including qualitative analyses of marker/bioactive compounds and the other major constituents, is a major challenge to scientists. Without consistent quality of a phytochemical mixture, a consistent pharmacological effect is not expected. Resurgence of interest and the growing market of herbal medicinal products necessitate strong commitment by the stakeholders to safeguard the consumer and the industry. Standardization is the first step for the establishment of a consistent biological activity, a consistent chemical profile, or simply a quality assurance program for production and manufacturing. Therefore, the EU has defined three categories of herbal products:

- Those containing constituents (single compounds or families of compounds) with known and experienced therapeutic activity that are deemed solely responsible for clinical efficacy.
- Those containing chemically defined constituents possessing relevant pharmacological properties which are likely to contribute to the clinical efficacy.
- Those in which no constituents have been identified as being responsible for the therapeutic activity.

Standardization as defined in the text for guidance on the quality of herbal medicinal products means adjusting the herbal drug preparation to a defined content of a constituents or group of substance with known therapeutic activity. The European Medicines Agency (EMA) makes the distinction between constituents with known therapeutic activity which can be used to standardize a biological effect and marker compounds which allow standardization on a set amount of the chosen compound. The EMA define marker compounds as chemically defined constituents of a herbal drug which are of interest for control purposes, independent of whether they have any therapeutic activity or not. Examples of markers are the valernic acids in *Valeriana officinalis* L., ginkgolides and flavonoids in *Ginko biloba* L., and hypertension in *Hypericum perforiatum* L.,

1.2.6. Stability testing of Herbal Drugs

Stability testing of herbal drugs is a challenging risk, because the entire herb or herbal products is regarded as the active matter, regardless of whether constituents with defined therapeutic activity are known. The purpose of a stability testing is to provide proof on how the quality of the herbal products varies with the

time under the influence of environmental factors such as temperature, light, oxygen, moisture, other ingredients or excipients in the dosage form, particle size of drug, microbial contamination, trace metal contamination, leaching from the container and to establish a recommended storage condition and shelf-life. Stability testing is necessary to ensure that the product is of satisfactory quality throughout its entire storage period. Stability studies should be performed on at least three production batches of the herbal products for the proposed shelf life, which is normally denoted as long term stability and is performed under natural atmospheric conditions. Stability data can also be generated under accelerated atmospheric condition of temperature, humidity and light, which is referred to as short term stability and the data so obtained is used for predicting shelf-life of the product. Stability testing should be conducted on the dosage form packaged in the container closure system proposed for marketing. With the help of modern analytical technique like spectrophotometry, HPLC, HPTLC and by employing proper guidelines it is possible to generate a sound stability data of herbal products and predict their shelf-life, which will help in improving global acceptability of herbal products (Bodhisattwa Maiti et al.,2011).

1.4. PHYTOCONSTITUENTS AND ITS ACTIONS

1.4.1. Alkaloids:

Alkaloids are derived from plant sources, they are basic, they contain one or more nitrogen atoms (usually in a heterocyclic ring) and they usually have a marked physiological action on man or other animals.

Alkaloids are mainly used as antitumor (vincristine, vinblastin), anticholinergic (atropine), stimulant (caffeine), antimalarial and antipyretic (quinine), cough medicine and analgesic (codein) etc....

1.4.2. Flavonoids:

Flavonoids are the largest class of polyphenols. Chemically, they may be defined as a group of polyphenolic compounds consisting of substance that have two substituted benzene rings connected by the chain of three carbon atoms and an oxygen bridge.

Flavonoids possess anti-bacterial, anticancer, anti-inflammatory actions and used in the treatment of cardiovascular diseases.

1.4.3. Glycosides:

Glycosides may be defined, in general, as the organic compounds from plants or animal sources which on enzymatic or acid hydrolysis give one or more sugar (glycon) moieties along with non-sugar (aglycon) moiety.

Glycosides are used as cardio tonic, purgative, anti-rheumatic and analgesic, demulcent.

1.4.4. Tannins:

Tannins are chemically defined as the mixture of complex organic substance where in polyphenols are present with O-dihydroxy or O-trihydroxy groups on a phenyl ring.

Tannins are used as mild antiseptic, in treatment of diarrhea and to forestall minor hemorrhage.

1.4.5. Carbohydrates:

Carbohydrates may be defined as polyhydroxy aldehyde or ketones or compounds which produce them on hydrolysis.

Carbohydrates are mainly used as demulcent, laxative, anti-diarrheal, pharmaceutical agent etc....

1.4.6. Fixed oils and fats:

These are the reverse food materials of plants and animals. Those, which are liquid at 15.5°C to 16.5°C are called as fixed oils; while those which are solid or semisolid at above temperature are termed as fats. Fixed oil is mainly used as rubifacient, counter irritant, laxative, and spermicidal, ointment base, suppository base etc....

1.4.7. Mucilage:

These are polysaccharide complexes of sugar and uronic acids, usually formed from the cell wall. They are insoluble in alcohol but swell or dissolve in water.

They are used as emulsifiers, suspending agent, demulcent etc...

1.4.8. Proteins and amino acids:

Proteins are complex nitrogenous organic substances of plant and animal origin. They are of great importance in the functioning of living cells. They contain carbon, hydrogen, oxygen, nitrogen and rarely sulfur. The ultimate products of complete hydrolysis of proteins, either by chemical reagents or enzymes, are amino acids.

Amino acids are group of organic compounds containing two functional groups-amino and carboxyl. The amino group is basic while the carboxyl group is acidic in nature.

Proteins are mainly used as digest ant, anti-inflammatory agent, anticoagulant,nutritive,dietary supplement (Kokatae *et al.*,2012).

1.5. INTRODUCTION TO DIABETES

1.2.1. History of diabetes mellitus

The earliest description of diabetes was documented in the writings of Hindu scholars as long as in 1500 BC. They had already described “*a mysterious disease causing thirst, enormous urine output, and wasting away of the body with flies and ants attracted to the urine of people.*”

The term diabetes was probably coined by Apollonius of Memphis around 250 BC, which literally meant “to go through” or siphon as the disease drained more fluid than a person could consume. Later on, the Latin word “mellitus” was added because it made the urine sweet. (MacCracken J. 1997).

Sushruta, Arataeus, and Thomas Willis were the early pioneers of the treatment of diabetes. Greek physicians prescribed exercise, preferably on horseback, to “employ moderate friction” and alleviate excess urination. Wine, overfeeding to compensate for loss of fluid weight, starvation diet, potato therapy, and oat cure were some of the other curious forms of remedy suggested for the therapy of diabetes in olden days (MacCracken J.1997).

Sir William Osler, in the year 1915, is said to have even recommended opium! Early research linked diabetes to glycogen metabolism, and the islet cells of pancreas were discovered by Paul Langerhans, a young German medical student. In 1916, Sharpey-Shafer of Edinburgh suggested that a single chemical was missing from the pancreas and proposed its name as “insulin.” The term insulin originates from the word Insel, which is German for an islet or island.

Researchers like E.L. Scott and Nikolae Paulesco were successful in extracting insulin from the pancreas of experimental dogs. The key breakthrough, though, came from the Toronto University with the discovery of insulin in 1921. FG Banting and JJR Macleod were awarded the Nobel Prize for Physiology or Medicine in 1923 (Bliss M.1982.).

1.2.2. Definition of Diabetes mellitus

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The abnormalities in carbohydrate, fat, and protein metabolism that are found in diabetes are due to deficient action of insulin on target tissues.

If ketones are present in blood or urine, treatment is urgent, because ketoacidosis can evolve rapidly (American diabetes association, 2001).

1.2.3. Causes

Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin, or alternatively, when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood sugar, hyperglycemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to

serious damage to many of the body's systems, especially the nerves and blood vessels.

1.2.4. Symptoms of diabetes mellitus

Common symptoms include the following:

- Frequent urination
- Excessive thirst
- Unexplained weight loss
- Extreme hunger
- Sudden vision changes
- Tingling or numbness in the hands or feet
- Feeling very tired much of the time
- Very dry skin
- Sores that are slow to heal
- More infections than usual

Some people may experience only a few symptoms that are listed above. About 50 percent of people with type 2 diabetes don't experience any symptoms and don't know they have the disease. (www.ucsfhealth.org)

1.2.5. Pathophysiology of diabetes mellitus

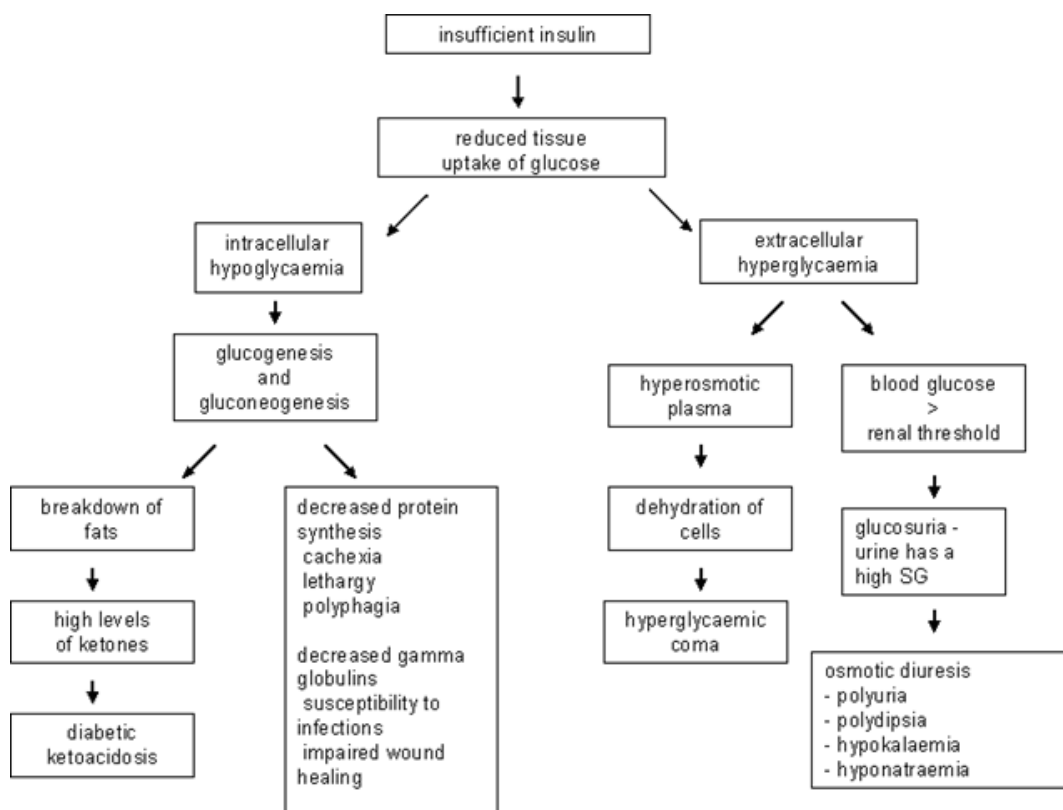


Figure No: 1. Pathophysiology of diabetes mellitus

1.3. CLASSIFICATION OF DIABETES MELLITUS

Diabetes mellitus (or diabetes) is a chronic, lifelong condition that affects your body's ability to use the energy found in food. There are three major types of diabetes: type 1 diabetes, type 2 diabetes, and gestational diabetes. (www.webmd.com)

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

Type 1 diabetes represents a heterogeneous and polygenic disorder, with a number of non-HLA loci contributing to disease susceptibility (Lernmark A, 1998). Though this form of diabetes accounts for 5 to 10% of all diabetics, yet there is no

identified agent substantially capable of preventing this type of disease (Atkinson MA,2001). The WHO and the American Diabetics Association (WHO;1999 and ADA;2001) have proposed that type 1 diabetes can be divided into autoimmune/immune-mediated diabetes (Type 1A) and idiopathic diabetes with β -cell obstruction (Type 1B). This type of diabetes mellitus requires exogenous insulin to prevent Diabetic ketoacidosis.

