2.0 REVIEW OF LITERATURE

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion or insulin action (Jayakar and Suresh, 2003). The number of people suffering from diabetes worldwide is increasing at an alarming rate. It is predicted that about 366 million people are likely to be diabetic by the year 2030 (Wild et al., 2004).

Hyperglycemia and hyperlipidemia are the two important characters of diabetes mellitus in which diabetic patients experience various vascular complications such as atherosclerosis, coronary heart disease, diabetic nephropathy and diabetic neuropathy (Sheetz, 2002). Experimental diabetes in animals has provided considerable insight into the physiological and biochemical derangement of the diabetic state (Sochar et al., 1985). Increasing evidences from both experimental and clinical studies suggest that oxidative stress plays a major role in the pathogenesis of diabetes mellitus (Maritim et al., 2003). Abnormally high level of free radicals and the simultaneous decline in antioxidant defense mechanisms may lead to the damage of cellular organelles and enzymes, increased lipid peroxidation and development of insulin resistance (EI–Naggar et al., 2005).

2.1. Prevalence of diabetes around the world

Diabetes is a metabolic disorder critically affecting the population of both developed and developing countries. According to the Diabetes Atlas, the global prevalence of diabetes is estimated to be 4.6% representing 151 million people and is expected to go up to 333 million people by 2025. Recent reports have estimated an increase in these figures, with the global prevalence reaching up to 6.6% representing 285 million people in 2010 and by 2030, it will increase globally up to 7.8% (438 million people). Also individual national prevalence rates from over 1% to almost 31% have been reported in severally affecting developing countries and more specifically, the lower socio-economic groups.

According to the International Diabetes Federation (IDF), the overall cost assessment for the global prevention and treatment of diabetes will run up to 490 billion
US dollars by 2030 (Colagiwri, 2010). The occurrence of diabetes is higher in men than in women and a notable increase in the proportion of people suffering from diabetes with more than 65 years of age is also reported (Wild et al., 2004).

Table I. Top 10 countries with the highest number of estimated diabetes cases for the year 2000 and 2030

<table>
<thead>
<tr>
<th>Rank</th>
<th>Country</th>
<th>2000</th>
<th>2030</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>People with diabetes (millions)</td>
<td>People with diabetes (millions)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>India</td>
<td>31.7</td>
<td>79.4</td>
</tr>
<tr>
<td>2</td>
<td>China</td>
<td>20.8</td>
<td>42.3</td>
</tr>
<tr>
<td>3</td>
<td>United States</td>
<td>17.7</td>
<td>30.3</td>
</tr>
<tr>
<td>4</td>
<td>Indonesia</td>
<td>8.4</td>
<td>21.3</td>
</tr>
<tr>
<td>5</td>
<td>Japan</td>
<td>6.8</td>
<td>13.9</td>
</tr>
<tr>
<td>6</td>
<td>Pakistan</td>
<td>5.2</td>
<td>11.3</td>
</tr>
<tr>
<td>7</td>
<td>Russian Federation</td>
<td>4.6</td>
<td>11.1</td>
</tr>
<tr>
<td>8</td>
<td>Brazil</td>
<td>4.6</td>
<td>8.9</td>
</tr>
<tr>
<td>9</td>
<td>Italy</td>
<td>4.3</td>
<td>7.8</td>
</tr>
<tr>
<td>10</td>
<td>Bangladesh</td>
<td>3.2</td>
<td>6.7</td>
</tr>
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</table>

(Wild et al., 2004)

The prevalence of Type II diabetes worldwide is set to increase from the present level of 150 million to 225 million by the end of the decade and to as many as 300 million by 2025 (Zimmet, 2003). Diabetes mellitus is occurring in epidemic proportions in many countries. The Type II diabetes is associated with significant morbidity and mortality which is increasing in prevalence a worldwide. The rapid increase in the prevalence of Type II diabetes represents a major challenge for health care delivery worldwide (Pittas, 2003).

2.2. Diabetes in India

India has more diabetics than any other country in the World, according to the International Diabetes Foundation. Diabetes affects more than 50 million Indians and kills 1 million Indians per year. Studies have indicated a shocking rise in the prevalence of diabetes in India (Modak et al., 2007).
According to WHO, India had 31.7 million diabetic subjects in the year 2000 and this number would increase up to 79.4 million by the year 2030 (Wild et al., 2004). Currently, India has the highest number of diabetic patients and India is being called the "Diabetic Capital of the World" (Mohan et al., 2007). The high incidence of diabetes is attributed to a combination of genetic susceptibility and adoption of a high calorie, low activity lifestyle by India's growing middle class people. Diabetes Research Centre showed that the prevalence of diabetes has steadily increased among Urban India from 5.2% in 1984 to 13.9% in 2000. National Survey of India also showed that the prevalence of diabetes is high in Urban India. Even in America, the incidence of diabetes among Indians were 10-15 times more than other population groups (Khader, 2001).

2.3. History of Diabetes Mellitus

Diabetes was one of the first diseases described (Brian et al., 2011) with an Egyptian manuscript from C. 1500 BCE mentioning "too great emptying of the urine". The first described cases were believed to be Type I diabetes. Indian physicians around the same time identified the disease and classified it as "Madhuma" or "Honey urine, noting the urine would attract ants. The term "diabetes" or "to pass through" was first used in 230 BCE by the Greek Apollonius of Memphis. The disease was considered as rare during the time of the Roman empire, Galen commenting that he had only seen two cases during his career. This is possibly due to the diet and the life-style of the ancient people or because the clinical symptoms were observed during the advanced stage of the disease. Galen named the disease as "diarrhoea of the urine"(Diarrhoea uricosa).

Type I and Type II diabetes were identified as separate conditions for the first time by the Indian physicians Sushruta and Charka in 400-500 BCE with Type I associated with youth and Type II with being overweight. The term "mellitus" or "from honey" was added by `Briton John Rolle' in the late 1700's to separate the condition from "Diabetes insipidus", which is also associated with frequent urination. Effective treatment was not developed until the early part of the 20th century, when Canadian 'Frederick Banting' and 'Charles Herbert best' isolated and purified insulin in 1921 and 1922. This was followed by the development of the long-acting insulin NPH in the 1940's (Poretsky, 2009).
2.4. Etiology of Diabetes Mellitus

The cause of diabetes depends on the type of diabetes mellitus. Type I diabetes is partly inherited and then triggered by certain viral infections like mumps, congenital rubella and coxsackie, which may infect pancreatic beta cells and induce antibody attack (Reddy, 2001). A genetic element in individual susceptibility to some of these triggers has been traced to particular HLA genotype. However, even in those who have inherited the susceptibility, Type I diabetes mellitus seems to require an environmental trigger. The onset of Type I Diabetes is unrelated to life style.

Table II. Comprehensive list of other causes of diabetes

<table>
<thead>
<tr>
<th>S.No</th>
<th>Causes</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Genetic defects of beta cell function</td>
<td>• Maturity onset diabetes of the young</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Mitochondrial DNA mutations</td>
</tr>
<tr>
<td>2</td>
<td>Genetic defects in insulin processing or insulin action</td>
<td>• Defects in pro insulin conversion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Insulin gene mutations</td>
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<tr>
<td></td>
<td></td>
<td>• Insulin receptor mutations</td>
</tr>
<tr>
<td>3</td>
<td>Exocrine pancreatic defects</td>
<td>• Chronic pancreatitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Pancreatectomy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Pancreatic neoplasia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Cystic fibrosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Hemochromatosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Endocrinopathies</td>
</tr>
<tr>
<td>4</td>
<td>Growth hormone excess</td>
<td>• Acromegaly</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Cushing syndrome</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Hyperthyroidism</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Pheochromocytoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Glucagonoma</td>
</tr>
<tr>
<td>5</td>
<td>Infections</td>
<td>• Cytomegaloviral infection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Coxsackie viral B infection</td>
</tr>
<tr>
<td>6</td>
<td>Drugs</td>
<td>• Glucocorticoids</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Thyroid hormone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• β-adrenergic agonists</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Statins (Satter et al., 2010)</td>
</tr>
</tbody>
</table>
Type II diabetes is primarily due to lifestyle factors and genetics (Riserus, 2009). Increasing body weight is associated with increasing risk for Type II diabetes and 80% of people with Type II diabetes are overweight or obese (Hensrud, 2001). Overeating during pregnancy may increase the risk of diabetes for the unborn foetus in later life (Dixit, 2001). A study on the analysis of blood sample of 1038 men in Finland reported that those with high iron stores were 2.4 times more likely to develop diabetes than men with low iron stores (Salonen et al., 1999). Marginal to low chromium intake in elderly persons may also contribute to an increased risk for developing diabetes (Morris, 1994). The adoption of unhealthy dietary patterns, growing socio-economic and racial disparities in chronic diseases, low level of physical activity and unidentified genetic and environmental determinants have led to increased incidence of diabetes mellitus (Pradhan et al., 2002).

