1. NEED FOR THE STUDY

Accumulation of lipids in diabetes is mediated through specially deficiency hence, diabetic patient more prone to hypertriglyceridaemia and hypercholesterolaemia (Kasiappan et al., 2005). One of the major pathogenesis of lipid metabolism disturbances in diabetes is the increased mobilization of fatty acids from adipose tissue and secondary elevation of free fatty acid level in the blood.

Hyperlipidemia is the major risk factor for Congestive Heart Failure (CHF) and it is one of the leading cause for cardiac complication like angina pectoris, myocardial infarction. Worldwide so many people dies because of diabetes associated with hyperlipidemia.

Our Indian meditional plant have large source for curing this type of serious diseases and use of meditional plant to treat diabetes associated with hyperlipidemia is devoid of dangerous side effect. Because of this reason traditional medicinal plant is selected for the present study. Present study is evaluated for antidiabetic and antihyperlipidemic activity.

*Euphorbia neriifolia* Linn. is also known as Common Milk Hedge in English, Sehund and Thohar in Hindi belonging to family Euphorbiaceae. (Gaur et al., 2009). *Euphorbia neriifolia* Linn. is traditionally used to treat diabetes in different rural regions of India (Irfan et al., 2005) Traditional use of this plant in diabetes and no scientific and research data is reported to treat diabetes regarding this plant.

Our attempt is to establish the scientific data of this plant as common alternative antidiabetic agent.
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1.2 Diabetes

1.2.1 Definition:

Diabetes mellitus, often simply referred to as diabetes is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). (Leon S. et al., 2004)

1.2.2 Types Of Diabetes

There are three main types of diabetes:

- **Type 1 diabetes**: results from the body’s failure to produce insulin, and presently requires the person to inject insulin. (Also referred to as insulin-dependent diabetes mellitus, IDDM for short, and juvenile diabetes.)

- **Type 2 diabetes**: results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency. (Formerly referred to as non-insulin-dependent diabetes mellitus, NIDDM for short, and adult-onset diabetes.)

- **Gestational diabetes**: is when pregnant women, who have never had diabetes before, have a high blood glucose level during pregnancy. It may precede development of type 2 DM.

Other forms of diabetes mellitus include congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids, and several forms of monogenic diabetes. (Leon S. et al., 2004)
1.2.3 Signs and Symptoms

(A) Type 1 diabetes

Primary symptom is in the form of nausea and vomiting. In later stage, which leads to diabetic ketoacidosis, a state of metabolic dysregulation characterized by the smell of acetone, the body starts breaking down the muscle tissue and fat for producing energy hence, causing fast weight loss. Dehydration is also usually observed due to electrolyte disturbance. In advanced stages, coma and death is witnessed

(B) Type 2 diabetes

- Increased fatigue
- Excessive thirst (polydipsia)
- Excessive urination (polyuria)
- Excessive eating (polyphagia):
- Poor wound healing
- Infections
- Blurry vision
- Altered mental status: Agitation, unexplained irritability, inattention, extreme lethargy, or confusion can all be signs of very high blood sugar, ketoacidosis, hyperosmolar, nonketotic syndrome hyperglycemia, or hypoglycemia. (Leon S. et al., 2004).

1.2.4 Causes of Diabetes

The precise Etiology of most cases of diabetes is uncertain, although certain contributing factors are as follows.
(A) Type 1 Diabetes

Type 1 Diabetes is autoimmune disease that affects 0.3% on average. It is result of destruction of beta cells due to aggressive nature of cells present in the body. Some of the Etiology and Risk factors which may trigger type 1 diabetes may be genetic, poor diet (malnutrition) and environment (virus affecting pancreas). Secondly, in most of the cases, diabetes occurs because there is abnormal secretion of some hormones in blood which act as antagonists to insulin.

Example- Adrenocortical hormone, Adrenaline hormone and Thyroid hormone.

(B) Type 2 Diabetes

Type 2 Diabetes is also called non insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes. It occurs when the body produces enough insulin but cannot utilize it effectively. This type of diabetes usually develops in middle age. A general observation says that about 90% of people suffering with diabetes are type 2; about 80 percent are overweight. It is more common among people who are older; obese; have a family history of diabetes; have had gestational diabetes. More the Etiology and Risk factors carried by an individual, the higher the risk for developing diabetes.

Following are the Causes of Diabetes

- **Hereditary or Inherited Traits**: It is strongly believed that due to some genes which passes from one generation to another, a person can inherit diabetes. It depends upon closeness of blood relationship as mother is diabetic, the risk is 2 to 3%, father is diabetic, the risk is more than the previous case and if both the parents are diabetic, the child has much greater risk for diabetes.

- **Age**: Increased age is a factor which gives more possibility than in younger age. This disease may occur at any age, but 80% of cases occur after 50 year, incidences increase with the age factor.

- **Poor Diet (Malnutrition Related Diabetes)**: Improper nutrition, low protein and fiber intake, high intake of refined products are the expected reasons for developing diabetes.
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- **Obesity and Fat Distribution**: Being overweight means increased insulin resistance, that is if body fat is more than 30%, BMI is more than 25, waist girth 35 inches in women or 40 inches in males.

- **Sedentary Lifestyle**: People with sedentary lifestyle are more prone to diabetes, when compared to those who exercise thrice a week, are at low risk of falling prey to diabetes.

- **Stress**: Either physical injury or emotional disturbance is frequently blamed as the initial cause of the disease. Any disturbance in Cortiosteroid or ACTH therapy may lead to clinical signs of the disease.

- **Drug Induced**: Clozapine, olanzapine, risperidone, quetiapine and ziprasidone are known to induce this lethal disease.

- **Infection**: Some of the staphylococci are supposed to be responsible factor for infection in pancreas.

- **Sex**: Diabetes is commonly seen in elderly especially males but, strongly in women and those females with multiple pregnancy or suffering from Polycystic Ovarian Syndrome.