1.3.2.Immune mediated diabetes

This form of diabetes, previously encompassed by the terms insulin dependent diabetes, or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the β -cells of the pancreas.(American diabetes association,2012)

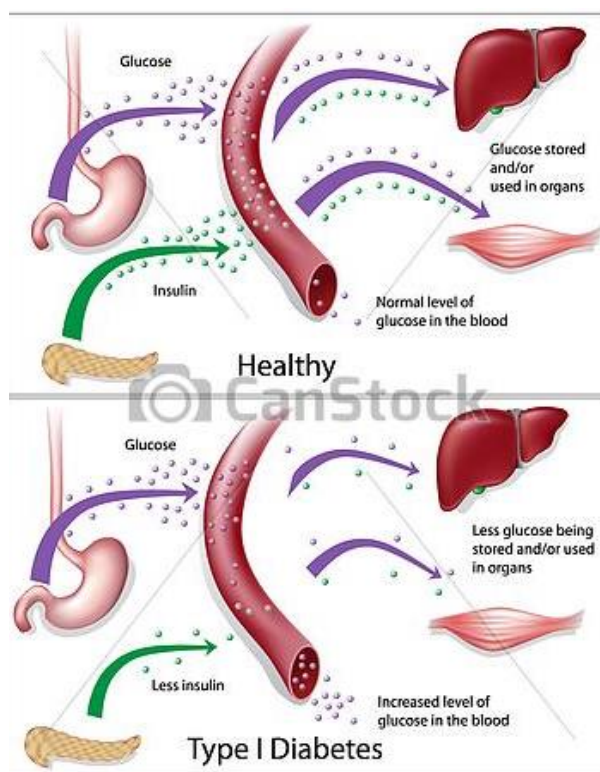


Figure No: 2. Immune mediated diabetes

Type 1 diabetes is an autoimmune condition. It's caused by the body attacking its own pancreas with antibodies. In people with type 1 diabetes, the damaged pancreas doesn't make insulin.

This type of diabetes may be caused by a genetic predisposition. It could also be the result of faulty beta cells in the pancreas that normally produce insulin.

A number of medical risks are associated with type 1 diabetes. Many of them stem from damage to the tiny blood vessels in your eyes (called diabetic retinopathy), nerves (diabetic neuropathy), and kidneys (diabetic nephropathy). Even more serious is the increased risk of heart disease and stroke.(www.webmd.com)

1.3.3. Idiopathic diabetes

Some forms of type1 diabetes have no known etiologies. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity.Although only a minority of Patients with type 1 diabetes fall into this category, of those who do, most are of African or Asian ancestry.

Individuals with this form of diabetes suffer from episodic ketoacidosis and exhibit varying degrees of insulin deficiency between episodes. This formof diabetes is strongly inherited, lacks immunological evidence for β -cell autoimmunity(Americandiabetes association,2012).

Treatment for type 1 diabetes involves taking insulin, which needs to be injected through the skin into the fatty tissue below. (www.webmd.com)

1.3.4. Type 2 diabetes

Type 2 diabetes is far more common and results from a combination of defects in insulin secretion and insulin action, either of which may predominate. People with type 2 diabetes are not dependent on exogenous insulin, but may require it for the control of blood glucose levels if this is not achieved with diet alone or with oral hypoglycemic agents. This type of diabetes accounts for 90 to 95% of all diabetic patients (DeFronzo RA, 1997).

All forms of diabetes are characterized by chronic hyperglycemia and the development of diabetes-specific microvascular pathology in the retina, renal glomerulus, and peripheral nerve. As a consequence of its microvascular pathology, diabetes is a leading cause of blindness, end-stage renal disease, and a variety of debilitating neuropathies.

When islet b-cell function is impaired, insulin secretion is inadequate, leading to overproduction of glucose by the liver and under-utilization of glucose in peripheral tissue (Bergman RN *et al*, 1989).

Type 2 diabetes is made up of different forms, each of which is characterized by a variable degree of insulin resistance and β -cell dysfunction and which together lead to hyperglycemia (American Diabetes Association, 2001).

At each end of this spectrum are single gene disorders that affect the ability of the pancreatic β cell to secrete insulin (Fajans SS *et al*, 2001 and Owen K *et al*, 2001) or the ability of muscle, fat, and linear cells to respond to insulin action (Taylor SI *et al*, 1999 and Barroso I *et al*, 1999).

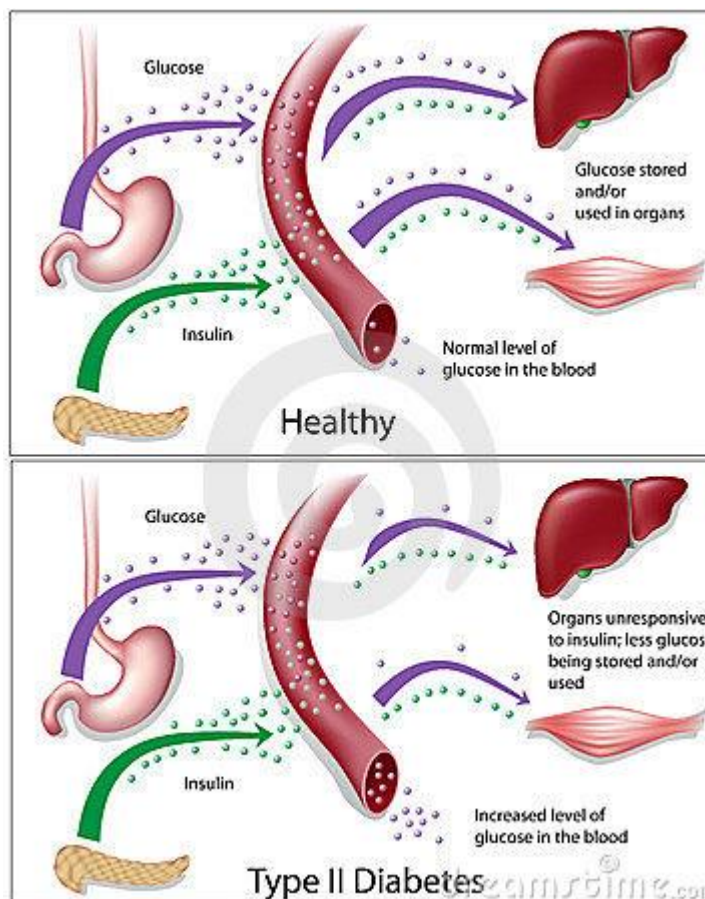


Figure No: 3. Type 2 diabetes

With Type 2 diabetes, the pancreas usually produces some insulin. But either the amount produced is not enough for the body's needs, or the body's cells are resistant to it. Insulin resistance, or lack of sensitivity to insulin, happens primarily in fat, liver, and muscle cells.

People who are obese more than 20% over their ideal body weight for their height are at particularly high risk of developing type 2 diabetes and its related medical problems. Obese people have insulin resistance. With insulin resistance, the pancreas has to work overly hard to produce more insulin. But even then, there is not enough insulin to keep sugars normal. (www.webmd.com)

There is no cure for diabetes. Type 2 diabetes can, however, be controlled with weight management, nutrition, and exercise. Unfortunately, type 2 diabetes tends to progress, and diabetes medications are often needed(www.webmd.com).

1.3.5. Gestational Diabetes

Diabetes that's triggered by pregnancy is called gestational diabetes (pregnancy, to some degree, leads to insulin resistance). It is often diagnosed in middle or late pregnancy. Because high blood sugar levels in a mother are circulated through the placenta to the baby, gestational diabetes must be controlled to protect the baby's growth and development.

According to the National Institutes of Health, the reported rate of gestational diabetes is between 2% to 10% of pregnancies. Gestational diabetes usually resolves itself after pregnancy. Having gestational diabetes does, however, put mothers at risk for developing type 2 diabetes in later. Up to 10% of women with gestational diabetes develop type 2 diabetes. It can occur anywhere from a few weeks after delivery to months or years later.

With gestational diabetes, risks to the unborn baby are even greater than risks to the mother. Risks to the baby include abnormal weight gain before birth, breathing problems at birth, and higher obesity and diabetes risk later in life. Risks to the mother include needing a cesarean section due to an overly large baby, as well as damage to heart, kidney, nerves, and eye.