2.5. Classification of Diabetes mellitus

Diabetes mellitus is classified into four broad categories: Type I, Type II, Gestational diabetes and other specific types. The term Type I diabetes has replaced several former terms, including Childhood-onset diabetes, Juvenile diabetes and Insulin Dependent Diabetes Mellitus (IDDM). Likewise, the term Type II diabetes has replaced several former terms, including Adult-onset diabetes, Obesity-related diabetes and Non-Insulin Dependent Diabetes Mellitus (NIDDM).

(i) Type I Diabetes Mellitus

Type I Diabetes mellitus is characterized by loss of insulin – producing beta cells of the islets of langerhans in the pancreas, leading to insulin deficiency. This type can be further classified as immune – mediated or idiopathic. The majority of Type I diabetes is of the immune - mediated nature, in which beta cell loss is a T-cell mediated autoimmune attack (Rother, 2007).

Most affected people are healthy and on a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type I diabetes can affect children or adults, but was traditionally termed “Juvenile diabetes” because a majority of these diabetes cases were children (O’donovan and Fernandes, 2004). There are many reasons for Type I diabetes to be accompanied by irregular and unpredictable hyperglycemia, frequently with ketosis and sometimes serious
hypoglycemia including an impaired counter regulatory response to hyperglycemia, occult infection, gastroparesis which leads to erratic absorption of dietary carbohydrates and endocrinopathies (eg. Addison’s disease).

**Fig 1. Glucose Metabolism In Type-I Diabetes**

(ii) **Type II diabetes mellitus**

Type II diabetes mellitus is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion. The defective responsiveness of body tissues to insulin is believed to involve the insulin receptors. However, the specific defects are not known. Type II diabetes is the most common type. In the early stage of Type II diabetes, the predominant abnormality is reduced insulin sensitivity. At this stage, hyperglycemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver (David et al., 2011).
(iii) Gestational diabetes

Gestational diabetes is a condition characterized by glucose intolerance recognized during pregnancy (Metzger, 1991). In common with other maternal disorders, gestational diabetes is associated with an increase in prenatal mortality and morbidity (Pettit et al., 1985). Women with gestational diabetes are also likely to be affected by pre-eclampsia (Garner et al., 1990) and are more prone to develop Type II diabetes mellitus in later life and at the risk of increased cardiovascular morbidity (Roberts and Redman, 1993; Mc Carthy et al., 1993).

(iv) Other specific types of Diabetes mellitus

Prediabetes, a condition that occurs when a person’s blood glucose levels are higher than normal but not high enough for a diagnosis of Type II diabetes mellitus. Many people destined to develop Type II diabetes mellitus in a state of prediabetes for many years (Handelsman Yehuda, 2001).

- Malnutrition-Related Diabetes Mellitus (MRDM)

This type of diabetes mellitus is mainly seen in tropical countries like India and it occurs in young people between 15-30 years of age. People with MRDM are lean and undernourished. In this type of diabetes, the pancreas fails to produce adequate insulin (Raghuram et al., 1993).
• Latent Autoimmune Diabetes of Adults (LADA)

This is a condition in which Type I diabetes mellitus develops in adults. Adults with LADA are frequently misdiagnosed as having Type II diabetes mellitus, based on age rather than etiology.

• Some cases of diabetes are caused by the body’s tissue receptors not responding to insulin. This form is very uncommon. Genetic mutations can lead to defects in beta cell function. Any disease that cause extensive damage to the pancreas may lead to diabetes (for chronic pancreatitis and cystic fibrosis). Many drugs may impair insulin secretion and some toxins may damage beta cells of pancreas and cause diabetes.

2.6. Risk factors of Diabetes Mellitus

(i) Obesity

Non-insulin dependent diabetes mellitus is not a major cause of death in normal weight people, but it is an important contributor to morbidity and mortality in obese people. A man with average weight of more than 140% is 5.2 times more likely to develop diabetes and die than a person with normal weight and for women, the mortality ratio is 7.9 times for a similar degree of overweight (Guogeon et al., 1997).

(ii) Hypertension

Diabetes and hypertension are common chronic conditions which frequently coexist. Hypertension is approximately twice as common in individuals with diabetes when compared to those without it (Sugantri and Saradha, 1999).

(iii) Lack of Exercise

Lack of exercise and physical activity will cause obesity. Studies have suggested that regular physical activity may prevent the severity of Non-insulin dependent diabetes mellitus. Regular physical activity and exercise can reduce the risk for this type of diabetes by about one third (Wiley, 1997).

(iv) Age

The risk of diabetes increases with age mainly because the number of beta cells in the langerhans of pancreas that produce insulin decreases (Guogeon et al., 1997).
(v) Psycho – Social stress

Stress may be a diabetogenic factor that cannot be ignored and is perhaps one of the factors in the causation of diabetes. Some hormones released during stress may block the effect of insulin on the cells and cause diabetes. During stress, hormones such as adrenaline, nor-adrenaline and cortisol are released and increase the blood glucose levels (Srilakshmi, 1995).

(vi) Smoking

Cigarette smoking is associated with accelerated macro vascular disease. Presence of diabetes along with smoking will increase the severity of diabetes. The nicotine in tobacco smoke constricts the small blood vessels and reduce the supply of blood to the tissues. Continuous smoking associated with diabetes lead to hypercholesterolemia and cardiovascular diseases (Adams, 1998).

(vii) Alcohol Consumption

Alcohol may lower blood sugar level below certain critical normal limits and result in hypoglycemia. Alcohol provides empty calories as it does not contain proteins or fats. The extra calories may make a diabetic overweight or obese. Alcohol, when consumed for a long period of time, causes damage to liver, pancreas, heart and induce diabetes mellitus (Hopaur, 1992).

(viii) Heredity

Genetic factors are crucial in the development of diabetes mellitus. If both parents have diabetes, the chances for getting diabetes in their children may go up to 90%. Even if the parents are free from diabetes, a person may inherit it from distant relatives (Warren, 1990).

2.7. Metabolic changes in Diabetes mellitus

Diabetes mellitus is primarily due to deficiency of insulin production. Hence the metabolism of carbohydrate, fat and protein is impaired, as insulin is essential for the utilization of glucose in the body (Swaminathan, 1994).
(i) Carbohydrate metabolism

In diabetes, lack of insulin produces some fundamental changes in carbohydrate metabolism and may lead to hyperglycemia. The changes include

- Reduced entry and oxidation of glucose in muscle and other tissues.
- Reduced formation of glycogen in liver.
- Decreased synthesis of fat from carbohydrates.
- Release of more glucose into blood due to increased breakdown of glycogen in liver.
- Low level of glucose in body cells and high level in blood and other extra cellular fluids.

(ii) Fat Metabolism

In diabetic condition, fat is used as a source of energy, as the tissues cannot oxidize sufficient quantities of glucose to meet the energy needs. As a result, fatty acids are released from the cells and are metabolized by the liver into ketone bodies. The accumulation of ketone bodies in large amounts in blood produces a condition called ‘ketosis’ which may lead to diabetic coma in severe diabetics (Swaminathan, 1994).

(iii) Protein Metabolism

There is a breakdown of tissue proteins in diabetes which may lead to negative nitrogen balance and wasting of muscles. Due to insulin deficiency, the synthesis of tissue protein is decreased in diabetics. Consequently, the concentration of amino acids is increased in blood and liver (Swaminathan, 1994).

2.8. Signs and symptoms of Diabetes mellitus

The classic symptoms of untreated diabetes are loss of weight, polyuria (frequent urination), polydypsia (increased thirst) and polyphagia (increased hunger).

Symptoms may develop rapidly (weeks or months) in Type I diabetes, while they usually develop much more slowly or absent in Type II diabetes. Prolonged high blood glucose can cause glucose absorption in the lens of the eye, which leads to change in its
shape, resulted in vision change. Blurred vision is a common complaint in Type I diabetes. A number of cases may be observed with skin rashes, known as diabetic dermadromes in diabetes (Cooke and Plotnick, 2008).

2.9. Complications of Diabetes mellitus

Diabetes is a corrosive, troublesome wasting disease, which can affect the heart, kidneys, eyesight, liver, digestive system and the nervous system of the body. The diabetic complications include

(i) Diabetic retinopathy

Diabetic retinopathy is a complication of diabetes that affects the eyes. Blindness due to diabetic retinopathy is the leading cause of blindness in people between 20-74 years of age (Albert, 1997). About 12,000 to 24,000 people lose their eyesight as a result of diabetes (Eriksson et al., 1999).