- **Hypertension**: In many studies there is direct relation between high systolic pressure and diabetes.

- **Serum lipids and lipoproteins**: High triglyceride and cholesterol level in the blood is related to high blood sugars, in some cases it has been studied that risk is involved even with low HDL levels in circulating blood. (Laurance et al., 1997)

### 1.2.5 Diagnostics Tests Of Diabetes

Doctors use special tests in diagnosing diabetes and also in monitoring blood sugar level control in known diabetics. Diabetes mellitus is characterized by recurrent or persistent hyperglycemia, and is diagnosed by demonstrating any one of the following

- Fasting plasma glucose level $\geq 7.0$ mmol/L (126 mg/dL).
- Plasma glucose $\geq 11.1$ mmol/L (200 mg/dL) two hours after a 75 g oral glucose load as in a glucose tolerance test.
- Glycated hemoglobin (Hb A1C) $\geq 6.5%$.

A number of laboratory tests are available to confirm the diagnosis of diabetes.
(A) Fingerstick blood glucose:

This is a rapid screening test that may be performed anywhere, including community-based screening programs.

A finger stick blood glucose test is not as accurate as testing the patient’s blood in the laboratory but is easy to perform, and the result is available right away.

The test involves sticking the patient’s finger for a blood sample, which is then placed on a strip. The strip goes into a machine that reads the blood sugar level. These machines are only accurate to within about 10% of true actual laboratory values.

Fingerstick blood glucose values may be inaccurate at very high or very low levels, so this test is only a preliminary screening study. (William et al., 1997).

(B) Fasting plasma glucose:

The patient will be asked to eat or drink nothing for 8 hours before having blood drawn (usually first thing in the morning). If the blood glucose level is greater than or equal to 126 mg/dL without eating anything, they probably have diabetes.

If the result is abnormal, the fasting plasma glucose test may be repeated on a different day to confirm the result, or the patient may undergo an oral glucose tolerance test or a glycosylated hemoglobin test (often called "hemoglobin A1c") as a confirmatory test.

If fasting plasma glucose level is greater than 100 but less than 126 mg/dL, then the patient has what is called impaired fasting glucose, or IFG. This is considered to be pre-diabetes. The patient does not have diabetes, but they are at high risk of developing diabetes in the near future.

(C) Oral glucose tolerance test:

This test involves drawing blood for a fasting plasma glucose test, then drawing blood for a second test at two hours after drinking a very sweet drink containing 75 grams of sugar.

If the blood sugar level after 2 hour the sugar drink is greater than or equal to 200 mg/dL, the patient has diabetes.

If the blood glucose level is between 140 and 199, then the patient has impaired glucose tolerance (IGT), which is also a pre-diabetic condition.
(D) Glycosylated hemoglobin or hemoglobin A1c:

This test is a measurement of how high blood sugar levels have been over about the last 120 days (the average life-span of the red blood cells on which the test is based). Excess blood glucose hooks on to the hemoglobin in red blood cells and stays there for the life of the red blood cell. The percentage of hemoglobin that had excess blood sugar attached to it can be measured in the blood. The test involves having a small amount of blood drawn.

A hemoglobin A1c test is the best measurement of blood sugar control in people known to have diabetes. A hemoglobin A1c result of 7% or less indicates good glucose control. A result of 8% or greater indicates that blood sugar levels are too high for too much of the time. The hemoglobin A1c test is less reliable to diagnose diabetes than for follow-up care. Still, a hemoglobin A1c result greater than 6.1% is highly suggestive of diabetes. Generally, a confirmatory test would be needed before diagnosing diabetes.

The hemoglobin A1c test is generally measured about every three to six months for people with known diabetes, although it may be done more frequently for people who are having difficulty achieving and maintaining good blood sugar control. (Graname-Smith et al., 2002)

1.3 EPIDEMIOLOGY OF DIABETES

1.3.1 Epidemiology of Diabetes In World

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Table 1.1: Top 10 countries for number of people aged 20-79 years with diabetes in 2000; 2010 and 2030 (Shaw JE, et al., 2010)

1.3.2 Epidemiology Of Diabetes In India

Nowhere is the diabetes epidemic more pronounced in India as the World Health Organization (WHO) reports show that 32 million people had diabetes in the year 2000 (Wild S, et al., 2000). The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025 (Sieree R, et al., 2006)
northern India (New Delhi), 11.6%; and western India (Mumbai), 9.3%. The study also suggested that there was a large pool of subjects with impaired glucose tolerance (IGT), 14% with a high risk of conversion to diabetes.

Figure 1.3: Recent population based studies of prevalence of type 2 diabetes in different parts of India
Figure 1.4: Differences in mortality rates among diabetic and non-diabetic individuals.

The Chennai urban population study (CUPS) cohort showed that the overall mortality rates were nearly three-fold higher (18.9 per 1000 person-years) in people with diabetes compared to non diabetic subjects (5.3 per 1000 person-years, $P=0.004$) (Mohan V. et al., 2006). The hazard ratio (HR) for all cause mortality for diabetes was found to be 3.6 compared to non diabetic subjects. The study also showed that mortality due to cardiovascular (diabetic subjects: 52.9% vs. non diabetic subjects 24.2%, $P=0.042$) and renal (diabetic subjects 23.5% vs. non diabetic subjects 6.1%, $P=0.072$) causes was higher among diabetic subjects.