Treatment during pregnancy includes,

- Careful meal planning to ensure adequate pregnancy nutrients without excess fat and calories

- Daily exercise
- Controlling pregnancy weight gain
- Taking diabetes insulin to control blood sugar levels if needed(www.webmed.com)

1.3.6.Other specific types of diabetes

a) Genetic defects of the β -cell

Several forms of diabetes are associated with monogenetic defects in β -cell function. These forms of diabetes are frequently characterized by onset of hyperglycemia at an early age (generally before age 25 years). They are referred to as maturity onset diabetes of the young (MODY) and are characterized by impaired insulin secretion with minimal or no defects in insulin action.

b) Genetic defects in insulin action

There are unusual causes of diabetes that result from genetically determined abnormalities of insulin action. The metabolic abnormalities associated with mutations of the insulin receptor may range from hyperinsulinemia and modest hyperglycemia to severe diabetes.

c) Diseases of the exocrine pancreas

Any process that diffusely injures the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatectomy, and pancreatic carcinoma. With the exception of that caused by cancer, damage to the pancreas must be extensive for diabetes to occur; adenocarcinomas that involve only a small portion of the pancreas have been associated with diabetes. This implies a

mechanism other than simple reduction in β -cell mass. If extensive enough, cystic fibrosis and hemochromatosis will also damage β -cells and impair insulin secretion.

d) Endocrinopathies

Several hormones (e.g., growth hormone, cortisol, glucagon, epinephrine) antagonize insulin action. Excess amounts of these hormones (e.g., acromegaly, Cushing's syndrome, glucagonoma, pheochromocytoma, respectively) can cause diabetes. This generally occurs in individuals with preexisting defects in insulin secretion, and hyperglycemia typically resolves when the hormone excess is resolved.

e) Drug- or chemical-induced diabetes

Many drugs can impair insulin secretion. These drugs may not cause diabetes by themselves, but they may precipitate diabetes in individuals with insulin resistance. In such cases, the classification is unclear because the sequence or relative importance of β -cell dysfunction and insulin resistance is unknown. Certain toxins such as Vacor (a rat poison) and intravenous pentamidine can permanently destroy pancreatic β -cells.

f) Infections

Certain viruses have been associated with β -cell destruction. Diabetes occurs in patients with congenital rubella, although most of these patients have HLA and immune markers characteristic of type 1 diabetes. In addition, coxsackievirus B, cytomegalovirus, adenovirus, and mumps have been implicated in inducing certain cases of the disease. (American diabetes association, 2012)

1.4. Evolution of insulin

Insulin is a natural hormone and as essential as air, water, and light. Amongst all the antidiabetic medications, it is the most potent agent that reduces blood glucose levels. Its benefits exceed beyond the realms of glycemic control.

It reduces glucotoxicity and lipotoxicity, reverses insulin resistance, preserves beta cell function, and it improves lipid profile, endothelial dysfunction, anti-inflammatory effects, and anti-platelet effects as well as the quality of life. In spite of all these benefits, there is an inherent clinical inertia before initiating insulin therapy, especially for patients with type 2 diabetes mellitus. (Galloway JA *et al*;1994.)

1.4.1. Structure of insulin

Insulin was purified and crystallized by Abel within a few years of its discovery. Sanger established the amino acid sequence of insulin in 1960, the protein was synthesized in 1963, and Hodgkin and coworkers elucidated insulin's three-dimensional structure in 1972. Insulin was the hormone for which Yalow and Berson first developed the radioimmunoassay (Kahn and Roth, 2004).

The β cells of pancreatic islets synthesize insulin from a single-chain precursor of 110 amino acids termed preproinsulin. After translocation through the membrane of the rough endoplasmic reticulum, the 24-amino-acid N-terminal signal peptide of preproinsulin is cleaved rapidly to form proinsulin. Thereafter, proinsulin folds, and the disulfide bonds form. During conversion of human proinsulin to insulin, four basic amino acids and the remaining connector or C peptide are

removed by proteolysis. This gives rise to the A and B peptide chains of the insulin molecule, which contains one intra-subunit and two inter-subunit disulfide bonds.

The A chain usually is composed of 21 amino acid residues, and the B chain has 30; the molecular mass is thus about 5734 daltons. Although the amino acid sequence of insulin has been highly conserved in evolution, there are significant variations that account for differences in both biological potency and immunogenicity (De Meyts, 1994)

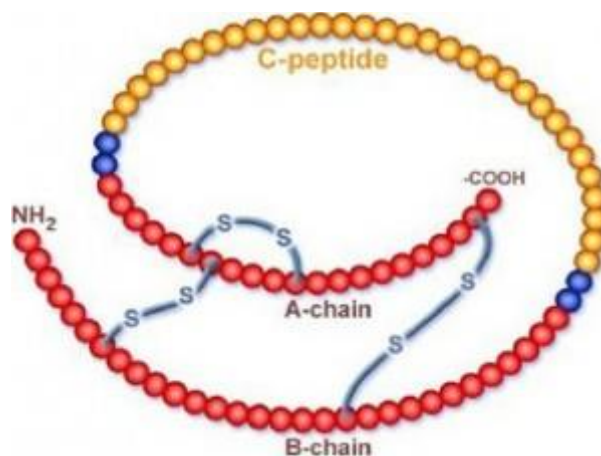


Figure No: 4. Structure of insulin

There is a single insulin gene and a single protein product in most species. However, rats and mice have two genes that encode insulin and synthesize two molecules that differ at two amino acid residues in the B chain.

The crystal structure reveals that the two chains of insulin form a highly ordered structure with α -helical regions in each of the chains. The isolated chains of insulin are inactive.

In solution, insulin can exist as a monomer, dimer, or hexamer. Two molecules of Zn^{2+} are coordinated in the hexamer, and this form of insulin presumably is stored in the granules of the pancreatic β cell. It is believed that

Zn²⁺ has a functional role in the hexamer formation and that this process facilitates the conversion of proinsulin to insulin and storage of the hormone.

Traditional insulin is hexameric in most of the highly concentrated preparations used for therapy. When the hormone is absorbed and the concentration falls to physiological levels (nanomolar), the hormone dissociates into monomers, and the monomer is most likely the biologically active form of insulin. Monomeric insulin is now available for therapy. (Goodman and Gillman, 2006)

Insulin is a member of a family of related peptides termed *insulinlike growth factors* (IGFs). The two IGFs (IGF-1 and IGF-2) have molecular masses of about 7500 daltons and structures that are homologous to that of proinsulin. However, the short equivalents of the C peptide in proinsulin are not removed from the IGFs.

In contrast with insulin, the IGFs are produced in many tissues, and they may serve a more important function in the regulation of growth than in the regulation of metabolism. These peptides, particularly IGF-1, are the presumed mediators of the action of growth hormone, and they originally were called *somatomedins*. The uterine hormone *relaxin* also may be a distant relative of this family of polypeptides, although the relaxin receptor clearly is distinct from those for insulin and IGF-1.

The receptors for insulin and IGF-1 are also closely related (Nakae *et al.*, 2001). Thus, insulin can bind to the receptor for IGF-1 with low affinity and *vice versa*. The growth-promoting actions of insulin appear to be mediated in part through the IGF-1 receptor, and there may be discordance between the metabolic potency of an insulin analog and its ability to promote growth.

1.4.2.Synthesis of Insulin

Like other peptide hormones insulin is synthesized as a precursor (preproinsulin) in the rough endoplasmic reticulum. Preproinsulin is transported to the Golgi apparatus, where it undergoes proteolytic cleavage first to proinsulin and then to insulin plus a fragment of uncertain function called C-peptide.¹

Insulin and C-peptide are stored in granules in β cells, and are normally cosecreted by exocytosis in equimolar amounts together with smaller and variable amounts of proinsulin. The main factor controlling the synthesis and secretion of insulin is the blood glucose concentration. β cells respond both to the absolute glucose concentration and to the rate of change of blood glucose.

Other stimuli to insulin release include amino acids (particularly arginine and leucine), fatty acids, the parasympathetic nervous system, peptide hormones for the gut and drugs that act on *sulfonylurea receptors*.

There is a steady basal release of insulin and also a response to an increase in blood glucose. This response has two phases: an initial rapid phase reflecting release of stored hormone, and a slower, delayed phase reflecting both continued release of stored hormone and new synthesis (Bolli G B *et al* 2000).

1.4.3.Regulation of Insulin Secretion

Insulin secretion is a tightly regulated process designed to provide stable concentrations of glucose in blood during both fasting and feeding. This regulation is achieved by the coordinated interplay of various nutrients, gastrointestinal hormones, pancreatic hormones, and autonomic neurotransmitters. Glucose, amino

acids, fatty acids, and ketone bodies promote the secretion of insulin. The islets of Langerhans are richly innervated by both adrenergic and cholinergic nerves. Stimulation of α_2 adrenergic receptors inhibits insulin secretion, whereas β_2 adrenergic receptor agonists and vagal nerve stimulation enhance release. In general, any condition that activates the sympathetic branch of the autonomic nervous system (such as hypoxia, hypoglycemia, exercise, hypothermia, surgery, or severe burns) suppresses the secretion of insulin by stimulation of α_2 adrenergic receptors. Predictably, α_2 adrenergic receptor antagonists increase basal concentrations of insulin in plasma, and β_2 adrenergic receptor antagonists decrease them. (Goodman and Gillman, 2006)

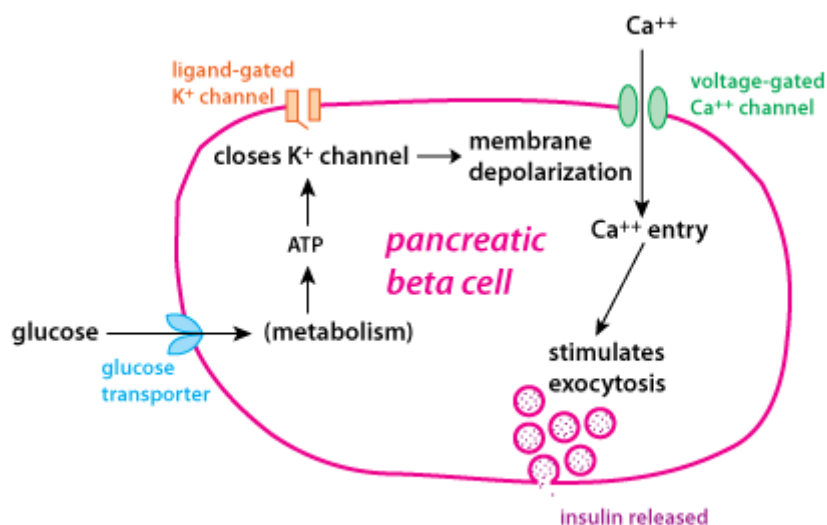


Figure No: 5. Regulation of Insulin Secretion

1.4.4. Distribution and Degradation of Insulin

Insulin circulates in blood as the free monomer, and its volume of distribution approximates the volume of extracellular fluid. Under fasting conditions, the pancreas secretes about 40 mg (1 unit) of insulin per hour into the portal vein to achieve a concentration of insulin in portal blood of 2 to 4 ng/ml (50

to 100 min/ml) and in the peripheral circulation of 0.5 mg/ml (12 minuits/ml) or about 0.1 nm.

After ingestion of a meal, there is a rapid rise in the concentration of insulin in portal blood, followed by a parallel but smaller rise in the peripheral circulation. A goal of insulin therapy is to mimic this pattern, but this is difficult to achieve with subcutaneous injections.

The half-life of insulin in plasma is about 5 to 6 minutes in normal subjects and patients with uncomplicated diabetes. This value may be increased in diabetics who develop anti-insulin antibodies.