(ii) Diabetic Cardiomyopathy

Diabetic cardiomyopathy is a disorder of the heart muscle in people with diabetes. Most of the heart failure in people with diabetes results from coronary artery disease. Poor control of diabetes is thought to predispose the development of degenerative arterial disease in the coronary, cerebral and peripheral arteries. This has been attributed to hyperlipidemia and hypercholesterolemia of the chronic diabetic state (Cyril et al., 1992). The prevalence of coronary heart disease, cardiovascular and cerebrovascular diseases may be as much as three times more frequent in Type II diabetes mellitus. Increased body fat increases the risk of ischemic stroke and raised plasma insulin concentration may lead to this increased risk of ischemic stroke (Folsom et al., 1999).

(iii) Diabetic nephropathy

Diabetic nephropathy refers to a characteristic structural and functional kidney abnormalities in patients with diabetes (Reeves and Andreoli, 2000). Patients with diabetes undergoing dialysis have a 15% - 22% higher mortality, when compared with patients without diabetes (Kikkawa et al., 2003).
(iv) **Diabetic neuropathy**

Diabetic neuropathy is the impact of diabetes on the nervous system, causing tingling pain in the feet and increased risk of skin damage due to altered sensation. Neuropathy also contribute to the risk of diabetes related foot problems (Diabetic foot ulcers) that can be difficult to treat and occasionally require amputation (Boussageon *et al.*, 2011).

(v) **Diabetic Ketoacidosis**

Diabetic ketoacidosis (DKA) is a life-threatening complication in patients with diabetes mellitus. DKA results from a shortage of insulin, which may cause burning of fatty acids, producing acidic ketone bodies that may lead to most of the symptoms and complications of diabetes (Kitabchi *et al.*, 2009). There are two major hyperglycemic crisis associated with diabetes namely diabetic ketoacidosis and hyper osmotic hyper glycemic state (Chaithongdi *et al.*, 2011).

DKA is a medical emergency and without treatment, it can lead to death. DKA was first described in 1886. Until the introduction of insulin therapy in 1920’s, DKA was universally fatal and now a mortality of less than 1% is observed due to adequate and timely treatment (Eledrisi *et al.*, 2006).

2.10. **Diagnosis of Diabetes Mellitus**

**Blood glucose measurement**

The diagnosis of diabetes is based on the three methods of blood glucose measurement.

(i) **Measurement of fasting blood glucose**

Diabetes can be diagnosed, if the patient has a fasting blood glucose level of 126mg/dl (7.0 mmol/L) (American Diabetes Association, 2010). The limitation of the test include the need for an eight-hour fast before the blood draw, a 12-15% day-today variance in fasting blood glucose values and a slightly lower sensitivity for predicting micro vascular complications (Petersen *et al.*, 2005; Saudek *et al.*, 2008).
(ii) Measurement of random blood sugar

Diabetes can also be diagnosed with a random blood glucose level of 200mg/dl (11.1mmol/L) or greater, if classic symptoms of diabetes such as polyuria polydypsia, blurred vision, weight loss and fatigue are present. Lower random blood glucose levels (140-180mg/dl [7.8 – 10.0 mmol/L]) have a fairly high specificity of 92-98%. Therefore, patients with these values should undergo more definite testing. A low sensitivity of 39-55% limits the use of random blood glucose testing (Saudek et al., 2008).

(iii) Oral glucose tolerance Test (OGTT)

OGTT is considered as a first line diagnostic test. Limitations include poor reproducibility and patient compliance because an eight-hour fast is needed before the 75g glucose load, which is followed two hours later by a blood draw. The criterion for diabetes is a serum blood glucose level of greater than 199 mg/dl (11.0mmol/L) (Ko et al., 1998). Impaired glucose tolerance continues to be defined as a blood glucose level between 140 and 199 mg/dl (7.8 and 11.0mmol/L) two hours after a 75g glucose load. Patients meeting either of these criteria are at significantly higher risk of progression to diabetes and should be counselled on effective strategies to lower their risk such as weight loss and exercise (Knowler et al., 2002).

(iv) Glycosylated hemoglobin (HbA1c) measurement

HbA1c measurement has been recently endorsed as a diagnostic and screening tool for diabetes. One advantage of using HbA1c measurement is the ease of testing, because it does not require fasting condition. The HbA1c level of greater than 6.5 percent is considered as a diagnostic of diabetes (International Expert Committee report, 2009). Limitations of HbA1c testing include low sensitivity, possible racial disparities and interference by anaemia and some medications (Little et al., 2001).

2.11. Treatment of diabetes mellitus

Anti-diabetic medication

Anti-diabetic drugs are used to treat diabetes mellitus by lowering glucose levels in the blood. They are administered orally and are thus also called oral hypoglycemic agents or oral anti-hyperglycemic agents. There are different classes of anti-diabetic drugs and their selection depends on the nature of the diabetes, age and other factors.
Type I diabetes mellitus is a disorder, caused by the lack of insulin hence insulin must be injected to treat Type I diabetes. Type II Diabetes mellitus is a disorder of insulin resistance by cells and treatments include

a. agents that increase the amount of insulin secreted by the pancreas  
b. agents that increase the sensitivity of target organs to insulin  
c. agents that decrease the rate at which glucose is absorbed from the gastrointestinal tract.

**Insulin**

Insulin is usually given subcutaneously either by injection or by an insulin pump. There are three types of insulin characterized by the rate at which they are metabolized by the body. They are rapid acting insulin, intermediate acting insulin and long acting insulin.

**2.12. Standard Drug used for the study - Glibenclamide**

Glibenclamide also known as Glyburide is an anti-diabetic drug in a class of medications known as sulfonylureas, closely related to sulfadrugs (Marble, 1971).

**Chemical structure of Glibenclamide**

[Chemical structure image]

**Systematic name**

5-Chloro-N-(2-{4-[(cyclohexylcarbamoyl)sulfamoyl]phenyl}ethyl)-2-methoxybenzamide
Clinical Data

Trade names: Diabeta, Glynase, Micronase, Daonil, Semi – Daonil, Euglucon, Delmide

Legal status: POM

Routes: oral

Pharmacokinetic data

Protein binding: Extensive

Metabolism: Hepatic hydroxylation

Half life: 10 hours

Excretion: Renal and biliary

Chemical Data

Formula: C$_{23}$ H$_{28}$ C$_{1}$ N$_{3}$ O$_{5}$ S

Mol Mass: 494.004 g /mol

Among the various synthetic drugs, glibenclamide has been widely used in the management of non-insulin dependent diabetes mellitus (Figueroa – Valverde et al., 2012). It is one of the two oral anti-diabetic drugs in the World Health organization model list of essential medicines (the other being metformin) (WHO expert committee, 2011) and it is the most popular sulfonylurea (Riddle, 2003).

(i) Mechanism of action of Glibenclamide

The drug works by binding to and activating the sulfonylurea receptor 1 (SUR1), the regulatory subunit of the ATP – sensitive potassium channels (KATP) on the β - cells of the pancreas (Serrano – Martin et al., 2006). This inhibition causes cell membrane depolarization opening voltage – dependent calcium channel. This results an increase in intracellular calcium level in the β - cells and subsequent stimulation of insulin release.
(ii) Side effects and contraindications

Glibenclamide is a major cause of drug-induced hypoglycemia and may be contraindicated for those with glucose-6-phosphate dehydrogenase deficiency and may cause acute haemolysis (Meloni and Meloni, 1996). Glibenclamide is associated significantly with higher annual mortality, when combined with metformin than other insulin–secreting medications, after correcting for other potentially confounding patient characteristics (Monami et al., 2006). Glibenclamide has been demonstrated to block the protection offered by myocardial preconditioning in dogs (Gross and Auchampach, 1992).

2.13. Importance of Medicinal Plants

Medicinal plants offer alternative remedies with tremendous opportunities to generate income, employment and foreign exchange for developing countries (Ragavan and Krishnakumari, 2006). Many traditional healing herbs and their parts have been shown to have medicinal value and can be used to prevent, alleviate or cure several human diseases (Pal et al., 1999; Dhar et al., 1999). Consumption of herbal medicine is widespread and increasing in recent years and approximately 80% of the people in developing countries depend on traditional medicines for primary health care needs (Farnsworth et al., 1985). Out of the 3,50,000 plant species identified so far, about 35,000 are used worldwide for medicinal purpose and less than about 0.5% of these have phytochemically investigated (Comer and Debus, 1996).

(i) Medicinal Plants in India

India is one of the leading countries in Asia in terms of the wealth of traditional knowledge systems related to herbal medicine and employs a large number of plant species, which include Ayurveda (2000 species), Siddha (1121 species), Unani (751 species) and Tibetan (337 species) (Kala and Mathur, 2002).