1.4 PATHOPHYSIOLOGY OF DIABETES

The pathophysiology of diabetes mellitus (all types) is related to the hormone insulin, which is secreted by the beta cells of the pancreas. This hormone is responsible for maintaining glucose level in the blood. It allows the body cells to use glucose as a main energy source. However, in a diabetic person, due to abnormal insulin metabolism, the body cells and tissues do not make use of glucose from the blood, resulting in an elevated level of blood glucose or hyperglycemia. Over a period of time, high glucose level in the bloodstream can lead to severe complications, such as eye disorders, cardiovascular diseases, kidney damage and nerve problems.
(A) Type 1 diabetes (DM-1)

The pancreas cannot synthesize enough amount of insulin hormone as required by the body. It may due to following cause:

1. **Immune dysregulation:** caused by genetic susceptibility and environmental modifiers, leads to development of autoantibodies against various islet cell components, including glutamic acid decarboxylase antibodies (GAD-65), islet cell antibodies (ICA512/IA-2) and insulin antibodies (IAA). These antibodies serve as marker for DM-1. Indeed, the best predictor for future development of DM-1 is the expression of multiple autoantibodies. Beta cell destruction is thought to be primarily a T-cell mediated process, as evidence by the presence of intense insulits in newly diagnosed patients. Beta cell destruction is variable being more rapid in younger individuals and slower in older individuals. Type 1 diabetes is associated with other autoimmune disorders including Graves’ disease, Addison's disease and autoimmune polyendocrine syndromes.

<table>
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<td>80%</td>
<td>99%</td>
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<tr>
<td>ICA512/IA-2</td>
<td>ICA512/IA-2 Ab</td>
<td>50%</td>
<td>99%</td>
</tr>
<tr>
<td>Insulin</td>
<td>IAA</td>
<td>50%</td>
<td>99%</td>
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Table 1.2: Major islet-cell autoantigen-specific antibodies in type 1 diabetes

2. **Genetic predisposition.** The capacity of the β islet cell to produce insulin and to adapt to the increasing demands of the insulin resistance state is genetically predetermined to a great extent. Rare monogenic defects in insulin synthesis or secretion have been described:

- Mutant insulin gene (resulting in dysfunctional insulin)
- Abnormal processing of pro-insulin
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- Defects in glucose-mediated insulin secretion by the beta islet cell. These autosomal dominant conditions are known as Maturity Onset Diabetes of the Young (MODY) syndromes. The following genetic defects have been described:
  - MODY1 - Mutant transcription factor, Hepatic Nuclear Factor-4 α (HNF-4α)
  - MODY2 - Impaired beta cell Glucokinase activity
  - MODY3 - HNF-1 α
  - MODY4 - IPF1 (necessary for normal beta cell development and function).

Although these defects provide us with important insight into the physiology of beta cell function, these candidate genes only account for a small number of adult diabetes (1-2%).

3. Environmental exposure. These include virus, toxin, stress obesity, nutrition, and physical activity, which are closely associated with each other.

Figure 1.5: Pathogenesis of DM-1. Various factors cause β cells destruction and insulin deficiency is responsible for DM-1.
(B) Type 2 diabetes (DM-2)
Previously known as NIDDM or adult-onset diabetes, is the most prevalent form of diabetes, accounting for over 90% of all cases of diabetes. Type 2 diabetes is characterized by varying degrees of insulin resistance and insulin deficiency. Resistance to the action of insulin will result in impaired insulin mediated glucose uptake in the periphery (by muscle and fat), incomplete suppression of hepatic glucose output and impaired triglyceride uptake by fat. To overcome the insulin resistance (and therefore prevent abnormal fuel metabolism and maintain normal glucose and lipid levels), beta islet cells will increase the amount of insulin secreted. Higher circulating insulin levels will overcome the impedance to the action of insulin. Abnormal fuel metabolism (hyperglycemia and dyslipidemia) occurs when there is a mismatch between insulin requirements, as dictated by insulin resistance, and insulin supply, as dictated by beta cell function. Therefore, For DM-2 to develop, two defects are necessary: insulin resistance and insulin deficiency relative to the resistance. (Carol et al., 2005)
Figure 1.6: Pathogenesis of DM-2. Insulin resistance causes a decrease in peripheral uptake of glucose, abnormal metabolism, hypertension, and hypercoagulability in the body, which may cause cardiovascular disease (CVD).

1.5 HIGH FAT DIET AND TYPE II DIABETES

Prospective studies of the natural history of type 2 diabetes have shown that the pre-diabetic state is characterized by resistance to insulin-mediated glucose disposal and compensatory hyperinsulinemia (Lillioja et al., 1993 & Warram et al., 1990). The transition from prediabetes to type 2 diabetes occurs when the secretory capacity of the pancreatic B cell is no longer able to compensate for the insulin resistance (Reed et al., 2000).

Insulin resistance plays a primary role in the development of type-2 diabetes (Defronzo et al., 1988) and is a characteristic feature of other health disorders including obesity, dyslipidemias, hypertension, and cardiovascular disease (Reaven et al., 1988). It is widely known that an elevation in circulating free fatty acid (FFA) levels impairs insulin action and leads to insulin resistance in animals and humans (Boden et al., 1997; Chakley et al., 1998 & Boden et al., 1994). This may represent a physiologic mechanism of insulin resistance because elevated FFA levels are generally observed in most human insulin-resistant states (Ling et al., 2006; Hevener et al., 2001 & Defronzo et al., 1992).

The HFD (58% calories as fat) causes insulin resistance in rats. The feeding of HFD for a period of 2 weeks produced rats with insulin resistance syndrome as was characterized by the increased body weight (obesity), mild hyperglycemia, hypertriglyceridemia, hypercholesterolemia and compensatory hyperinsulinemia (Reaven et al., 1988; Frayne et al., 1993; Perseghin et al., 1997).

High fat-fed rats with high dose of Streptozotocin (45 and 55 mg/kg) resembled more like type 1 diabetes. In contrast, STZ (25 mg/kg) did not produce significant hyperglycemia in NPD as well as HFD-fed rats (Srinivasan K. et al., 2004). Interestingly, the dose of STZ (35 mg/kg, i.p.) that produced frank hyperglycemia in HFD-fed rats failed to produce the same in NPD-fed rats. The HFD rat model with low dose of STZ (35 mg/kg) represent the pathophysiological state of type 2 diabetes and was accompanied by marginal increase in body weight in contrast to the catabolic loss of body weight,
characteristic of diabetic condition produced by high dose of STZ. Hence, HFD in combination with low dose of STZ (35 mg/kg) is the best model for study of type-II diabetes (Srinivasan et al., 2004).