Degradation of insulin occurs primarily in liver, kidney, and muscle . About 50% of the insulin that reaches the liver *via* the portal vein is destroyed and never reaches the general circulation. Insulin is filtered by the renal glomeruli and is reabsorbed by the tubules, which also degrade it.

Severe impairment of renal function appears to affect the rate of disappearance of circulating insulin to a greater extent than does hepatic disease. Hepatic degradation of insulin operates near its maximal capacity and cannot compensate for diminished renal breakdown of the hormone. Peripheral tissues such as fat also inactivate insulin, but this is of less significance quantitatively (Goodman and Gillman,2006).

1.4.5.Mechanism of Insulin

Insulin binds to a specific receptor on the surface of its target cells. The receptor is a large trans-membrane glycoprotein complex belonging to the kinase-linked type 3 receptor super families and consisting of two α and two β subunits.

Occupied receptors aggregate into clusters, which are subsequently internalized in vesicles, resulting in down-regulation. Internalized insulin is degraded in lysosomes, but the receptors are recycled to the plasma membrane.

- The signal transduction mechanisms that link receptor binding to the biological effects of insulin are complex. Receptor autophosphorylation -the first step in signal transduction-is a consequence of dimerization, allowing each receptor to phosphorylate the other.
- Insulin receptor substrate (IRS) proteins undergo rapid tyrosine phosphorylation specifically in response to insulin and insulin-like growth factor-1 but not to other growth factors. The best characterized substrate is IRS-1, which contains 22 tyrosine residues that are potential phosphorylation sites.
- It interacts with proteins that contain a so-called SH2 domain, thereby passing on the insulin signal. Knockout mice lacking IRS-1 are hypo responsive to insulin (insulin-resistant) but do not become diabetic because of robust B-cell compensation with increased insulin secretion. By contrast, mice lacking IRS-2 fail to compensate and develop overt diabetes, implicating the IRS-2 gene as a candidate for human type 2 diabetes.
- Activation of phosphatidylinositol 3-kinase by interaction of its SH2 domain with phosphorylated IRS has several important effects, including recruitment of insulin-sensitive glucose transporters (Glut-4) from the Golgi apparatus to the plasma membrane in muscle and fat cells.

- The longer-term actions of insulin entail effects on DNA and RNA, mediated partly at least by the Rassignalling complex. Ras is a protein t growth and cycles between an active GTP-bound form and an inactive GDP-bound form.
- Insulin shifts the equilibrium in favor of the active form, and initiates a phosphorylation cascade that results in activation of mitogen-activated protein kinase, which in turn activates several nuclear transcription factors, leading to the expression of genes that are involved both with cell growth and with intermediary metabolism. Regulation of the rate of mRNA transcription by insulin provides an important means of modulating enzyme activity. (rang and dale)

1.4.6. Cellular action of Insulin

Insulin is the main hormone controlling intermediary metabolism, having actions on liver, muscle and fat. It is an anabolic hormone: its overall effect is to conserve fuel by facilitating the uptake and storage of glucose, amino acids and fats after a meal. Acutely, it reduces blood sugar. Consequently, a *fall* in plasma insulin increases blood glucose. The biochemical pathways through which insulin exerts its effects and molecular aspects of its mechanism are discussed below.

1.4.7. Effect of insulin on Carbohydrate metabolism

Insulin influences glucose metabolism in most tissues, especially the liver, where it inhibits glycogenolysis (glycogen breakdown) and gluconeogenesis (synthesis of glucose from non-carbohydrate sources) while stimulating glycogen synthesis. It also increases glucose utilization (glycolysis), but the overall effect is to increase hepatic glycogen stores.

In muscle, unlike liver, uptake of glucose is slow and is the rate-limiting step in carbohydrate metabolism. The main effects of insulin are to increase facilitated transport of glucose via a transporter called Glut-4, and to stimulate glycogen synthesis and glycolysis.

Insulin increases glucose uptake by Glut-4 in adipose tissue as well as in muscle, enhancing glucoseol, which is esterifies with fatty acids to form triglycerides, thereby affecting fat metabolism metabolism. One of the main end products of glucose metabolism in adipose tissue is glycerol, which is esterifies with fatty acids to form triglycerides, thereby affecting fat metabolism.

1.4.8.Effect of insulin on fat metabolism

Insulin increases synthesis of fatty acid and triglyceride in adipose tissue and in liver. It inhibits lipolysis, partly via dephosphorylation (and hence inactivation) of lipases. It also inhibits the lipolytic actions of adrenaline, growth hormone and glucagon by opposing their actions on adenylatecyclase.

1.4.9.Effect of insulin on protein metabolism

Insulin stimulates uptake of amino acids into muscle and increases protein synthesis. It also decreases protein catabolism and inhibits oxidation of amino acids in the liver.

1.4.10.Other metabolic effect of insulin

Other metabolic effects of insulin include transport into cells of K^+ , Ca^{2+} , nucleosides and inorganic phosphate

1.4.11. Long term effect of insulin

In addition to its rapid effects on metabolism, exerted via altered activity of enzymes and transport proteins, insulin has long-term actions via altered enzyme synthesis. It is an important anabolic hormone during fetal development. It stimulates cell proliferation and is implicated in somatic and visceral growth and development.

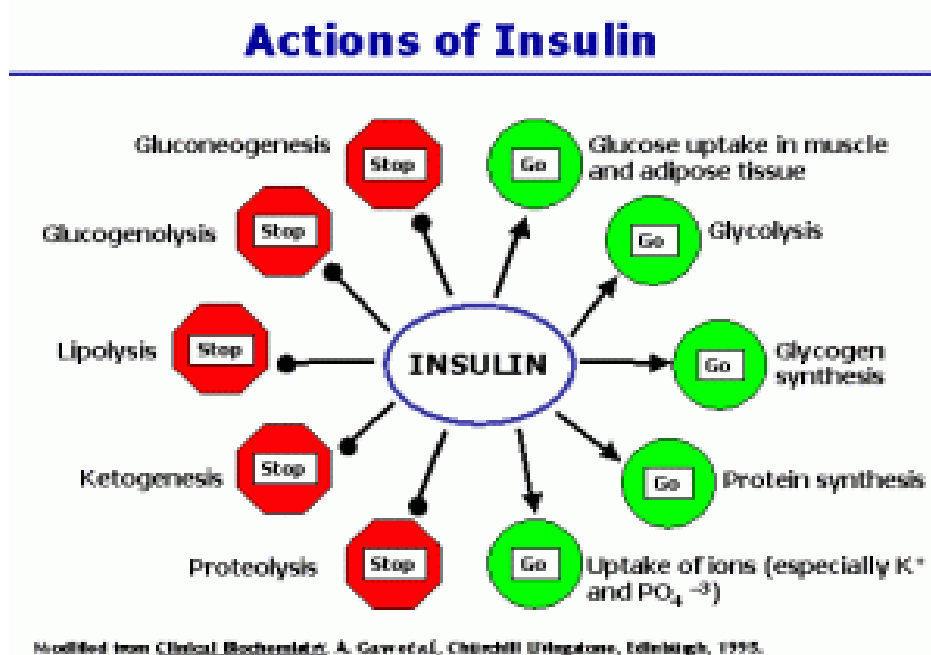


Figure No: 6. Long term effect of insulin

1.4.12. Insulin administration

Because insulin is a polypeptide, it is degraded in the gastrointestinal tract if taken orally. It therefore is generally administered by subcutaneous injection. [Note: In a hyperglycemic emergency, regular insulin is injected intravenously.] Continuous subcutaneous insulin infusion has become popular, because it does not require multiple daily injections.

Insulin preparations vary primarily in their times of onset of activity and in their durations of activity. This is due to differences in the amino acid sequences of the polypeptides. Dose, site of injection, blood supply, temperature, and physical activity can affect the duration of action of the various preparations. Insulin is inactivated by insulin-degrading enzyme (also called insulin protease), which is found mainly in the liver and kidney.

1.4.13. Adverse reactions to insulin

The symptoms of hypoglycemia are the most serious and common adverse reactions to an overdose of insulin. Long-term diabetics often do not produce adequate amounts of the counter-regulatory hormones (glucagon, epinephrine, cortisol, and growth hormone), which normally provide an effective defense against hypoglycemia. Other adverse reactions include weight gain, lipodystrophy (less common with human insulin), allergic reactions, and local injection site reactions. Diabetics with renal insufficiency may require adjustment of the insulin dose.

1.5. Consequences of diabetes mellitus

People living with diabetes may have to deal with short-term or long-term complications as a result of their condition.

- Short-term complications include hypoglycaemia diabetic ketoacidosis (DKA), and hyperosmolar hyperglycaemic state (HHS).
- Long-term complications include how diabetes affects your eyes (retinopathy), heart (cardiovascular disease), kidneys (nephropathy), and nerves and feet (neuropathy).

1.5.1. Acute complications

These include diabetic keto acidosis (DKA) and non-ketotic hyper-osmolar state (NKHS). While the first is seen primarily in individuals with type 1 DM, the latter is prevalent in individuals with type 2 DM. Both disorders are associated with absolute or relative insulin deficiency, volume depletion, and altered mental state.

1.5.2. Chronic complications

The chronic complications of diabetes mellitus affect many organ systems and are responsible for the majority of morbidity and mortality. Chronic complications can be divided into vascular and nonvascular complications. The vascular complications are further subdivided into microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular complications (coronary artery disease, peripheral vascular disease, and cerebrovascular disease). Nonvascular complications include problems such as gastroparesis, sexual dysfunction, and skin changes.

➤ Diabetic retinopathy

Diabetic retinopathy occurs in 3/4 of all persons having diabetes for more than 15 years and is the most common cause of blindness. There is appearance of retinal vascular lesions of increasing severity, culminating in the growth of new vessels.

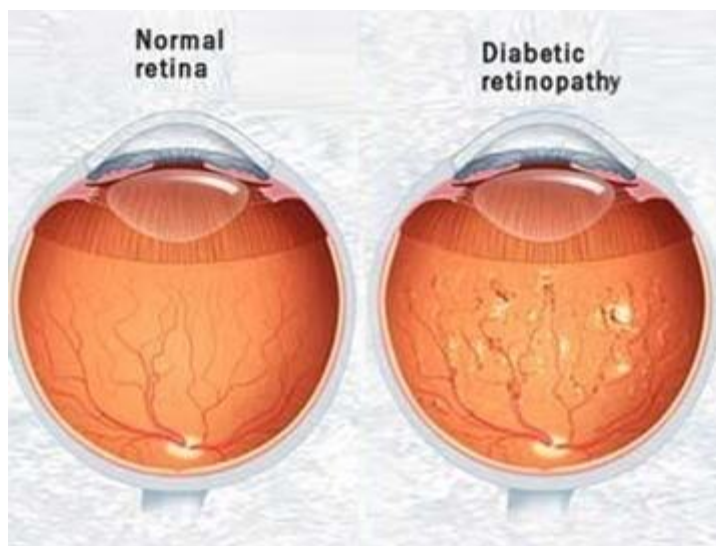


Figure No: 7. Diabetic retinopathy

Diabetic retinopathy is classified into two stages: non proliferative and proliferative.