In India, medicinal plants are widely used by all sections of the population with an estimated 7500 species of plants used by several ethnic communities and it is known that India has the second largest tribal population in the world after Africa (Kala, 2005;
Jagtap et al., 2006). Even today, tribal communities in India still collect and preserve locally available wild and cultivated plant species and practice herbal medicine to treat a variety of diseases and disorders (Mahishi et al., 2005).

India has about 45,000 plant species and among them, several thousands have been claimed to possess medicinal properties. Research conducted in last few decades on plants mentioned in ancient literature or used traditionally for diabetes have shown anti-diabetic property. Grover et al. (2002), reported 45 plants and their products (active, natural principles and crude extracts) that have been used in the Indian traditional system of medicine and showed experimental and clinical anti-diabetic activity.

(ii) Hypoglycemic constituents from plants

Indian plants which are most effective and the most commonly studied in relation to diabetes and their complications are Allium cepa, Allium sativum, Aloe vera, Cajanus cajan, Coccinia indica, Caesalpinia bonducella, Ficus bengalensis, Gymnema sylvestre, Momordica charantia, Ocimum sanctum, Pterocarpus marsupium, Swertia chirayita, Syzygium cumini, Tinospora cordifolia and Trigonella foenum-graecum. All these plants have shown varying degree of hypoglycemic and anti-hyperglycemic activity. Many kinds of natural products such as terpenoids, alkaloids, flavonoids, phenolics have shown anti-diabetic potential. Particularly, schulzeines A, B, C, radicamines A and B, 2,5,-imino - 1,2,5, trideoxy-L-glucitol, beta-homo-fuconojirimycin, myriciacitrin IV, 4 (alpha - rhamnophranosyl) ellagic acid and 1,2,3,4,6 - pentagalloylglucose have shown significant anti-diabetic activities as reported by Jung et al. (2006).

Many active compounds have been isolated from the various plant species of India. These active principles are dietary fibres, alkaloids, flavonoids, saponins, amino acids, steroids, peptides and others. These have produced potent hypoglycemic, anti-hyperglycemic and glucose suppressive activities (Saxena et al., 2006). The above effects achieved by either insulin release from pancreatic beta cells, inhibited glucose absorption in the gut, stimulated glycogenesis in liver or increased glucose utilization by the body (Grover et al., 2002; Saxena and Vikram, 2004). These compounds also exhibited their antioxidant, hypolipidemic, anti-cataract activities, restored enzymatic functions, repair and regeneration of pancreatic islets and the alleviation of liver and renal
damage (Mukherjee et al., 2006). Some active constituents have been obtained from plants possess insulin like activity and could provide alternate source for insulin therapy. Bellahcen et al. (2013) reported that the anti- diabetic activity of Virgin oil obtained from the fruits of *Argania spinosa* in diabetic rats may be related to its high content of tocopherol and phenolic compounds.

**(iii) Anti-hyperglycemic activity of plants**

Herbal drugs constitute a glorious chapter in Indian system of medicine and were used in the treatment of diabetes mellitus. Extracts of bark and flowers of neem, reduced blood glucose and glycogen levels in rats (Dixit et al., 1992). Oral administration of aqueous extracts of *Syzigium cumini* seeds showed a significant blood sugar lowering effect (Prince et al., 1998). Oral hypoglycemic effect of *Caesalpinia bonducella* studied in alloxan and streptozotocin-induced diabetic rats showed a significant reduction in blood sugar level (Biswa et al., 1997). Extracts of plants *viz Aegle marmelos* and *Syzygium cumini* exhibited significant antihyperglycemic effect in experimental diabetic animals (Anandharajan et al., 2006). Ekaidem et al. (2007) reported that the ethanol extract of neem leaves have shown to demonstrate anti-lipid peroxidative, anti- hyperglycemic and anti-hypercholesterolemic activities as well as to reduce the triglyceride serum levels in a diabetic rat model. Arumugam et al. (2008) reported anti-diabetic activity of leaves and callus extracts of *Aegle marmelos* in rabbits.

A daily dose of *Bidens pilosa* water extract given once for 28 days significantly reduced blood glucose levels and increased serum insulin levels in db/db mice. Besides, 28-day treatment with *Bidens pilosa* water extract significantly improved glucose tolerance, decreased HbA1c levels and protected islet structure in db/db mice. *Bidens pilosa* water extract stimulated insulin secretion via pancreatic islets (Hsu et al., 2009). Aqueous extracts of *Momordica charantia* and *Trigonella foenum-graecum* exhibited antiperoxidative and antioxidant activities in heart tissues of alloxan- induced diabetic rats by decreasing the levels of lipid peroxidation products and increasing the levels or activities of key antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT),
glutathione-s-transferase (GST) and reduced glutathione (GSH) content. These plants could exert beneficial effects against diabetes and associated free radicals complications in heart tissue. (Umanath and Deepak, 2009).

*Annona muricata* (Adeyemi et al., 2010), *Salacia reticulata* (Arunakumara and Subasinghe, 2010), *Catharanthus roseus* (Karuna et al., 2010), *Teucrium stans* and *Teucrium cubense* (Angel Josabad et al., 2010), *Ficus exasperata* (Adewole et al., 2012), *Gymnema sylvestre* (Kang et al., 2012), *Momordica charantia* (Md Alamgir et al., 2012) and *Coccinia indica* (Shibib et al., 2012) plant extracts exhibited anti-hyperglycemic effect in experimental diabetic animals. Azahar et al. (2012) showed that aqueous extract of *Octomeles sumatrana* is beneficial for improvement of hyperglycemia in streptozotocin-induced diabetic rats.

Ethanolic extract of aerial parts of *Melothria heterophylla* and its active constituents significantly normalized blood glucose levels and serum biochemical parameters as compared to those of STZ controls. Both Gallic acid (4mg kg\(^{-1}\) b.w) and Rutin (4mg kg\(^{-1}\) b.w) exhibited maximum glucose lowering effect (69.1% and 66.7%, respectively) in diabetic rats compared to the other dose (2mg kg\(^{-1}\) b.w) at the end of the study. The selected ethanolic plant extract, gallic acid and rutin also showed significant increase in serum insulin and body weight of streptozotocin-induced diabetic rats (Mondal et al., 2012).

Nain et al. (2012) reported that oral administration of hydromethanolic extract of *Emblica officinalis* leaves at a concentration of 100, 200, 300 and 400 mg kg\(^{-1}\) b.w. daily for 45 days showed a significant decrease in fasting blood glucose, increase in insulin level, reduction in all biochemical parameters like serum creatinine, serum urea, SGOT, SGPT and lipid profile as compared with diabetic rats. The treatment also resulted in a significant increase in antioxidant enzymes such as reduced glutathione, glutathione peroxidase, superoxide dismutase, catalase and decrease in LPO level in the liver and kidney of diabetic rats. The results exerted protective role against lipid peroxidation by scavenging free radicals and reduced the risk of diabetic complications.

Dada et al. (2013) revealed the hypoglycemic and antioxidant activity of hydroethanolic leaf extract of *Byrsocarpus coccineus* in alloxan-induced diabetic rats.
The extract of *Byrsocarpus coccineus* at a dose of 800 mg kg$^{-1}$ b.w on the 10th day preserved *in vivo* antioxidant levels in the kidneys, heart and liver, increased the level of high density lipoprotein and insulin, and reduced the level of triglycerides and low density lipoprotein compared to diabetic control. They also suggested that the plant extract possesses antidiabetic activity possibly mediated through inhibition of intestinal glucose absorption, *in vivo* antioxidant activity and enhancement of regeneration of beta cells of the pancreas and insulin secretion.

Petroleum ether extract of *Streblus asper* stem barks significantly normalized blood glucose levels and serum biochemical parameters as compared with those of STZ controls. α-Amyrin acetate, a compound isolated from stem bark (75 mg kg$^{-1}$ b.w) exhibited maximum glucose lowering effect (71.10 %) in diabetic rats compared to the other dose (25, 50 mg kg$^{-1}$ b.w) at the end of the study. Both petroleum ether extract of *Streblus asper* and α-Amyrin acetate demonstrated remarkable antidiabetic activity in STZ-induced diabetic rats (Karan *et al.*, 2013).