It is interesting and noteworthy that the development of diabetes occurs only in insulin-resistant HFD-fed rats but not in NPD-fed normal rats following low dose of STZ (35 mg/kg, i.p.). The reasons for the high degree of glycemic difference induced by STZ (35 mg/kg, i.p.) between these two groups might be that HFD-fed rats were already insulin resistant together with compensatory hyperinsulinemia to maintain glucose homeostasis, and hence, even the slight insult by low dose of STZ that could compromise the beta cell function might lead to drastic hyperglycemic effect as against the NPD-fed normal animals where in the effect could be compensated by normal defense homeostasis mechanisms. Furthermore, in the case of HFD-fed rats, they were already mildly hyperglycemic due to insulin resistance, thus enhancing their susceptibility to diabetogenic effect of STZ.

HFD has been shown to induce insulin resistance by different mechanisms but considered mainly through Randle or glucose–fatty acid cycle (Randle et al., 1963). Briefly, the presence of high level of triglycerides due to excess fat intake could constitute a source of increased fatty acid availability and oxidation.

Increased plasma FFA concentrations are typically associated with many insulin-resistant states, including obesity and type 2 diabetes mellitus (Reaven et al., 1988; & Frayne KN. 1993). In a cross-sectional study of young normal weight offspring of type 2-diabetes patients, altered FFA metabolism was found as a contributing factor in causing the insulin resistance. (Perseghin et al., 1997).(Randle et al., 1963) Demonstrated that FFAs compete with glucose for substrate oxidation in isolated rat heart muscle and rat diaphragm muscle, and speculated that increased fat oxidation might cause the insulin resistance associated with obesity. The proposed mechanism pointed to FFAs causing elevation of the intramitochondrial acetyl-coenzyme A/coenzyme A and NADH/NAD1 ratios, with subsequent inactivation of pyruvate dehydrogenase. This in turn would cause citrate concentrations to increase, leading to inhibition of phosphofructokinase, a key rate-controlling enzyme in glycolysis. Subsequent accumulation of Glucose-6-Phosphate
would inhibit hexokinase II, resulting in decreased glucose uptake (Srinivasan et al., 2004).

Apart from glucose, these fat-fed, insulin-resistant STZ animals also showed abnormalities in lipid metabolism as evidenced from increased Plasma triglyceride and cholesterol levels, as in case of human type 2 diabetic patients which might contribute to various cardiovascular complications. The hypertriglyceridemia observed in these fat-fed/STZ rats may be due to increased absorption and formation of triglycerides in the form of chylomicrons following exogenous consumption of diet rich in fat or through increased endogenous production of TG-enriched hepatic very low density lipoprotein (VLDL) and decreased TG uptake in peripheral tissues (Srinivasan, et al., 2004). Hypercholesterolemia may be attributed to increased dietary cholesterol absorption from the small intestine following the intake of HFD in a diabetic condition (Shafrir et al., 2003 & Colca et al., 1991).

The elevated glucose concentrations were relatively stable over a period of 10 weeks when tested on this model (Srinivasan. et al., 2004) Hence, this model could also be useful for the long term studies on diabetic complications, such as neuropathy, nephropathy and hypertension.

1.6 DIABETIC VASCULAR COMPLICATIONS

Diabetes mainly cause two type of following vascular complication.

1) **Micro vascular** like retinopathy,
   nephropathy,
   neuropathy
   foot complications

2) **Macro vascular** like cardiovascular complications
   cerebrovascular complications
   peripheral arterial complications
1.7 THERAPEUTIC STRATEGIES FOR DIABETES

Therapies for Diabetes may be divided into

1.7.1 Insulin preparation
1.7.2 Oral Hypoglycemic drugs
1.7.3 Herbal medicines and nutraceuticals

1.7.1 Insulin preparation

Use of strict control of glucose in case of Type-I diabetes

1.7.1.1 Rapid-acting and short-acting insulin preparations

Regular insulin is a short-acting, crystalline zinc insulin and soluble. Regular insulin is a short-acting, soluble, crystalline zinc insulin. Regular insulin is usually given subcutaneously (or intravenously in emergencies), and it rapidly lowers blood glucose. Regular insulin, lispro insulin and aspart insulin are pregnancy category. Glulisine has not been studied in pregnancy. Due to its short duration of action and their rapid, lispro, aspart and glulisine forms of insulin are classified as rapid acting insulins. These agents offer more flexible treatment regimens and may lower the risk of hypoglycemia. Insulin lispro differs from regular insulin in that lysine and proline at positions 29 and 28 in the B chain are reversed. This results in more rapid absorption after subcutaneous injection than is seen with regular insulin; as a consequence, insulin lispro acts more rapidly. Peak levels of insulin lispro are seen at 30 to 90 minutes after injection, as compared with 50 to 120 minutes for regular insulin. Insulin lispro also has a shorter duration of activity. Insulin aspart and insulin glulisine have pharmacokinetic and pharmacodynamic properties similar to those of insulin lispro. They are administered to mimic the prandial (mealtime) release of insulin, and they are usually not used alone but, rather, along with a longer-acting insulin to assure proper glucose control. Like regular insulin, they are administered subcutaneously. Insulin lispro is usually administered 15 minutes prior to a meal or immediately following a meal, whereas glulisine can be taken either 15 minutes prior a meal or within twenty minutes after starting a meal. Aspart insulin must be ministered just prior to the meal. All of the rapid-acting formulations are
suitable for intravenous administration, although regular insulin is most commonly used when the intravenous route is needed. Insulin lispro, insulin aspart, and insulin glulisine may also be used in external insulin pumps.