The non-proliferative stage is marked by retinal vascular microaneurysms, blotshemorrhages and cotton-wool spots and includes loss of retinal pericytes, increased retinal vascular permeability and alterations in regional blood flow, and abnormal retinal microvasculature, all of which lead to retinal ischemia.

In proliferative retinopathy there is the appearance of neovascularization in response to retinal hypoxia. The newly formed vessels may appear at the optic nerve and/or macula and rupture easily, leading to vitreous hemorrhage, fibrosis, and ultimately retinal detachment (Aiello LP *et al*,1998).

➤ **Neuropathy**

About half of all people with diabetes have some degree of neuropathy, which can be polyneuropathy, mono-neuropathy and/or autonomic neuropathy.

In polyneuropathy there is loss of peripheral sensation which, when coupled with impaired microvascular and macrovascular junction in the periphery, can contribute to non-healing ulcers, the leading cause of non-traumatic amputation. There is thickening of axons, decrease in microfilaments, and capillary narrowing involving small myelinated or non-myelinated C-fibers.

It can occur both from direct hyperglycemia-induced damage to the nerve parenchyma and from neuronal ischemia leading to abnormalities of microvessels, such as endothelial cell activation, pericyte degeneration, basement membrane thickening, and monocyte adhesion.

Mono-neuropathy is less common than polyneuropathy and includes dysfunction of isolated cranial or peripheral nerves. Autonomic neuropathy can involve multiple systems, including cardiovascular, gastrointestinal, genitourinary, sudomotor, and metabolic systems (Chen YD *et al*, 1997).

➤ **Nephropathy**

This is a major cause of end-stage renal disease. There are glomerular hemodynamic abnormalities resulting in glomerular hyper-filtration, leading to glomerular damage as evidenced by microalbuminuria. There is overt proteinuria, decreased glomerular filtration rate, and end-stage renal failure.

Dysfunction of the glomerular filtration apparatus is manifested by microalbuminuria and is attributed to changes in synthesis and catabolism of various glomerular basement membrane macromolecules such as collagen and proteoglycans, leading to an increase in glomerular basement thickening.

Another possible mechanism to explain the increase in permeability of the glomerulus is the increase in renal VEGF levels, since VEGF is both an angiogenic and a permeability factor (Ritz E *et al*,1999).

➤ **Cardiovascular morbidity and mortality**

In diabetes mellitus there is marked increase in several cardiovascular diseases, including peripheral vascular disease, congestive heart failure, coronary artery disease, and myocardial infarction, and a one- to fivefold increase in sudden death. The absence of chest pain (silent ischemia) is common in individuals with diabetes, and a thorough cardiac evaluation is indicated in individuals undergoing major surgical procedures.

Despite proof that improved glycemic control reduces microvascular complications in diabetes mellitus, it is possible that macrovascular complications may be unaffected or even worsened by such therapies.

An improvement in the lipid profiles of individuals in the intensive group (lower total and low-density lipoprotein cholesterol, lower triglycerides) suggested that intensive therapy may reduce the risk of cardiac vascular mortality.

In addition to coronary artery disease, cerebrovascular disease is increased in individuals with diabetes mellitus (threefold increase in stroke).

Individuals with DM have increased incidence of congestive heart failure (diabetic cardiomyopathy). The etiology of this abnormality is probably multifactorial and includes factors such as myocardial ischemia from atherosclerosis, hypertension, and myocardial cell dysfunction secondary to chronic hyperglycemia.

Though DM itself does not increase levels of LDL, LDL particles found in type 2 DM are more atherogenic and are more easily glycosylated and susceptible to oxidation (Grundy SM *et al*, 1999).

➤ **Hypertension**

Hypertension can accelerate other complications of diabetes mellitus, particularly cardiovascular disease and nephropathy. (Tripathi B.K *et al*)

1.6. ANIMALS USED FOR THE SCREENING OF ANTIDIABETIC DRUG

1. Obese mouse
2. Diabetic mouse
3. Sand mouse (*Psammomysobesus*)
4. Spiny mouse (*Acomyscahirinus*)
5. BB rats
6. KK mouse
7. Yellow mouse
8. Yellow KK mouse
9. New Zealand obese mouse
10. Tuco-tuco (*Ctenomys talarum*)- these are burrowing rodents from Argentina.
11. Chinese hamster (*Cricetus griseus*)
12. NUDE mouse
13. Japanese wistar rat (Goto rat) etc. (www.Pharma.info.net)

1.6.1. CHEMICALS USED TO INDUCE DIABETES MELLITUS

A. Irreversible beta cytotoxic agents

1. Alloxan
2. Streptozotocin
3. Diphenylthiocarbazine
4. Diphenylthiocarbazine
5. Oxine-9-hydroxyquinolone
6. Vacor

B. Reversible beta cytotoxic agents

1. 6-amino nicotinamide
2. L-aspartase
3. Azide
4. Cyanide
5. Cyproheptadine
6. Phenytoin (www.Pharmainfo.net).

Commonly used diabetes inducing agents are,

1. Alloxan monohydrate
2. Streptozotocin (STZ)

Alloxan Monohydrate

The name alloxan is derived from Allantoin, a product of uric acid excreted by the fetus into the allantois and oxaluric acid derived from oxalic acid and urea, found in urine.

Biological effects

Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas (β cells) when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (Alloxan Diabetes) in these animals, with characteristics similar to type I diabetes in humans.

Mechanism of action

Alloxan is selectively toxic to insulin-producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the GLUT2 glucose transporter. Alloxan, in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid.

The beta cell toxic action of alloxan is initiated by free radicals formed in this redox reaction. One study suggests that alloxan does not cause diabetes in humans. Other studies show some correlation between alloxan plasma level and diabetes Type I in children. (www.wikipedia.com)

Streptozotocin

Streptozotocin or Streptozocin or Izoctazin or Zanosar (STZ) is a synthetic antineoplastic agent that is classically an anti-tumor antibiotic and chemically is

related to other nitrosureas used in cancer chemotherapy. Streptozotocin sterile powders are provided and prepared a chemotherapy agent.

Each vial of sterilized Streptozotocin powder contains 1 gr. of Streptozotocin active ingredient with the chemical name, 2-Deoxy-2-[[[(methylnitrosoamino) carbonyl] amino]-D-glucopyranose and 200 mg. citric acid. Streptozotocin was supplied by Pharmacia Company. Streptozotocin is available for intravenous use as a dry-frozen, pale yellow, sterilized product. Pure Streptozotocin has alkaline pH. When it is dissolved inside the vial in distilled water as instructed, the pH in the solution inside the vial will be 3.5-4.5 because of the presence of citric acid. This material is prepared in 1-gr vials and kept in cold store and refrigerator temperature (2-8 °C) away from light. (A. Akbarzadehet *al*, 2007)

Glibenclamide

Glibenclamide is a popular antidiabetic drug, belonging to class of sulfonylureas. The drug is widely used for treating type II diabetes. (www.neisslabs.com)

Mechanism of action

The drug works by inhibiting ATP-sensitive potassium channels in pancreatic β cells. This inhibition causes cell membrane depolarization, opening of voltage dependent calcium channels, thus triggering an increase in intracellular calcium into the beta cell which stimulates insulin release. (www.neisslabs.com)

Dosage

Dosage should be adapted to each individual patient and is determined by results of medical examinations. In general the initial dose is 2, 5 mg daily (half a *glibenclamide* tablet). The daily dose can then be raised gradually in steps of half tablets, but only after repeating medical examination. Raising the dose beyond three tablets daily dose not produce any increased response. When changing over from another oral antidiabetic preparation, with a similar mode of action, the dosage of *Glibenclamide* is determined by the amount of the previously administered dose and the medical examination. (www.neisslabs.com)

CHAPTER 2

LITERATURE REVIEW

Literature review is the first and most important step for the proper selection of plants and it also forms basis for the planning of any scientific work that has to be performed. Due to this reason, the review of literature regarding *Pouteria campechiana* has been done under various divisions like Pharmacognostical, Phytochemical, Pharmacological, Ethno medical and also miscellaneous reviews (www.indianmedicine.eidoc.ub.rug.nl/).

- Neire.M.De Gouveia *et al.*, ***Pouteria ramiflora* extract inhibits salivary amylolytic activity and decrease glycemic level in mice** in Annals of the Brazilian Academy of Sciences. February 27, 2013. ISSN 0001-3765.
- Moustafa H Baky *et al.*, reported **A Review on phenolic compounds from family sapotaceae** in Journal of pharmacognosy and phytochemistry 2016; 5(2): 280-287. E-ISSN 2278-4136, P-ISSN 2349-8234.
- Jun Ma *et al.*, reported **Analysis of Polyphenolic from the Fruits of Three *Pouteria* Species by selected on Monitoring Liquid Chromatography – Mass Spectroscopy** in Journal of Agriculture and Food Chemistry 2004, 52, 5873-5878.
- Adiyaman P *et al.*, reported **Identification and qualification of polyphenolic compounds in underutilized fruits (star fruit & Egg fruit) using HPLC** in Indian journal of traditional knowledge, Vol 15(3) July 2016 pp.487-493.
- Christine L.C. *et al.*, reported **Isolation and evaluation of antimutagenic activity of phenolic compounds from *pouteria campechiana*** in Philippine journal of science 137(1) ;1-10. June 2008 ISSN 0031-7683.

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- Aly M Elsayed *et al.*, reported **Chemical composition and biological activities of *Pouteria campechiana*** in Journal of medicinal plant research Vol.10(16) pp.209-215. April 25 2016. ISSN 1996-0875.
 - Raffi R. Isah *et al.*, reported **Potential cholesterol lowering activity of selected plant** in Science Diliman 28:2, 83-91, July-December 2016. ISSN 0115-7809.
 - H.Mehraj *et al.* , reported **Plant physiology and fruit secondary metabolites of Canistel (*Pouteria campechiana*)** in World applied science journal 33(12) :1908-1914,2015. ISSN 1818-4952.
 - Maduka S.de Lanerolle reported **The carotenoids of *Pouteria campechiana*** in Journal of the National science foundation Srilanka 2008.36(1):95-98.
 - Sufia Syed *et al.*, reported **A Comprehensive Review on Herbal Hepatoprotective in an International Journal of Pharmaceutical Research(Pharmacoerena)** Vol.1 Issue 2,December – 2015.ISSN 2455 – 2402.