Oral administration of the aqueous extract of *Gmelina arborea* decreased the plasma glucose level significantly in the diabetic rats. Additionally, the aqueous extract also reduced loss of body weight and significantly decreased food and water intake in the diabetic animals (Kulkarni and Veeranjaneyulu, 2013). Agnihotri and Singh (2013) reported that the alcoholic extracts of *Tamarindus indica* and *Cassia fistula* significantly decreased the blood glucose level in alloxan-induced diabetic rats. Serum cholesterol, serum triglyceride, serum creatinine, serum albumin, total proteins and body weight were recovered to normal levels by both the extracts. Aqueous extract of *Leptadenia hastata* roots (Sanda *et al.*, 2013) and extract of *Pterocarpus marsupium* heart wood (Devgan *et al.*, 2013) also exhibited anti-hyperglycemic activity in experimental diabetic rats.

### 2.14. Study Plant- *Acalypha indica* Linn

(i) **Description of the plant**

*Acalypha indica* Linn. is an annual herb found in various parts of India, Bangladesh, Sri Lanka, the Philippines and tropical Africa (Kirtikar and Basu, 1999). It belongs to the Family Euphorbiaceae (Pal *et al.*, 1999) locally known as Khokali or Kuppi in Hindi, Indian copper leaf in English and Kuppaimeni in Tamil. It is a common herb, grows up to 75cm tall.
with ovate leaves. Flowers are green unisexual found in catkin florescence. In West Africa, the leaves are cooked and eaten as a vegetable (Schmelzer and Gurib–Fakim, 2008). It generally occurs as a troublesome weed in gardens, backyards of houses and waste places, roadsides and throughout the plains of India (Praveen et al., 2007).

(ii) Previous investigations of A. indica

Mahesh et al. (1984) showed that the seeds of Acalypha indica possesses laxative and carminative properties and improve appetite. Vaishnava et al. (1993) reported that 50% ethanolic extract of pods of A. indica exhibited anti-fertility activity in female albino rats. Barthakur et al. (1995) reported that the seed powder of A. indica is used for constipation and has cathartic properties. The root extracts are useful in the treatment of diabetes, inflammation, liver disorders, eye disorders and as an antipyretic, abortifacient and demulcent agent (Misra et al., 1996). Aqueous residue of A. indica has been reported to show antibacterial activity against Aeromonas hydrophila and Bacillus cereus (Perumalsamy et al., 1999).

Previous phytochemical investigations on this species revealed the presence of acalyphamide (as acetate), aurantiamide and its acetate, succinamide, calypho-lactate, 2-methyl anthraquinone, tri-o-methylellagic acid, β-sitosterol and its β-D-glucoside (leaves), cyanogenic glycoside, two alkaloids namely acalyphine and triacetonamine, an essential oil n-octacosanol, kaempferol, quebrachitol, β - sitosterol acetate and tannin (whole plant) and stigmasterol (root) (Raj and Singh, 2000). Rastogi and Mehrotra (2004) showed that the roots of A. indica are useful in the treatment of heart diseases and fever and leaf juice for skin diseases. Recently, four kaempferol glycosides mauritianin, clitorin, nicotiflorin and biorobin have also been isolated from the flowers and leaves of this plant (Nahrstedt et al., 2006). Extract of the plant root barks with alcohol can be used for backward fever and the leaves are used as laxative, as emollient, a poultice in insect bites, swelling, rheumatism and facial paralysis (Kirtikar and Basu, 2006). According to Chopra et al. (2006), the roots of Acalypha indica are used for chest pain, joint pain, migraine and dysentery.

The roots of A. indica is prescribed as a tonic, astringent, febrifuge and as a strong purgative (Khare, 2007). Govindarajan et al. (2008) reported antibacterial activity of hexane, chloroform, ethyl acetate and methanol extracts from the leaves of A. indica against Staphylococcus epidermidis, Bacillus cereus, Streptococcus faecalis, Klebsiella
pneumoniae and Proteus vulgaris. The hexane, chloroform and methanol extracts of aerial parts of A. indica showed antibacterial activity against Staphylococcus aureus and Pseudomonas aeruginosa. Nadkarni (2009) reported that the leaves of A. indica possess anti-periodic and laxative properties and the leaves are used in jaundice, piles, rheumatism, ulcer, skin eruptions, ring worms, eczema and insect bites. The ashes from burnt pods of A. indica mixed with little salt and honey relieve cough (Ben et al., 2009).

The ethanol and aqueous extracts of roots of A. indica exhibited significant anti-oxidant activity, which may be related to the flavonoid and phenolic contents of the plant (Balaksrishnan et al., 2009). The leaf extract of this plant reduced mutagenicity in Escherichia coli (Gupta, 2010). Rahman et al. (2010) showed that the methanolic extract of whole plant of A. indica exhibited analgesic and anti-inflammatory activities. The chloroform and methanol extracts of A. indica showed inhibition zones against Aspergillus flavus and Clostridium albican and also reported significant anti-oxidant and anticancer activities of aerial parts of A. indica (Sanseera et al., 2010). Madhuri et al. (2011) reported that the petroleum ether extract of A. indica leaves was found to be a potent vasoconstrictor, due to the presence of steroids in the plant.

2.15. Phytochemical analysis

(i) Qualitative analysis of phytochemical components

Phytochemical analysis have attracted the attention of plant scientists due to the development of new and sophisticated techniques. These techniques played a significant role in giving the solution to systematic problems on the one hand and in search for additional resources of raw materials for pharmaceutical industry on the other hand. Plants synthesize a wide variety of chemical compounds, which can be sorted by their chemical class, bio synthetic origin and functional groups in to primary and secondary metabolites. Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information be of value in disclosing new resources of such chemical substances (Mojab, 2003).

Traditional indigenous medicine is limited to small tribal and geographical area called “little traditions” are an excellent repository of knowledge about medicinal properties of botanical sources. Komboj (2000) stated that the bioactive extract should be
standardized on the basis of phytochemical compounds. Phytochemical screening of medicinal plants are very important in identifying new sources of therapeutically and industrially important compounds. It is imperative to initiate an urgent step for screening of plants for secondary metabolites.

Ethanolic extracts of 45 Indian medicinal plants traditionally used in medicine were studied for its phytocompounds qualitatively. The study inferred that all the plants found to possess common phytocompounds including phenols, tannins and flavonoids as major active constituents (Ahmad and Beg, 2001).

Lavanya and Hemalatha (2002) isolated phytochemical compounds such as carotenoids, anthocyanins and flavonoids from Vinca rosea flower extract. The flavonoids such as isoflavones, chalcones and aurones were isolated, purified and characterized from petals of Cassia angustifolia flowers (Kavitha and Hemalatha 2002). Sekar (2002) isolated anthroquinones from the methanolic extract of Cassia obtusa roots. Malini and Hemalatha (2002) isolated flavonoids, terpenoids and carotenoids from the leaves of Centella asiatica.

Ramana et al. (2003) reported the preliminary phytochemical analysis of Eupatorium odoratum showed the presence of glycosides, flavonoids, steroids, saponins and tannins. Chromatographic fragmentation of seeds of P. zenkeri led to the isolation and characterization of the major constituents viz coumarins, umbelliprenin, imperatoren, bergaptan, isopimpinellin and byaangelicin. New triterpene glycosides were isolated from the stems of Anomospermum grandifolium by Plaza et al. (2003).

Ten medicinal plants belonging to different families viz Cleome nutidosperma, Emilia coccinea, Euphorbia heterophylla, Physalis angulata, Richardia bransitensis, Scopania dulcis, Sida acuta, Spigelia anthelmia, Stachytarpheta cayennensis and Tridax procumbents were screened for their phytocomstituents by Edeoga et al. (2005). All the plants were found to contain alkaloids, tannins, flavonoids except for the absence of tannins in S. acuta and flavonoids in S. cayennsis, respectively.

Bark of Bauhinia variegata was defatted with petroleum ether. The non-defatted plant material as well as the defatted plant material was then individually extracted in different solvents with increasing polarity viz. 1,4-dioxan, acetone, methanol,
dimethylformamide (DMF) and distilled water, respectively. The extractive value of *B. variegata* for non-defatted extracts ranged from 0.7-13% and for defatted extracts the extractive value ranged from 1-10.5% (Parekh *et al*., 2006).

Gilani *et al.* (2007) stated that the medicinal plant *Rhazya stricta* found to contain more than 100 alkaloids. Out of which only few of these compounds were screened for their pharmacological activities. The phytochemical screening of the ethanolic extract of *Carica papaya* leaves, *Mangifera indica* stem bark, *Psidium guajava* leaves and *Vernonia amygdalina* leaves showed the presence of flavonoids, terpenoids, saponins, tannins and reducing sugars. *M. indica* did not contain cardiac glycosides and alkaloids while *P. guajava* also showed the absence of alkaloids and anthroquinones. Anthroquinones were similarly absent in *V. amygdalina* (Ayoola *et al*., 2008).