1.7.1.2 Intermediate-acting insulin

Neutral protamine Hagedorn (NPH) insulin is a suspension of crystalline zinc insulin combined at neutral pH with a positively charged polypeptide, protamine. [Note: Another name for this preparation is insulin isophane.] Its duration of action is intermediate. This is due to delayed absorption of the insulin because of its conjugation with protamine, forming a less-soluble complex. NPH insulin should only be given subcutaneously (never intravenously). It is used for basal control and is usually given along with rapid- or short-acting insulin for mealtime control. [Note: A similar compound called neutral protamine lispro (NPL) insulin, has been prepared that is used only in combination with insulin lispro.

1.7.1.3 Long-acting insulin preparations

- Insulin glargine: The isoelectric point of insulin glargine (GLAR-geen) is lower than that of human insulin, leading to precipitation at the injection site, thereby extending its action. It is slower in onset than NPH insulin and has a flat, prolonged hypoglycemic effect. Like the other insulins, it must be given subcutaneously.

- Insulin detemir: Insulin detemir has a fatty-acid side chain. The addition of the fatty-acid side chain enhances association to albumin. Slow dissociation from albumin results in long-acting properties similar to those of insulin glargine.

Synthetic Amylin Analog

- Pramlintide is a synthetic amylin analog. By acting as an amylinomimetic, pramlintide delays gastric emptying, decreases postprandial glucagon secretion and improvement satiety. Pramlintide is administered by subcutaneous injection and should be injected immediately prior to meals. Pramlintide may not be mixed in the same syringe with any insulin preparation. Adverse effects are mainly gastrointestinal and consist of nausea, anorexia, and vomiting. Pramlintide should
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not be given to patients with diabetic gastroparesis (delayed stomach emptying) or a history of hypoglycemic unawareness.

1.7.2 Oral hypoglycemic agents

1.7.2.1 Sulfonylureas

These agents are classified as insulin secretagogues, because they promote Mechanisms of action of the sulfonylureas: These include 1) From the beta cells stimulation of insulin release occurs by blocking the ATP-sensitive K⁺ channels, resulting in depolarization and Ca²⁺ influx; 2) reduction in hepatic glucose production; and 3) increase in peripheral insulin sensitivity.

- Pharmacokinetics and fate: These drugs bind to serum proteins, are metabolized by liver and are excreted by the liver or kidney. Tolbutamide has shorten duration of action where as the second generation agents last for 24 hours.
- Adverse effects: These drugs should be used with caution in patients with hepatic or renal insufficiency, because late removal of the drugs results in its accumulation may cause hypoglycaemia. Renal impairment is a particular problem in the case of those agents that are metabolized to active compounds, such as glyburide. Glyburide has minimal transfer across the placenta and may be a reasonably safe alternative to insulin therapy for diabetes in pregnancy.

1.7.2.2 Meglitinide analogs

This class of agents includes repaglinide and nateglinide. Although they are not sulfonylureas, they have common actions.

- Mechanism of action: Like the sulfonylureas, their action is dependent on functioning pancreatic cells. They bind to a distinct site on the sulfonylurea receptor of ATP-sensitive potassium channels, thereby initiating a series of reactions culminating in the release of insulin. However, in contrast to the sulfonylureas, the meglitinides have a rapid onset and a short duration of action. They are particularly effective in the early release of insulin that occurs after a
meal and, thus, are categorized as postprandial glucose regulators. Combined therapy of these agents with metformin or the glitazones has been shown to be better than monotherapy with either agent in improving glycemic control. Meglitinides should not be used in combination with sulfonylureas due to overlapping mechanisms of action.

- Pharmacokinetics and fate: Drugs are well absorbed orally after one to thirty minutes before meals. Both are metabolized to inactive products by CYP3A4 and are excreted via bile.
- Adverse effects: Repaglinide has been reported to cause severe hypoglycemia in patients who are also taking the lipid-lowering drug gemfibrozil. Weight gain is less of a problem with the meglitinides than with the sulfonylureas.

1.7.2.3 Biguanides

Metformin, the only currently available biguanide, is classed as an insulin sensitizer; that is, it increases glucose uptake and utilization by target tissues, thereby decreasing insulin resistance. Like the sulfonylureas, metformin requires insulin for its action, but it differs from the sulfonylureas in that it does not promote insulin secretion. Hyperinsulinemia is not a problem. Thus, the risk of hypoglycemia is far less than that with sulfonylurea agents, and it may only occur if caloric intake is not adequate or exercise is not compensated for calorically.

- Mechanism: Metformin also slows intestinal absorption of sugars and improves peripheral glucose uptake and utilization. A very important property of this drug is its ability to modestly reduce hyperlipidemia LDL, VLDL and HDL. These effects may not be apparent until 4 to 6 weeks of use. The patient often loses weight because of loss of appetite. Metformin may be used alone or in combination with one of the other agents, as well as with insulin. Hypoglycemia has occurred when metformin was taken in combination.
- Pharmacokinetics and fate: Orally absorbed and is not bound to serum proteins and is not metabolized.
Chapter 1 Introduction and literature review

- Adverse effects: These are largely gastrointestinal. It should be used with precaution in the patients greater than eighty years of age or in those with a history of CHF or alcohol abused. It should be temporarily discontinued in patients undergoing diagnosis require i.v radiographic contrast agents. Rarely, potentially fatal lactic acidosis has occurred. Long-term use may interfere with vitamin $B_{12}$ absorption.

1.7.2.4 Thiazolidinediones or glitazones

Another group of agents that are insulin sensitizers are the thiazolidinediones (TZDs) or, more familiarly the glitazones. Although insulin is required for their action, these drugs do not promote its release from the pancreatic cells; thus, hyperinsulinemia does not result.