CHAPTER 3

AIM AND PLAN OF WORK

3.1 AIM OF PRESENT STUDY

In recent year there has been a tremendous increase in demand for herbal drugs due to its safety, efficacy and better therapeutic results and also due to its economic pricing as compared to synthetic or allopathic drugs, which have several therapeutic complications.

The selection of this plant, *Pouteria campechiana* was made on the basis of its

- ✓ High therapeutic value
- ✓ Easy availability
- ✓ Degree of research work which is not done

Very less pharmacological studies have been carried out on the leaves of Hence, I have decided to choose the *Pouteria campechiana* project on Which detailed studies on Preliminary Phytochemical and Pharmacological activities of Oral glucose tolerance, and In-vivo Anti- diabetic studies is done.

3.2 THE PLAN OF WORK

The plan of work for the study of *Pouteria campechiana* carried out as follow.

Collection and authentication of raw material

1. Preliminary phytochemical studies
 - a. Preparation of extract
 - b. Qualitative phytochemical studies

2. Pharmacological studies

- a. Acute oral toxicity study
- b. Oral glucose tolerance test
- c. Screening of Anti- diabetic activity

➤ *In-vivo* study of anti-diabetic activity (Alloxan induced diabetic in high fat diet Rats)

CHAPTER 4

PLANT PROFILE

Plant Classification :

Kingdom : Plantae

Order : Ericales

Family : Sapotaceae

Genus : Pouteria

Species : Campechiana

Common names : Egg Fruit, Canistel, Lavulu, Kunth, Baehni.

Tree of *Pouteria campechiana*



Leaves and fruits of *Pouteria campechiana***Description:**

The *Pouteria campechiana* is an erect tree and generally not more than 8m tall, reach height 27-30 m and the trunk may attain diameter of 1 m. Slender in habit or with a spreading crown, it has brown, furrowed bark and abundant white, gummy latex. Young branches are velvety brown.

The evergreen leaves, alternate but mostly grouped at the branch tips, are relatively thin, glossy, short to long-stemmed, oblanceolate, lanceolate-oblong, or obovate, bluntly pointed at the apex, more sharply tapered at the base; 11.25-28 cm long, 4-7.5 cm wide.

Fragrant, bisexual flowers, solitary or in small clusters, are borne in the leaf axils or at leafless nodes on slender pedicles. They are 5 or 6 lobed, cream coloured, silky-hairy, about 8-11 mm long.

The fruit, extremely variable in form and size, may be nearly round, with or without a pointed apex or curved beak, or may be somewhat oval, ovoid, or spindle-shaped. Length varies from 7.5-12.5 cm and width from 5-7.5 cm. On ripening, the skin turns lemon yellow, golden- yellow or pale orange-yellow, is very smooth and glossy.(Negreros-Castillo*etal.*,)

CHAPTER 5

MATERIALS AND METHODS

5.1 COLLECTION AND IDENTIFICATION

5.1.1 Collection of specimen:

The species for the proposed study that is leaves of collected *Pouteria campechiana* carefully from the Ponnani, Malappuram Dt, Kerala.

5.1.2 Taxonomical identification:

The plant was positively identified by Dr.Prabhukumar.K.M Senior Scientist and Head, Plant Systematics and Genetic Resource Division and CMPR Herbaria, Centre for Medicinal Plant Research, Arya Vaidya Sala, Kottakkal. The plant was authenticated as *Pouteria campechiana* (Kunth) Baehni of *Sapotaceae* family.

5.1.3 Shade drying:

After collection, the leaves of *Pouteria campechiana* are washed thoroughly with water to remove the dirt particles and other foreign material adheres to leaves. Then after, the leaves were wiped off with cotton cloth and transferred to newspaper and evenly spreader on to paper.

The *Pouteria campechiana* leaves were subjected to shade drying to treat fungus until complete dryness of leaves. Then the dried leaves were powdered by mixer grinder until to get coarse powder, which was used for further detailed studies, extraction with solvent and phytochemical studies.

5.2.2 PRELIMINARY PHYTOCHEMICAL ANALYSIS

Extraction of *Pouteria campechiana* leaves:

Ethanol extract:

About 250gm of air dried powdered material was taken in 3000ml soxhlet apparatus and extracted with petroleum ether until green colour disappear. At the end of day the powder was taken out and dried. After drying it was again packed and extracted by using ethanol (S.D. Fine Chemicals Ltd. Mumbai, India) as solvent, till colour disappeared. The temperature was maintained at 55°C-65°C. After that extract was concentrated by distillation and solvent was recovered. The final solution was evaporated to dryness. The colour, consistency and yield of ethanolic extract were noted.

Table: 5.1 Nature of extract of *Pouteria campechiana*

S.No.	Name of extract	Colour	Consistency	Yield% W/W
1.	Ethanolic extract	Dark Greenish	Sticky mass	8

5.3 CHEMICAL TESTS:

A) Test for carbohydrates:

1. Molisch's Test: It consists of treating the compounds of α -naphthol and concentrated sulphuric acid along the sides of the test tube.

Purple colour or reddish violet colour was produced at the junction between two liquids. (Kokate, C.K *et al.*, 2000)

2. **Fehling's Test:** Equal quantity of Fehling's solution A and B is added. Heat gently, brick red precipitate is obtained.

B) Test for Alkaloids:

1. **Dragendroff's Test:** To the extract, add 1ml of Dragendroff's reagent Orange red precipitate is produced.

2. **Wagner's test:** To the extract add Wagner reagent. Reddish brown precipitate is produced.

3. **Mayer's Test:** To the extract add 1ml or 2ml of Mayer's reagent. Dull white precipitate is produced.

4. **Hager's Test:** To the extract add 3ml of Hager's reagent yellow Precipitate is produced.

C) Test for Steroids and Sterols:

1. **Salkowski test:** Dissolve the sample of test solution in chloroform and add equal volume of conc. sulphuric acid. Bluish red cherry red and purple color is noted in chloroform layer, whereas acid assumes marked green fluorescence.

D) Test for Glycosides:

1. **Bortrager test:** Add a few ml of dilute sulphuric acid to the test solution. Boil, filter and extract the filtrate with ether or chloroform. Then organic layer is separated to which ammonia is added, pink, red or violet colour is produced in organic layer.

2. **Killer Killani test:** Sample is dissolved in acetic acid containing trace of ferric chloride and transferred to the surface of concentrated sulfuric acid. At

the junction of liquid reddish brown color is produced which gradually becomes blue.

E) Test for Saponins:

Foam test: About 1ml of alcoholic sample is diluted separately with distilled water to 20ml, and shaken in graduated cylinder for 15 minutes. 1 cm layer of foam indicates the presence of saponins.

F) Test for Flavonoids

Ferric chloride test – Test solution when treated with few drops of Ferric chloride solution, blackish red color indicating the presence of flavonoids.

Alkaline reagent Test – Test solution when treated with sodium hydroxide solution, shows increase in the intensity of yellow color which would become colorless on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids.

Lead acetate solution Test – Test solution when treated with few drops of lead acetate (10%) solution, no yellow precipitate is formed presence of flavonoids.

G) Test for Tri-Terpenoids:

In the test tube, 2 or 3 granules of tin was added, and dissolved in a 2ml of thionyl chloride solution and test solution is added. Pink colour is produced which indicates the presence of triterpenoids.

H) Tests for Tannins and Phenolic Compounds:

To 2-3 ml of extract, add few drops of following reagents:

- a) **5% FeCl₃ solution:** deep blue-black color.
- c) **Gelatin solution:** white precipitate not formed

K) Test for Proteins and Amino acids**a) Biuret test:**

Add 1 ml of 40% sodium hydroxide and 2 drops of 1% copper sulphate to the extract, a violet colour indicates the presence of proteins.

b) Ninhydrin test:

Add 2 drops of freshly prepared 0.2% Ninhydrin reagent to the extract and heat. A blue colour develops indicating the presence of proteins, peptides or amino acids.

c) Xanthoprotein test:

To the extract, add 20% of sodium hydroxide or ammonia. Orange colour indicates presence of aromatic amino acid.

5.5 PHARMACOLOGICAL EVALUATION:**5.4 TOXICOLOGICAL EVALUATION****Determination of LD₅₀ value of ethanolic extract of *Pouteria campechiana*****Acute Oral Toxicity Study:**

The procedure was followed by using OECD guidelines 423 (Acute toxic class method). The acute toxic class method is a step wise procedure with 3 animals

of single sex per step. Depending on the mortality and / or moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test animals while allowing for acceptable data based scientific conclusion.

The method uses defined doses (5, 50, 300, 2000mg/kg body weight) and the results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemical which cause acute toxicity.

Animals:

Female albino mice weighing 20-25g were used in the present study. All rats were kept at room temperature of 22-25°C in the animal house. All the animals were followed the internationally accepted ethical guidelines for the care of laboratory animals. Prior to the experiments, rats were fed with standard food for one week in order to adapt to the laboratory conditions. The experimental protocol has been approved by institutional animal ethics, committee, JKKMMRF College of Pharmacy, Proposal number – JKKMMRFCP/IAEC/2017/001 B.Komarapalayam, Namakkal.

Procedure:

Twelve animals Albino mice, (25-30gm) were selected for studies.

The starting dose of ethanolic extracts of *Pouteria campechiana* 300mg/kg, b.w, p.o, was administered.

Most of the crude extracts possess LD₅₀, value more than 2000mg/kg of the body weight of the animal used. Dose volume was administered 0.1ml/100gm body weight to the animal by oral route.

After giving the dose toxic signs were observed within 3-4 hours. Body weight of the animals before and after administration, onset of toxicity and signs of toxicity like changes in the skin and fur, eyes and mucous membrane and also respiratory, circulatory, autonomic and central nervous systems and somatomotor activity and behavior pattern, sign of tremors, convulsion, salivation, diarrhea, lethargy and sleep and coma was also to be noted, if any, was observed. The animal toxic or death was observed upto 14 days.

Observation

Acute toxicity studies and evaluation of dates are studied as per the guideline of OECD (423).

No toxicity or death was observed for these given dose levels, in selected and treated animals. So the LD₅₀ of the ethanolic extract of leaves of *Pouteria campechiana* was greater than 2000mg/kg (LD₅₀>2000mg/kg).

Hence the biological dose was fixed at three levels, 200 and 400mg/kg body weight for the extract.