Chitravadivu *et al.* (2009) performed the qualitative analysis of four important medicinal plants viz., *Acalypha indica*, *Cassia auriculata*, *Eclipta alba* and *Phyllanthus niruri* to detect the presence or absence of several bioactive compounds which are reported to cure different diseases and ailments. Some of the compounds detected were anthroquinone, alkaloids, catechols, flavonoids, phenolic compounds, saponins steroids, tannins and triterpenoids. Studies of Doss (2009) indicated that the medicinal plants viz., *Asteracantha longifolia*, *Passiflora edulis*, *Berberis tinctoria*, *Sphaeranthus indicus* and *Solanum trilobatum* were found to contain phenols, cardiac glycosides, steroids saponins and tannin except for the absence of flavonoids in *A. longifolia* and alkaloids in *P. edulis*, *A. longifolia*, *B. tinctoria* and *S. indicus*, respectively.

Phytochemical analysis of methanolic extract of four medicinal plants viz. *Plumbago zeylanica* (Root), *Acorus calamus* (Rhizome), *Hemidesmus indicus* (Stem) and *Holarrhena antidysenterica* (Bark) revealed the presence of major phytocompounds like alkaloids, glycosides, phenolics and saponins (Zahin *et al*., 2009).

Sathyaprabha *et al.* (2010) reported the presence of phytochemical constituents namely tannin, phlobatannins, saponin, flavonoids, steroids, terpenoids and cardiac glycosides in *Aloe vera* and *Cissus quadrangularis*. Preliminary phytochemical analysis of *Eclipta alba* and *Morinda citrifolia* extracts showed the presence of different groups
of secondary metabolites viz alkaloids, flavonoids, tannins, terpenoids and saponin which are of medicinal importance. The phenolic groups and flavonoids were rich in ethyl acetate extract when compared to other metabolites (Sharma and Sharma, 2010).

Praveenkumar et al. (2010) carried out the identification of phytochemicals, evaluation of total phenols, total flavonoids and antioxidant activity in Vitex negundo leaves. Total phenols were determined by Folin-Ciocalteu method and the phenolic content was 27.72 mg /100 g of gallic acid equivalent (GE).

Qualitative phytochemical screening of 18 important medicinal plants viz., Annona reticulata, Annona squamosa, Artabotrys hexapetalus, Bixa orellana, Cadaba indica, Capparis zeylanica, Clematis gouriana, Cleome viscosa, Cochlospermum religiosum, Cocculus hirsutus, Cyclea peltata, Dilienia indica, Nymphaea nelumbo, Polyalthia longifolia, Polyalthia pendula, Tinospora cardifolia, Cissampelos pareira, and Maeruna oblongifolia confirmed the presence of various phytochemicals like saponins, terpenoids, steroids, anthocyanins, coumarins, fatty acids, tannins, leucoanthocyanins and emodins (Savithramma et al., 2011).

Vaghasiya et al. (2011) investigated the total phenols and flavonoids content of 53 traditionally used medicinal plants of Western region of India. Qualitative phytochemical analysis was done for various phytoconstituents like alkaloids, tannins, cardiac glycosides, steroids and saponins. Tannins were present in more number of plants followed by cardiac glycosides and steroids. The Mangifera indica showed highest phenolic content, maximum content being in the methanol extract followed by Strychnos nux-vomica, Origanum marjoram. The Aristolochia bracteolate showed highest flavonoid content, maximum content being in the acetone extract followed by Phyllanthus reticulates, Argemone mexicana. The results suggested a positive correlation between total phenolic content and antioxidant activity and it can be stated that the Mangifera indica may possess good antioxidant property because of its high phenolic content.

Studies of Yadav and Munin (2011) in seven medicinal plants such as Bryophyllum pinnatum, Ipomea aquatica, Oldenlandia corymbosa, Ricinus communis, Terminalia bellerica, Tinospora cordifolia, and Xanthium strumarium showed the presence of phytochemicals, total phenolics and flavonoid contents. Proteins,
carbohydrates, phenols, tannins, flavonoids and saponins were detected in all of the plants tested. Total phenolic contents obtained were 18.4 mg/gm, 18.8 mg/gm, 11.6 mg/gm, 29.2 mg/gm, 29.6 mg/gm, 40.8 mg/gm, 12.8 mg/gm, 71.6 mg/gm of the extract and total flavonoid contents obtained were 8.4 mg/gm, 37.6 mg/gm, 4.4 mg/gm, 6 mg/gm, 42.8 mg/gm, 18 mg/gm, 6 mg/gm, 28.8 mg/gm of the extract in Bryophyllum pinnatum (Leaves), Ipomea aquatica (Leaves), Oldenlandia corymbosa (Whole plant), Ricinus communis (Roots), Terminalia bellerica (Leaves), Tinospora cordifolia (Leaves), Tinospora cordifolia (Stem), and Xanthium stramarium (Leaves), respectively.

Cardiospermum halicacabum and Cassia auriculata leaves were screened for the presence of secondary metabolites. Data revealed that the leaves of C. halicacabum found to contain saponins, flavonoids and steroids. Tannins, saponins, flavonoids and terpenoids were present in C. auriculata leaves (Senthilkumar and Vijayakumari, 2012 a). Phytochemical analysis of an underexplored plant, Melothria perpusilla indicated the presence of flavonoids, cardiac glycosides, triterpene, steroids and tannins (Menaga et al., 2012).

Iqbal (2012) reported the phytochemical screening of six native plants of Agra city i.e Achyranthus aspera, Acalypha indica, Euphorbia hirta, Lidenbergia indica, Parthenium hysterophorus and Peristrophe bicalyculata. The study indicated the presence of active compounds like alkaloids, tannins, saponins, glycosides, phenols, flavonoids, anthroquinone, terpenoids and steroids. Ethanolic extract of A. aspera showed all of these phytocompounds except tannins in comparison to other extracts. However ethanolic extracts of all plant species revealed the presence of most of the phytocompounds in comparison to other extracts tested.

The medicinal plant Cardiospermum halicacabum found to contain 38.04 mg g⁻¹ of total phenolics, 1.05 mg g⁻¹ of total flavonoids and 4.99 mg g⁻¹ of tannins (Senthilkumar and Vijayakumari, 2012 b).

The phytochemical screening revealed the extract richness in tannins, phlobatannin, saponins, flavonoids, steroids and alkaloids. Quantitative analysis of phenolics, alkaloids, saponins and flavonoids had revealed that Mentha spicata possessed maximum phenolics (80.41%), Gmelina arborea had the highest alkaloids (5.66%) and flavonoids (22.80%) and Trigonella foenum – graecum had the highest saponins...
(50.12%) contents (Soni and Sosa, 2013). Phytochemical analysis showed the presence of carbohydrates and saponins in all four samples viz. *Zingiber officinale, Curcuma longa, Cumminphora molmol* and *Pimpinella anisum*. Alkaloids were found in *Z. officinale* and *C. molmol* whereas flavonoids in *C. longa* and *P. anisum*. Steroids and tannins were found only in *Z. officinale* and *C. longa*, respectively (AI – Daihan et al., 2013). Studies of Wadood et al. (2013) in ten different medicinal plants, *Acacia nilotica, Psidium guajava, Luffa cylindrica, Morus alba, Morus nigra, Momordica charantia, Fagonia cretica, Punica granatum, Ficus palmatae and Prunus persica* showed the presence of terpenoids, phlobatannins, reducing sugars, flavonoids and alkaloids.

(ii) Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) of methanolic leaf extract of *Asparagus racemosus* revealed the presence of three bands with Rf values 0.40, 0.45 and 0.48 respectively, which indicate the presence of phenolic compounds (Asha et al., 2013). TLC analysis of *Caesalpinia sappan* leaves of methanol and ethanol extracts showed two spots for various phytocompounds at the resolution value of Rf 0.67, 0.90 for methanol extract and Rf value 0.60 and 0.95 for ethanol extract (Saravanakumar and Helan Chandra, 2013). Shreya et al. (2013) reported the presence of bioactive constituents in the methanolic extract of *Terminalia arjuna* bark extract. Phytochemical analysis of ethyl acetate extract of *Bridelia micrantha* showed that the non-polar BEA (Benzene/ethanol/ammonium hydroxide) system exhibited a much more separated profile of twelve compounds, while the non-polar EMV (Ethylacetate/Methanol/Water) system showed only four compounds (Anthonio and Roland, 2013).

(iii) Infra-red spectral analysis

Infra-red spectral analysis of methanolic extract of *Plumeria rubra* flowers and *Eucalyptus globus* leaf extracts revealed the presence of O-H, C=O, C-H, C=C, C-N and C-O bond stretching, indicating the presence of phytochemicals such as phenolic compounds with O-H and alkaloids with C-N (Egwaikhide et al., 2007). Shen and Laigeng (2011) demonstrated that NIR data can be applied to analyze the chemical contents including kalsen lignin, holo cellulose and α-cellulose content and S/G ratio, as well as to predict the digestibility of biomass materials from *Eucalyptus* trees. A study
conducted by Nazima and Adeel (2013) suggested that IR spectroscopy can be considered as a vital technique for identification, analysis, determination of the degree of fatty oils saturation and adulteration of plant origin.