- Pharmacokinetics and fate: Pioglitazone and rosiglitazone are well absorbed orally and extensively bind with plasma proteins. Above both drugs undergo metabolism by different cytochrome P450. Renal elimination of pioglitazone is negligible, with the majority of the active drug and metabolites excreted in the bile and eliminated in the feces. The metabolites of rosiglitazone are primarily excreted in the urine. No dosage adjustment is required in renal impairment. It is recommended that these agents not be used in nursing mothers.

- Adverse effects: Because there have been deaths from hepatotoxicity in patients taking troglitazone, it is recommended that liver enzyme levels of patients on these medications be measured initially and periodically thereafter. Very few cases of liver toxicity have been reported with rosiglitazone or pioglitazone. Weight increase can occur, possibly through the ability of TZDs to increase subcutaneous fat or due to fluid retention.

- Other uses: As with metformin, the relief of insulin resistance with the TZDs can cause ovulation to resume in premenopausal women with polycystic ovary syndrome.

1.7.2.5 Glucosidase Inhibitors
Acarbose and miglitol are orally active drugs

- **Mechanism:** Glucosidase inhibitors are taken at the beginning of meals. They act by delaying the digestion of carbohydrates, thereby resulting in lower postprandial glucose levels. Both drugs exert their effects by reversibly inhibiting membrane-bound glucosidase in the intestinal brush border. This enzyme is responsible for the hydrolysis of oligosaccharides to glucose and other sugars. Unlike the other oral hypoglycemic agents, these drugs do not stimulate insulin release, nor do they increase insulin action in target tissues. Thus, as monotherapy, they do not cause hypoglycemia. However, when used in combination with the sulfonylureas or with insulin, hypoglycemia may develop.

- **Pharmacokinetics:** Acarbose is absorbed poorly and it is metabolized primarily by intestinal bacteria and some of the metabolites are excreted and absorbed into the urine. Miglitol is very well absorbed but has no systemic effects and excreted in unchanged form by the kidney.

- **Adverse effects:** Major side effects are diarrhea, abdominal pain and flatulence. This drug is contraindicated in patient of inflammatory bowel disease.
1.7.3 Herbal Medicines And Neutraceuticals
<table>
<thead>
<tr>
<th>Plant/Drug</th>
<th>Effect/Activity</th>
<th>Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsicum frutescens</td>
<td>Diabetic neuropathy</td>
<td>High</td>
</tr>
<tr>
<td>Carica papaya</td>
<td>Diabetic wounds</td>
<td>Animal</td>
</tr>
<tr>
<td>Camellia sinensis</td>
<td>Diabetic nephropathy, Diabetic cataract, Diabetic retinopathy, Anti-oxidative effects</td>
<td>Animal</td>
</tr>
<tr>
<td>Centella asiatica</td>
<td>Gotu Kola, Diabetic microangiopathy and oedema, Diabetic wound healing</td>
<td>High, Animal</td>
</tr>
<tr>
<td>Cinnamomum cassia</td>
<td>Arteriogenic affect</td>
<td>Animal</td>
</tr>
<tr>
<td>Cinnamomum zeylanicum</td>
<td>Diabetic nephropathy</td>
<td>Medium</td>
</tr>
<tr>
<td>Colocasia esculenta</td>
<td>Dashen, Diabetic nephropathy</td>
<td>Animal</td>
</tr>
<tr>
<td>Curcuma longa</td>
<td>Turmeric, Anti-oxidative effect, Diabetic retinopathy</td>
<td>Animal, Animal</td>
</tr>
<tr>
<td>Dioscorea cayensis</td>
<td>Yam, Diabetic nephropathy</td>
<td>Animal</td>
</tr>
<tr>
<td>Eugenia jambolana/Syzygium cumini</td>
<td>Jam, Diabetic neuropathy, Diabetic nephropathy, Diabetic gastropathy, Diabetic cataract, Ulcer healing, Anti-oxidative effect</td>
<td>Animal, Animal</td>
</tr>
<tr>
<td>Fish oil</td>
<td>Endothelial function, Anti-oxidative effects, Diabetic nephropathy, Anti-inflammatory effect</td>
<td>High, Medium, Animal</td>
</tr>
<tr>
<td>Ganoderma lucidum</td>
<td>Lingzhi mushroom, Diabetic nephropathy</td>
<td>Animal</td>
</tr>
<tr>
<td>Ginkgo biloba</td>
<td>Diabetic retinopathy, Diabetic nephropathy, Endothelial dysfunction</td>
<td>Medium, High/Animal, High</td>
</tr>
</tbody>
</table>
Table 1.3: Medicinal Plants and Nutraceuticals for the Treatment of Diabetic Complications with Clinical and Animal Evidences

<table>
<thead>
<tr>
<th>Medicinal Plants/ Nutraceuticals</th>
<th>Common Name</th>
<th>Beneficial Effects in Diabetic Complications</th>
<th>Level of Scientific Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aloe vera</td>
<td>Aloe</td>
<td>• Anti-inflammatory effect</td>
<td>Animal studies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Diabetic wound healing</td>
<td>Animal studies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Diabetic nephropathy</td>
<td>Animal studies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Anti-oxidative effect</td>
<td>Animal studies</td>
</tr>
</tbody>
</table>

1.8 *Euphorbia neriifolia* Linn.

1.8.1 Introduction

Hedge euphorbia, Olaeander and Suhundah belongs to the family of Euphorbiaceae.

Properties of *Euphorbia neriifolia* Linn (Chatterjee et al., 1994).

- Katu (pung (Chatterjee et al., 1994).