5.5.1 ORAL GLUCOSE TOLERANCE TEST (OGTT):

The overnight fasted (18hr) normal rats were taken and divided into four groups consists of six animals. They were provided with drinking water only. Normal saline solution was administered to group I animals. Group II animals were received *Glibenclamide* (3mg/kg,b.w) as a standard. *Pouteria campechiana* ethanol

extract (200 and 400 mg/kg) was administered by oral route to group III and IV. Glucose (2mg/kg) load was fed 30 minutes after the administration of extracts. Blood was withdrawn from tail vein under mild ether anesthesia initial, 30,60 and 90 minutes after glucose administration and glucose level were estimated using glucose strips and a glucometer(Standard diagnostics Ltd). Blood glucose levels were noted and reported.

5.5.2 EVALUATION OF ANTI-DIABETIC ACTIVITY:

Animals:

Wistar albino rats (150-200g) were selected for either sex, for studies and they were kept in a standard polypropylene cage at room temperature of $27\pm 2^{\circ}\text{C}$, relative humidity 60-70% and well ventilated. They were fed a standard rat pellet and water ad libitum. Animals were deprived of food initially for 16 hrs but had free access to water. The experimental protocol has been approved by institutional animal ethics, committee, JKKMMRF College of Pharmacy, Proposal number – JKKMMRFCP/IAEC/2017/001 B.Komarapalayam, Namakkal.

Chemicals:

Alloxan monohydrate (LOBA Chemie, Mumbai, India) was purchased, preserved at 25°C and used for this study.

Glibenclamide is an oral antidiabetic preparation with an efficient hypoglycemic action. Daonil (Glibenclamide) (S.K.Prasad *et.al*, 2009) manufactured by Aventis Pharma Ltd. Goa, India, was collected from market and preserved at room temperature.

Induction of Experimental Diabetes:

Hyperglycemia / Diabetes was induced by single intraperitoneal injection of freshly prepared aqueous solution of alloxan monohydrate 150 mg/kg, to overnight fasted rats. After 48 hrs of alloxan injection, the animals which did not developed hyperglycemia i.e glucose level > 200mg/dl, were injected or replaced. Immediately after confirmation of diabetes, rats were classified into five groups of six rats each. Standard drug used for treatment, Glibenclamide, 5 mg/kg, ethanolic test extract were prepared, 200mg/kg and 400mg/kg in 2% Carboxy Methyl Cellulose(CMC) and were given orally. Taking six rats in each five groups did evaluation of antidiabetic effect.(G.Jyothi et al., 2013)

Experimental Design:

Experimental rats were divided into 5 groups of six animals each all the group of animals were induced diabetic except control and treated for 21 days as follows.

- Group I: Normal control rats fed with vehicles only. (Normal saline with 1% CMC)
- Group II: Diabetic controls rats (Alloxan monohydrate 120mg/kg body weight of rats, once i.p injection).
- Group III: Diabetic rats treated with standard drug, Glibenclamide 3mg/kg per oral body weight.
- Group IV: Diabetic rats treated with ethanolic extract of *Pouteria campechiana* 200mg/kg, per oral, dissolved in 1% carboxy methyl cellulose (CMC).
- Group V: Diabetic rats treated with ethanolic extract of *Pouteria campechiana* 400mg/kg, per oral, dissolved in 1% carboxy methyl cellulose (CMC).

Sample collection:

Fasting blood glucose (FBG) of all rats was determined before the start of the experiment. Blood sample was collected at weekly intervals from tail vein puncture till the end of study. In the continuous 21 days of drug treatment, a blood glucose level of all animals was determined at the 0, 7, 14, 21 day by using one touch glucometer (SD Check) method.

5.6 EVALUATION OF PARAMETERS:**1. Estimation of changes in body weight of the animals:**

Body weight of all rats was measured on starting day (0 day) of the experiment and 21st day of the experiment. Both initial and final body weights were noted and reported.

2. Estimation of blood glucose level:

Glucose level in plasma was estimated by glucose oxidase/ peroxidase method using a commercial kit from Med source Ozone Biomedicals Pvt Ltd followed by Trinder, p.(1969)Annals.Clin. Bio chem.6,24.

Reagents:

1. Enzyme reagent
2. Buffer solution
3. Glucose standard (100 mg%)

Procedure:

10 µl of plasma was added to 1.0 ml of working enzyme reagent, mixed well and incubated at 37°C for 15 min. The colour developed was read at 505 nm against blank containing distilled water instead of the sample. A standard was also processed similarly.

The level of glucose is expressed as mg/dl.

5.8 STATISTICAL ANALYSIS

All the values of body weight and fasting blood glucose level were expressed as mean \pm standard error of mean (S.E.M) and was analyzed for significance by ANOVA and groups were compared by Tukey-Kramer multiple comparison test. Differences between groups (p Value) were considered significant at $P < 0.05$ level.

CHAPTER 6

RESULTS AND DISCUSSION

Based on literature review the leaves of *Pouteria campechiana* were selected and project work was carried on *Pouteria campechiana* belonging to the family Sapotaceae was collected and authenticated. The result of the present study show that the ethanol extract of *Pouteria campechiana* exerts anti-diabetic effect against alloxan induced diabetes.

6.1 PHARMACOGNOSTICAL STUDIES

6.1.1 ANALYTICAL PARAMETERS

6.2 PRILIMINARY PHYTOCHEMICAL STUDIES

Table No.6.3: Weight of extract of *Pouteria campechiana*

Name of extract	Yield(%w/w)
Ethanol	8

The extract obtained were subjected to qualitative Phytochemical test to find out the active constituents.

Table No.6.4: Qualitative Phytochemical analysis of heart wood parts extract

TEST FOR PHYTOCONSTITUENTS	RESULT
Saponins	-
Alkaloids	+
Glycosides	+
Tannins and phenolic compounds	+, -
Carbohydrates	+
Proteins and aminoacids	+
Flavonoids	+
Steroids	+

(+) - Present (-) - Absent

DISCUSSION:

The preliminary Phytochemical studies were done in the ethanolic extract of *Pouteria campechiana* leaves result suggest that presence of **Alkaloids, Carbohydrate, Glycosides, Proteins and aminoacids, flavonoids, Steroids, and tannins.**

6.3 PHARMACOLOGICAL STUDIES**6.3.1 ACUTE ORAL TOXICITY STUDIES**

The acute oral toxicity of the ethanolic extract of *Pouteria campechiana* was carried out as per OECD 423-guidelines (Acute toxic class method). Acute toxicity studies revealed that $LD_{50} > 2000 \text{ mg/kg}$ for the extract. Hence, the biological dose was fixed at EEPC 200mg and 400mg of body weight for the extract.

6.3.2 EFFECT ON GLUCOSE TOLERANCE

In OGTT, the doses of EEPC 200 mg/kg and 400 mg/kg increased the tolerance for glucose suggesting increased peripheral utilization of glucose. The reduction in blood glucose level was dose dependent.

Table No.: 6.6 Effect of ethanolic extract of *Pouteria campechiana* and *Glibenclamide* on glucose tolerance of diabetic rats.

Groups	Treatment	Change in blood glucose levels(mg/dl)			
		Fasting	After 30 Minutes	After 60 minutes	After 90 minutes
1.	Glucose 2mg/kg	84.99±2.90	125.22±2.02	127.88±1.90	108.51±2.89
2.	<i>Glibenclamide</i> 3mg/kg	68.01±3.32	83.10±1.50 a	62.01±2.55 a	52.71±3.24 a
3.	EEPC 200mg/kg	67.05±1.49	113.08±6.99 b	113.76±3.02	96.42±2.98 a
4.	EEPC 400mg/kg	78.69±2.80	99.92±1.99 a	105.11±7.88 b	90.65±1.91 a

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a=*** = p<0.001; b= ** = p<0.01; c= * =p<0.05.EEAN. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests). Normal control group I was compared with group 2(std drug) and extract treated groups III , IV.

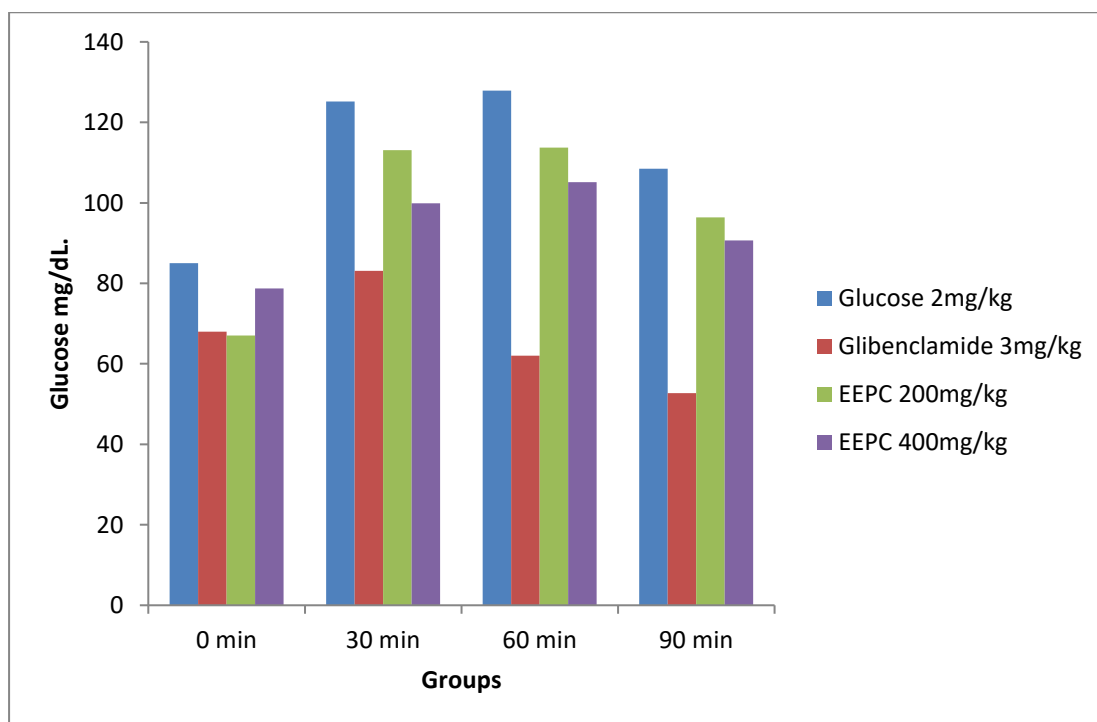


Figure No.6.1: Effect of ethanolic extract of *Pouteria campechiana* and *Glibenclamide* on glucose tolerance of diabetic rats.