(iv) GC-MS analysis

Gas Chromatography has a very wide field of applications. Chromatography is a term used to describe a separation technique in which a mobile phase carrying a mixture is caused to move in contact with the selectively absorbent stationary phase. The more precise information in qualitative analysis can be obtained by Gas Chromatography is coupled with Mass Spectrophotometry. Gas Chromatography (GC-MS) is normally used for the direct analysis of components existing in traditional medicines and medicinal plants. In recent years, GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non-polar components, volatile essential oil, fatty acid, lipids and alkaloids (Hanbali et al., 2005).

It has been shown that in vitro screening methods could provide the preliminary characterization necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations (Mathekaga and Meyer, 1998). Plants are used medicinally in different countries and they are the source of many potent and powerful drugs. Plants are the important source of popular folk medicine, useful for traditional remedies.

The chemical composition of the volatile oil constituent from the roots of *Pulicaria odora* has been analysed by GC –MS. About twenty-seven components were identified and the main constituents such as thymol (47.83%) and isobutyrate (30.05%) exhibited a significant antibacterial activity (Hanbali et al., 2005).

Lacikova et al. (2007) analysed four *Staphylea* plant species and identified four tocopherols, three sterols, amyrine, cyclourtenol, actinidiolide and linolenic acid by GS-MS method. GC-MS analysis of ethyl acetate extract of *Goniothalamus umbrosus* exhibited the presence of 1-butyl-2-cyclohexenol (46.84%), benzaldehyde (4.42%) and Globulol (4.07%) (Abdelwahab et al., 2009).
GC-MS analysis of the volatile components of the aerial parts of *Macfadyena unguis-cati* revealed the presence of 52 compounds with major components namely n-decane (12.12%) and phytol (12.19%) (Aboutab *et al.*, 2010).

GC-MS analysis of *Aloe vera* and *Cissus quadrangularis* showed the presence of squalene, oleic acid and dodecanoic acid and eugenol, n-Hexadecanoic acid, 1,2 Benzene dicarboxylic acid, diiso octyl ester, phenol and 2,4 bis (1-phenyl ethyl) respectively (Sathyaprabha *et al.*, 2010). The GC-MS study was carried out in *Vitex negundo* showed the presence of phytochemicals like 4 H-pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6 methyl – (RT : 6.17), phytol (RT : 19.67) and vitamin E (RT : 25.11) (Praveenkumar *et al.*, 2010).

A study in *Mussaenda frondosa* showed the presence of about 20 different chemical constituents by GC-MS analysis (Gopalakrishnan and Vadivel, 2011). Rajeswari *et al.* (2011) reported the presence of sesquiterpenoids, nitrogenous compounds, aldehydes, terpinolene and phenol constituents, which may be responsible for the anti-microbial, anti-tumour and antioxidant properties. Sarumathy *et al.* (2011) reported the presence of phytochemicals namely 9,12,15 octadecatrienoic acid, methyl esters, 2,2,2-n hexadecanoic acid, 1,2-benzenedicarboxylic acid and di-isooctyl esters in ethanolic extract of *Caesalpinia sappan*. GC-MS analysis of ethanolic extract of *Murraya koenigii* revealed the existence of 1-Methyl-pyrrolidine-2-carboxylic acid (69.00%), Ethyl-a-d-glucopyranoside (13.36%), Isolongifoline,4,5-dehydro- (3.68%), Himachalene (2.88%), 1,2-ethanediol, monoacetate (2.79%), 1,2 benzene dicarboxylic acid, diiso octyl ester (2.55 %) (Hema *et al.*, 2011).

GC-MS analysis of methanolic extract of *Justicia wynnaedensis* showed the presence of 24 phytocompounds. Hexadecanoic acid (synonym : palmitic acid), 2H-1-Benzopyran-2-one, 3,4-dihydro (synonym: Dihydrocoumarin/melitol) and 3,7,11,15- Tetramethyl-2-hexadecan-1-1 (synonym: phytol) were the major components in the extract. Palmitic acid is reported to be an antioxidant, a nematicide and a pesticide while melitol and phytol are said to be cancer-preventive. Other antioxidants present were tetradecanoic acid, hexadecanoic acid, heptadecanoic acid, 2,6,10,14, 18, 22 – tetracosa hexane, 2,6,10, 15, 19, 23 – hexamethl (synonym: squalene), gamma tocopherol, vit E, Ergot-5-en-3, Beta-ol (synonym:}
Campesterol) and stigmasta-5,22-dien-3 beta-ol (3, beta, 22E) - (synonym: stigmasterol). The presence of so many antioxidants in *J. wynaadensis* justifies the high antioxidant property observed (Ponnamma and Manjunath, 2012).

GC-MS analysis of methanolic extract of *Eupatorium triplinerve* showed the presence of ten compounds. The major compounds were hexadecanoic acid (14.65%), 2,6,10-trimethyl, 14-ethylene – 14-pentadecane (9.84%), heptanes (2.38%), decanoic acid (3.86%), 1-undecanol (7.82%), 1-hexyl-1-nitrocyclohexane (2.09%), 1, 14-tetra decanediol (6.78%), octadecanoic acid (19.18%) and 2-hydroxy-3 [(9E) -9-octadecanoyl oxy] propyl (9E)-9 octadecanoate (8.79%). The spectrum profile of GC-MS confirmed the presence of 10 major compounds with retention time 15.084, 15.75, 16.2, 16.40, 16.96, 17.15, 18.38, 19.986, 20.148 and 21.619, respectively. The three major compounds viz. hexadecanoic acid, tetradecanoic acid and octadecanoic acid have shown to have hypocholesterolemic activity, antioxidant and lubricative activity. Anticancer and antiproliferative activities were shown by tetradecanoic acid and 2,6,10 – trimethyl-14-ethylene-14-pentadecane, while 1-hexyl-1-nitro cyclohexane and 1,14- tetra decanediol have shown antimicrobial and anti-inflammatory activities (Selvamangai and Anusha, 2012).

In GC-MS analysis 13 bioactive phytochemical compounds were identified in the ethanolic extract of *Cassia auriculata* by comparing their retention indices and mass spectra fragmentation patterns with those stored on MS-computer library. The major constituents identified were 3, 0-methyl d-glucose (59.71%), 2H- cyclopropa(a) naphthalene-2-one, 1, 1 a, 4, 5, 6, 7, 7 a, 7 b-octahydro-1, 1, 7, 7a-tetramethyl (11.57%) and azulene (10.80%). The other important constituents identified were phytol, squalene and 1,2-Benzenedicarboxylic acid, diisooctyl ester (Senthilkumar and Vijayakumari, 2012d).

Eight bioactive phytochemical compounds were identified in the ethanolic extract of *Euphorbia hirta* by GC-MS analysis. The results indicated that the compounds viz., 1, 6,10, 14 - Hexa decatetra en-3-ol, 3, 7, 11, 15 - tetra methyl - (E,E), Phytol and diazoprogesterone were major components (Suresh et al., 2012 a). In GC-MS analysis nine bioactive compounds were identified in the ethanolic extract of *Boerhaavia diffusa*, which confirm the presence of myo-inositol 4-c methyl, 1,14 Tetra decanediol phytol and vitamin E acetate as major constituents (Umamenaka et al., 2012).
Abirami and Rajendran (2012), evaluated bioactive compounds from *Vernonia cinera* using GC-MS analysis and the major components were found to be n-hexadecanoic acid (42.88%) and 1, 2 benzene dicarboxylic acid diisoctyl ester (23%). Bagavathi and Neelamegam (2012), reported the GC-MS study of ethanolic extract of whole plant of *Polygonum chinense* exhibited the presence of squalene with anticancer and antioxidant properties. *Calortropis procera* on GC-MS analysis revealed the presence of 9 phytochemicals out of which 2,6 dimethyl tetra-1,5-decaene and 3, 7,11 – Trimethyl – 2,6,10,12 – pentadecatrien-l-ol are newly reported from the latex (Doshi *et al.*, 2012). The phytochemical investigation on *Tylophora indica* by GC-MS analysis led to the identification of 10 compounds mainly Phytol, Ethyl tridecanoate and Oleic acid (Gurav, 2012).