Characteristics

- Large succulent shrub
- Stipular thorns

Spread

- Through out India
- Deccan peninsula of India
- Widely distributed throughout the world

Synonyms (Veena et al., 2011).
1.8.2 Morphology

- Small erect glabrous shrub
- Jointed cylindric with sharp stipular thorns arranged in 5 irregular rows
- Succulent leaves (This plant is leafless except monsoon season)
- Flowers in a bunch, female and male
- three chambered, tricoccaus but deeply divided in such a way that,

2.8.3 Chemical constituents (Pracheta et al., 2011)

- Latex: 69-94% water soluble and cautcouch.
- Gum resin, traces of alkaloids, wax, chlorophyll, tannin, mucilage, calcium oxalate
- Phenolic substance like gallic acid quercetine and traces of essential oils
- Euphol, friedelan taraxerol, nriifoliol, euphorbol, ingenol triacetate and pelargonin
- Tritertenes like neriifolione and cycloartenol
- Triterpenoids like methylenecycloartenol, euphorbol hexacosonate
- Flavonoids and steroidal saponins
- Amyrin
- Atinsine diterpene antiquorin and neriifolene.
Euphorbia Neriifolia is used traditionally in the treatment of diabetes in different rural areas of India. Euphorbia Neriifolia shows meditional traditional pharmacological uses like antifungal, antibacterial, antiparasitic, antiviral, antidiabetic, antiarthritic, antioxidant anticonvulsant, radioprotective, wound healing, anticancer and spasmodic. (Pullaiah et al., 2002).
### 1.9

<table>
<thead>
<tr>
<th>Model Category</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Spontaneous diabetic animals</td>
<td>Development of type 2 diabetes is of spontaneous origin involving genetic factors and the animals develop characteristic features resembling human type 2 diabetes.</td>
<td>Highly inbred, homogenous and mostly monogenic inheritance and development of diabetes is highly genetically determined unlike heterogeneity seen in humans.</td>
</tr>
<tr>
<td></td>
<td>Mostly of inbred animal models in which the genetic background is homogeneous and environmental factors can be controlled, allow genetic dissection of this multifactorial disease easy</td>
<td>Limited availability and expensive for the diabetes study</td>
</tr>
<tr>
<td></td>
<td>Variability of results perhaps minimum and require small sample size</td>
<td>Mitochondria due to ketosis problem is high in case of animals with brittle pancreas (db/db, ZDF rat, P. obesus, etc.) and require insulin treatment in later stage for survival</td>
</tr>
<tr>
<td>II. Diet/Nutrition induced diabetic animals</td>
<td>Develop diabetes associated with obesity as a result of overnutrition as in diabetes syndrome of human population</td>
<td>Mostly require long period of dietary treatment</td>
</tr>
<tr>
<td></td>
<td>Toxicity of chemicals on other body vital organs can be avoided</td>
<td>No frank hyperglycaemia develops upon simple dietary treatment in genetically normal animals and hence become not suitable for screening antidiabetic agents on circulating glucose parameter</td>
</tr>
<tr>
<td>III. Chemical induced diabetic animals</td>
<td>Selective loss of pancreatic beta cells (alloxan/STZ) leaving other pancreatic alpha and delta cells intact</td>
<td>Hyperglycaemia develops primarily by direct cytoxic action on the beta cells and insulin deficiency rather than consequence of insulin resistance</td>
</tr>
<tr>
<td></td>
<td>Residual insulin secretion makes the animals live long without insulin treatment</td>
<td>Diabetes induced by chemicals is mostly less stable and at times reversible because of the spontaneous regeneration of beta cells. Hence, care must be taken to assess the pancreatic beta cell function during long-term experiments</td>
</tr>
<tr>
<td></td>
<td>Ketosis and resulting mortality is relatively less</td>
<td>Chemical produce toxic actions on other body organs as well besides its cytoxic action on beta cells</td>
</tr>
<tr>
<td></td>
<td>Comparatively cheaper, easier to develop and maintain</td>
<td>Variability of results on development of hyperglycaemia is perhaps high</td>
</tr>
<tr>
<td>IV. Surgical diabetic animals</td>
<td>Avoids cytoxic effects of chemical diabetogens on other body organs</td>
<td>Involvement of cumbersome technical and post operative procedures</td>
</tr>
<tr>
<td></td>
<td>Resembles human type 2 diabetes due</td>
<td>Occurrence of some other digestive</td>
</tr>
</tbody>
</table>
1.10 Streptozocin (Szkudelski et al., 2001)

Streptozocin is synthesized by streptomyces acromogenes and is used to induce diabetes in almost all the species. Diabetes dose varies with the species and the optimal dose required to produce diabetes in rat was found to be (50 – 60 mg/kg i.p. or i.v.), in mice (175-200 mg/kg i.p. or i.v.) and in dogs (15 mg/kg, for 3 days). Due to its low stability the rapid i.v. injection STZ induces hamster, monkey and guinea pigs. STZ diabetes can be induced by two ways either by single injection of STZ or by multiple low dose injection of STZ. Like alloxan, it shows triphasic fluctuation pattern in diabetes. Initial hyperglycemia is observed by 1 h after the injection followed by hyperglycemia and again a hyperglycemia state at 48 h, the elevated blood glucose level is observed by 48-72 h (peak effect) and is maintained thereafter. Different mechanism of action on the β-cells destruction by STZ have been proposed. Its main actions through free radical generations. Other report proposed that STZ exerts lethal damage by alkylting DNA or its phosphate backbone as well as glycolytic or mitochondrial enzyme. STZ also influence the immune system by suppressing the T-cell function associated with atrophy of the thymus and peripheral lymphoid tissue. Like alloxan, STZ also induces OFR induced lipid peroxidation and DNA strand breaking in pancreatic islet cell. STZ enter into the beta cells via glucose transporter (GLUT 2) and cause alkylation of DNA. Damage of DNA induces activation
Figure 2.15:
1.11 LITERATURE REVIEW

Arambewela L.S.R. et al., (2005) investigated the antidiabetic activity of *Piper betle* leaves. This was tested in normoglycaemic and streptozotocin (STZ)-induced diabetic rats using oral administration of hot water extract (HWE) and cold ethanolic extract (CEE). In normoglycaemic rats, both HWE and CEE significantly lowered the blood glucose level in a dose-dependent manner. In glucose tolerance test, both extracts markedly reduced the external glucose load and blood glucose level.