6.3.4 EVALUATION OF PARAMETERS

1. Changes in body weight:

Vehicles control animals were found to be stable in their body weight but significant reduction in diabetic control group during 21 days (Table. 6.7). Alloxan caused body weight reduction, which is slightly reversed by ethanolic extract of *Pouteria campechiana* treated (200mg/kg and 400mg/kg) groups after 21 days.

While, significant ($p < 0.01, p < 0.001$) increase in body weight was observed in rats treated with ethanolic extract of *Pouteria campechiana*. The EEPC treated diabetic rats (400mg/kg) were slightly increased the body weight level and showed in Table No: 6.7, Fig No: 6.2.

Table No: 6.7 Body weight changes in ethanolic extract of *Pouteria campechiana* and *Glibenclamide* on control and experimental groups of rats

Group	Treatment	Body weight changes (g)	
		Day 0	Day 21
I	Normal control rats (vehicles only)	145±7.67	204.15±11.94
II	Diabetic control rats	162.5±8.54 b	129.18±7.67 b
III	Diabetic group + Glibenclamide 5mg/kg	150±2.44 a	208.37±02.37 a
IV	Diabetic group + EEPC (200mg/kg)	154.17±7.67 b	200±6.46 b
V	Diabetic group + EEPC (400mg/kg)	162.6±04.05 a	210.6±17.98 c

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a=*** = $p < 0.001$; b= ** = $p < 0.01$; c=* = $p < 0.05$. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests). (Diabetic control group II was compared with Normal control group I, group III and extract treated groups IV, V compared with Diabetic control group II.)

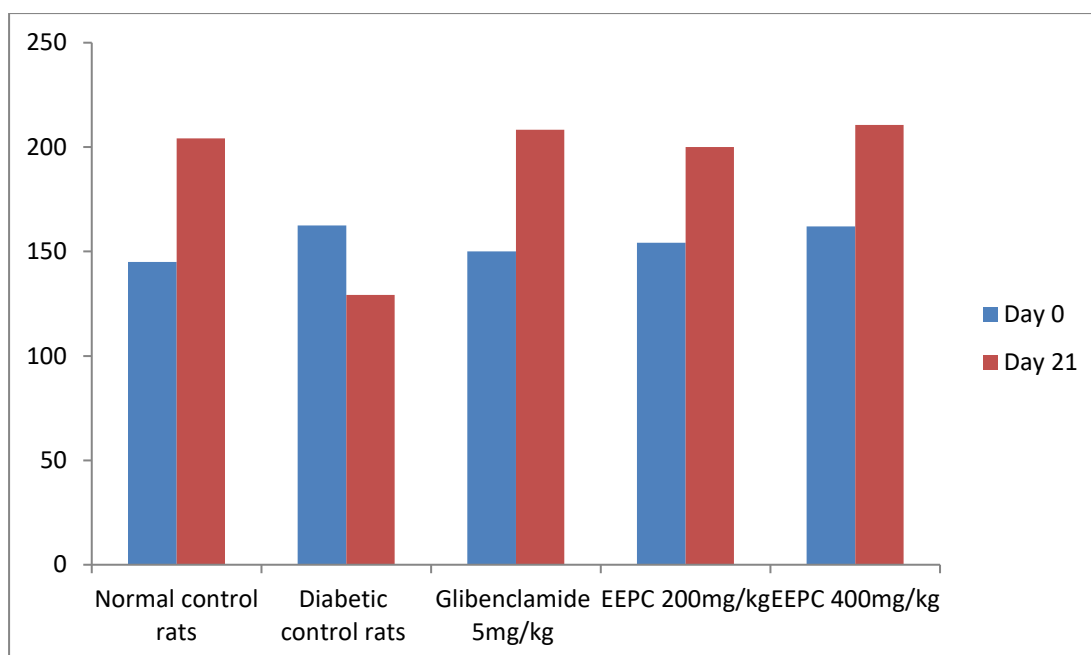


Figure: 6.2 Body weight changes in ethanolic extract of and *Pouteria campechiana* Glibenclamide on control and experimental groups of rats

Values are given as mean \pm S.E.M for groups of six animals each. Values are statistically significant at a=*** = $p < 0.001$; b= ** = $p < 0.01$; c=* = $p < 0.05$. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests). (Diabetic control group II was compared with Normal control group I, group III and extract treated groups IV, V compared with Diabetic control group II.)

2. Changes in blood glucose:

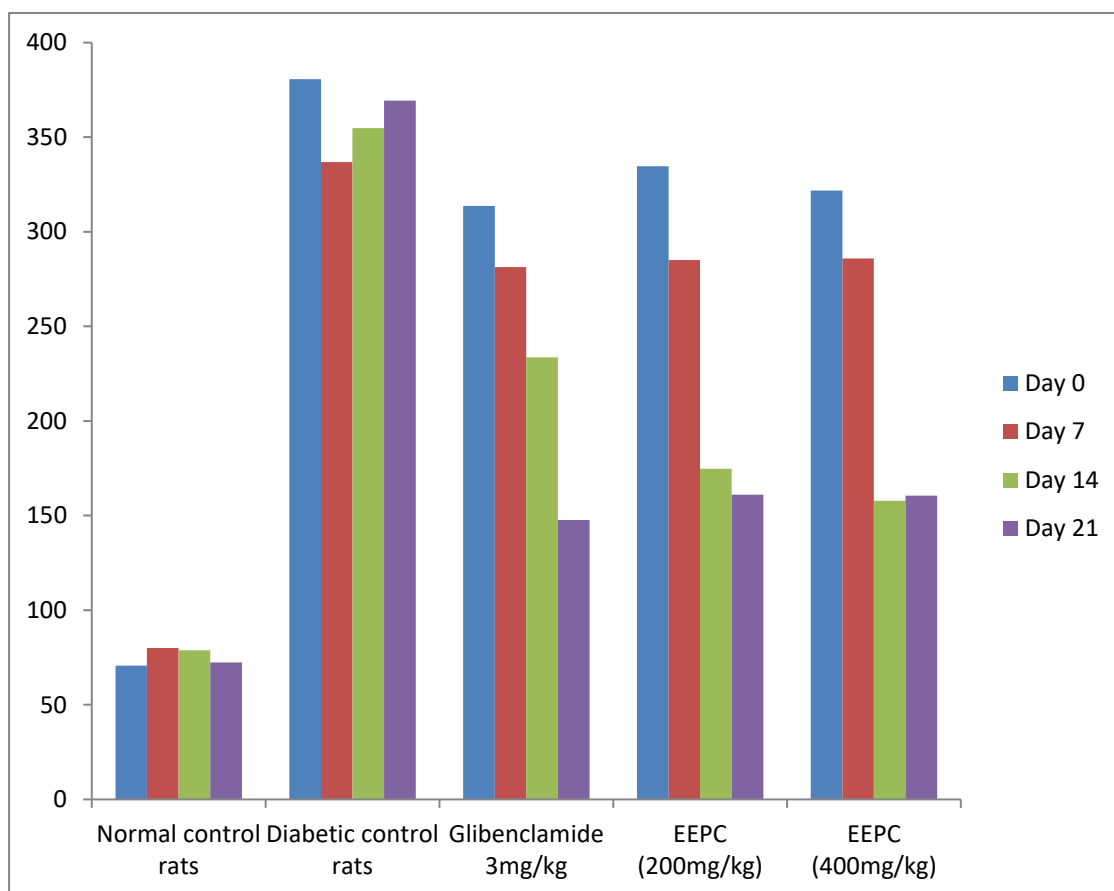
A significant increase in the level of blood glucose, was observed in diabetic control rats when compared to control rats. Administration of EEPC and *Glibenclamide* to diabetic rats significantly decreased the elevated level of blood glucose, near to control level. Showed Table No: 6.8, Figure No: 6.4.

Table No. 6.8. Effect of *Pouteria campechiana* ethanolic extract of and *Glibenclamide* on blood glucose level

Group	Treatment	Blood glucose level (mg/dl)			
		Day 0	Day 7	Day 14	Day 21
I	Normal control rats (vehicles only)	70.65±1.42	80±2.34	78.83±2.36	72.33±1.82
II	Diabetic control rats	380.6±1.57 a	336.84±2.18 a	354.84±2.81 a	369.32±1.09 a
III	Diabetic group + Glibenclamide 3mg/kg	313.6±2.09 a	281.34±1.56 a	233.65±1.42 a	147.67±1.05 a
VI	Diabetic group + EEPC (200mg/kg)	334.66±8.90 c	285±03.26 a	174.82±2.91 a	161±2.08 a
V	Diabetic group + EEPC (400mg/kg)	321.84±12.16 c	286±5.08 b	157.82±1.30 a	160.5±2.74 a

Values are expressed as Mean ±SE, n = 6 by Dunnett's t test; *P <0.01 Vs Control **P > 0.001 Vs Control.

Figure No: 6.4 Effect of ethanolic extract of *Pouteria campechiana* and Glibenclamide on blood glucose level.



Values are given as mean \pm S.E.M for groups of six animals each. Values are statistically significant at a=*** = $p < 0.001$; b= ** = $p < 0.01$; c=* = $p < 0.05$. (Analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests). (Diabetic control group II was compared with Normal control group I, group III and extract treated groups IV, V compared with Diabetic control group II.)

CHAPTER 7

SUMMARY AND CONCLUSION

The leaves of *Pouteria campechiana* belonging to the family sapotaceae has been examined to gain an insight of its phytochemical and pharmacological behavior.

The preliminary phytochemical investigation of powdered *Pouteria campechiana* showed the presence of Carbohydrates, Alkaloids, Glycosides, Steroid, Terpenoids, Flavonoids, Proteins and Amino acids.

The pharmacological and acute toxicity studies of ethanolic extract was performed by following, OECD-423 guidelines (Acute toxic class method). No mortality or acute toxicity was observed upto 2000mg/kg of body weight. The Biological dose of extract *Pouteria campechiana* dose was selected 200mg/kg and 400mg/kg in this dose possessed significant antidiabetic activity.

Alloxan causes a massive destruction of β -cells of the isletsof langher-hans, resulting in reduced synthesis and release of insulin. The function of the insulin suppressed, which leads to high level of hyperglycemic and eventually to death, but the different extracts of *pouteria campechiana* showed antidiabetic effect in alloxan induced diabetic rats and reduced the mortality rate significantly.

Overall, it can be concluded that ethanolic extract of *Pouteria campechiana* can be used as a natural source of anti-diabetic activity. Further pharmacological and biochemical studies are needed to isolate and characterize active compound responsible for the anti-diabetic activity.

CHAPTER 8

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