Ahmed O. et al., (2005) studied the effect of the butanol extract of *Zizyphus spina-christi* (L.), Wild (Rhamnaceae) leaves and its major saponin glycoside, christinain-A, on the serum glucose and insulin levels was studied in non-diabetic control, type-I (insulin-dependent) and type-II (non-insulin-dependent) diabetic rats. Pretreatment either with 100 mg/kg butanol extract or christinain-A potentiated glucose-induced insulin release in non-diabetic control rats. In type-II but not in type-I diabetic rats pretreatment with the butanol extract or christinain-A improved the oral glucose tolerance and potentiated glucose-induced insulin release.

Shokeen P. et al., (2008) investigated the antidiabetic activity of 50% ethanolic extract of roots of *Ricinus communis* (RCRE) along with its bioassay-guided purification. Five-hundred milligram per kilogram body weight appeared to be the effective dose as it caused the maximum lowering of the fasting blood glucose, both in normal as well as type 1 diabetic animals. The maximum hypoglycemic effect was always observed at the 8th h up to which the study has been conducted.

Pari M. et al., (2004) investigated the effects of daily oral administration of aqueous solution of *Boerhaavia diffusa* L. leaf extract (BLEt) (200 mg/kg) for 4 weeks on blood glucose concentration and hepatic enzymes in normal and alloxan induced diabetic rats. A significant decrease in blood glucose and significant increase in plasma insulin levels were observed in normal and diabetic rats treated with BLEt. Treatment with BLEt resulted in a significant reduction of glycosylated haemoglobin and an increase in total haemoglobin level.
Kannur D.M. et al. (2006) studied seed extracts of Caesalpinia bonduc cella were subjected to screening of antidiabetic activity in alloxan induced hyperglycemia. The oral administration of the extracts (300 mg/kg) produced significant antihyperglycemic action as well as it lowered the BUN levels significantly. In the same study the action of the extracts on diabetes induced hyperlipidemia was analyzed where the extracts significantly lowered the elevated cholesterol as well as LDL level. The antihyperglycemic action of the extracts may be due to the blocking of glucose absorption. The drug has the potential to act as antidiabetic as well as antihyperlipidemic.

Raut N.A. et al. (2006) investigated the effect of Cyperus rotundus on alloxan induced hyperglycemia in rats. Oral daily administration of 500 mg/kg of the extract (once a day for seven consecutive days) significantly lowered the blood glucose levels. This antihyperglycemic activity can be attributed to its antioxidant activity as it showed the strong DPPH radical scavenging action in vitro.

Jain S. et al. (2010) studied aqueous and ethanolic extracts (250 and 500 mg/kg body weight), administered orally to male Wistar albino rats. Alloxan monohydrate was used to induce diabetes mellitus. Total phenolic content was estimated in the extracts. The parameters studied included oral glucose tolerance test, fasting blood glucose, serum insulin and glycated haemoglobin levels, liver glycogen content, serum lipid profile, and changes in body weights. The results suggest that Paspalum scrobiculatum has antidiabetic activity, thereby justifying its traditional claim and augmenting it into the present day systems of medicine.

Nagappa A.N. et al. (2003) studied antidiabetic potential, effect of the petroleum ether, methanol, and aqueous extracts of Terminalia catappa Linn (combretaceae) fruit, on fasting blood sugar levels and serum biochemical analysis in alloxan-induced diabetic rats were investigated. All the three extracts of Terminalia catappa produced a significant antidiabetic activity at dose levels 1/5 of their lethal doses. Concurrent histological
studies of the pancreas of these animals showed comparable regeneration by methanolic and aqueous extracts which were earlier, necrosed by alloxan.

**Rao B.K. et al. (2003)** studied different doses of ethanolic fraction of fruits of *Terminalia pallida* for hypoglycemic and antihyperglycemic activity in normal and alloxan diabetic rats. The oral administration of ethanolic extract at a dosage of 0.5 g/kg body weight exhibited a significant antihyperglycemic activity in alloxan diabetic rats, whereas in normal rats no hypoglycemic activity was observed.

**Nagarajan N. et al. (2005)** studied petroleum ether and benzene extracts of *Clemo felina*, given orally at doses of 300 mg/kg/day for 30 days, were found to be antidiabetic and antihyperlipidemic on alloxan diabetic rats. Moreover, a significant decrease in the activities of serum enzymes like alkaline phosphatase, acid phosphatase and HMGCoA reductase activity in the liver was observed. However, treatment of rats with the extracts as well as standard antidiabetic drugs increased liver hexokinase activity and serum LDH activity.

**Arulmozhi S. et al. (2010)** evaluated the effect of ethanolic extract of the leaves of *A. scholaris* (known as EEAS) in streptozotocin-induced diabetic rats. The streptozotocin-induced diabetic rats were orally treated with vehicle (2% w/v Tween 80), glibenclamide (0.25 mg/kg) and EEAS (100, 200 and 400 mg/kg) to the respective treatment groups. The blood glucose level, body weight, glycosylated hemoglobin, muscle and liver glycogen, lipid profile, lipid peroxidation, antioxidant status were measured and histopathology of pancreas was performed after 6 weeks of treatment and compared to the control. It has been concluded that EEAS, in addition to the antidiabetic activity, also possess antihyperlipidemic and antioxidant activities in the streptozotocin-induced diabetic model.

**Eliza J. et al. (2009)** isolated eremanthin from Costus speciosus. The structure was identified using gas chromatography–mass spectrometry (GC–MS) analysis. Eremanthin was administered to streptozotocin (STZ) (50 mg/kg bw) induced diabetic male Wistar