GC- MS analysis of ethanolic extract of *Cardiospermum halicacabum* leaves found to contain 15 different bioactive components. Of which 3-0 methyl d glucose, $\alpha$- Amyrintrimethylsilyl ether, 1, 14 Tetradecanediol, vitamin E acetate and phytol were the major constituents followed by squalene and silane, 1, 4 phenylene bis (trimethyl) (Senthilkumar and Vijayakumari, 2012e). Studies of Suresh *et al.* (2012b) in ethanolic extract of *Tephrosia purpurea* by GC-MS method indicated the presence of myo- inositol 4- c methyl, squalene and phytol as major constituents.

GC-MS analysis of *Wedelia chinensis* leaf extract revealed the presence of 25 compounds and the major compounds include 9, 12, 15 octadeca trienoic acid, methyl ester, (z,z,z) (13.68%) and pentadecanoic acid, methyl ester (10.04%) (Rehana Banu and Nagarajah, 2013). Shirsat *et al.* (2013) reported the presence of bioactive compounds viz., Tetradecane (20.51%), 1-butoxy, 2-ethyl hexane (13.91%), betulin (10.84%) and 2-methyl benzoic acid (10.13%) in aerial parts of *Calotropis gigantea*.

### 2.16. Free Radicals and their mechanism of action

Free radical is a chemical compound, which contains an unpaired electron spinning on the peripheral layer around the nucleus. The family of free radicals generated from the oxygen is called as ROS (Reactive Oxygen Species), which cause damage to other molecules by extracting electrons from them in order to attain stability. ROS are ions, atoms or molecules that have the ability to oxidize reduced molecules. ROS are various forms of activated oxygen, which include free radicals such as superoxide anion radicals ($O_2^-$) and hydroxyl radicals (OH$^-$),
non-free radicals (H$_2$O$_2$) and singlet oxygen ($^1$O$_2$) (Halliwell, 1995). Superoxide anion radicals increase under stress conditions such as heavy exercise, certain drugs, infection and various disease states. During normal metabolic processes, human body generates more than 2 Kg of O$_2$ per year (Evans and Halliwell, 1999).

In the body, free radicals are from two sources: endogenous sources, e.g. nutrient metabolism, ageing process etc and exogenous sources e.g. tobacco smoke, ionizing radiation, air pollutants, organic solvents, pesticides etc (Buykokuroglu et al., 2001).

Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The propagation of free radicals can bring about thousands of reactions and may cause extensive tissue damage. Lipids, proteins and DNA are susceptible to attack by free radicals (Yu et al., 1992; Cotran et al., 1999). Most of the free radicals that occur \textit{in vivo} originate either from ROS (Reactive Oxygen Species) or Reactive Nitrogen Species (RNS). The majority of free radicals produced \textit{in vivo} are oxidants, which are capable of oxidizing a range of biological molecules including carbohydrates, fatty acids, amino acids and nucleotides (Knoncke et al., 1995; Cooper et al., 2002).

Free radicals are produced in the body during drug metabolism, exposure to ionizing radiation, UV light, pollution etc and cause chain of reactions which may magnify the cell damage by several folds (Naik et al., 2005). Free radicals are extremely reactive and unstable and can damage cells leading to cell mutation and destruction (Nencini et al., 2007). The free radicals mediated cell damage may play an important role in occurrence of many disorders viz Diabetes, Coronary Heart Disease CHD and Cancer (Srividya et al., 2009).

\section*{2.17. Reactive Oxygen Species (ROS)}

Reactive oxygen species are sometimes called as Active Oxygen Species (AOS). In living organisms, various ROS can be formed in different ways, including normal aerobic respiration stimulated polymorph nuclear leucocytes, macrophages and peroxisomes. They appear to be the main endogenous source of most of the oxidants produced by cells. Exogenous source of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents and pesticides (Naphade et al., 2009). ROS are ions, atoms or molecules that have the ability to oxidize reduced molecules and
are short lived entities that are continuously generated at low levels during the course of normal aerobic metabolism. ROS are various forms of activated oxygen which include singlet oxygen (\(1^1O_2\)), superoxide (\(O_2^-\)), hydrogen peroxide (H\(_2\)O\(_2\)), hydroxyl radical (OH•) etc. (Bickers and Ather, 2006)

**Fig. III. Formation of Free Radicals**

Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are collectively termed as free radicals and other non-radical reactive derivatives are known as oxidants. Free radicals are less stable than non-radical species, although their reactivity is generally stronger. Free radicals are formed from molecules via the breakage of chemical bonds to give another radical and also via redox reaction (Bahorun et al., 2006). Free radicals include Hydroxyl (OH•), Superoxide (O\(_2^+\)), Nitric oxide (NO•), Nitrogen dioxide (NO\(_2^+\)), Peroxyl (ROO•) and lipid peroxyl (LOO•) radicals. But Hydrogen peroxide (H\(_2\)O\(_2\)), Ozone (O\(_3\)), Singlet oxygen (\(1^1O_2\)), Hypochlorous acid (HOCl), Nitrous acid (HNO\(_2\)), Peroxynitrite (ONOO•), Dinitrogen trioxide (N\(_2\)O\(_3\)) and Lipid peroxide (LOOH) are not free radicals and generally known as oxidants. However these oxidants may lead to free radical reactions in living organisms (Genestra, 2007).
Reactive oxygen species (ROS) play an important role in physiological processes, but when being in excess ROS causes oxidative damage to molecules. Under physiological conditions, the production and detoxification of ROS are more or less balanced. Also in the thyroid, ROS and free radicals participate in physiological and pathological processes in the gland. For example, hydrogen peroxide (H$_2$O$_2$) is crucial for thyroid hormone biosynthesis, acting at different steps of the process. Additionally, H$_2$O$_2$ is believed to participate in the Wolff - Chaikoff’s effect, undergoing in conditions of iodide excess in the thyroid. Much evidence has been accumulated indicating that oxidative stress is involved in pathogenesis of thyroid functions, e.g. Graves’ disease, goitre formation or thyroid cancer (Karbownik and Lewinski, 2003).

When oxygen traps single electron, it becomes unstable and thus very reactive, since it generates harmful chain reactions against many biological molecules. The extreme toxicity of oxygen is related to its high capability of generating free radicals and in turn destroying many major biological molecules. They can attack lipids and proteins and destroy membranes. ROS can also damage DNA and lead to mutation and chromosomal damage. Oxidized cellular thiols abstract hydrogen atoms from unsaturated fatty acids to initiate the peroxidation of membrane lipids (Valko et al., 2006).

### 2.18. Beneficial activities of free radicals and oxidants

At low or moderate concentrations, ROS and RNS are necessary for maturation process of cellular structures and can act as weapons for the host defence system. Indeed, phagocytes such as neutrophils, macrophages and monocytes release free radicals to destroy invading pathogenic microbes as part of the body’s defence mechanism against infectious disease (Young and Woodside, 2001; Droge, 2002). At low or moderate concentration, some of the free radicals play beneficial physiological role in vivo which include energy production, cell growth, function in different cellular signalling systems and induction of mitogenic response (Poli et al., 2004).

At high concentrations, ROS can behave as an important mediator of damage to cell structures, nucleic acids, lipids and proteins (Valko et al., 2006; 2007). Superoxide radical (O$_2$•-) is responsible for lipid peroxidation and has the capability to decrease the activity of other antioxidant defence system of enzymes such as catalase (CAT) and
glutathione peroxidase (GP\textsubscript{X}). It causes damage to ribonucleotide, which is required for DNA synthesis. The protonated form of (O\textsubscript{2}*) is HO\textsubscript{2}*, which is more reactive and able to cross the membrane and damage the tissues.

OH\textsuperscript* radical is the most reactive chemical species. It is a potent cytotoxic agent and able to attack and damage almost every molecule present in living tissue. H\textsubscript{2}O\textsubscript{2} is not a radical, but produces toxicity to cells by causing DNA damage, membrane disruption and release calcium ions within cells, resulting in activation of calcium dependent photolytic enzymes. HOCl is produced by the enzyme myeloperoxidase in activated neutrophils and initiates the deactivation of latent proteases leading to tissue damage (Halliwell and Gutteridge, 1999). It has the ability to damage bio molecules, directly and also decomposes to liberate toxic chlorine. Metal induced generation of ROS attack DNA and other cellular components involving poly unsaturated fatty acid residues of phospholipids, which are extremely sensitive to oxidation (Siems et al., 1995).

2.19. Oxidative stress

Oxidative stress describes the condition, where the amount of ROS overpowers the amount of neutralizing agents or antioxidants (Baynes 1991; Chang et al., 1993; Young et al., 1995). Oxidative stress depicts the existence of products called free radicals and reactive oxygen species (ROS), which are formed under normal physiological conditions but become deleterious when not being eliminated by the endogenous systems. In fact, oxidative stress results from an imbalance between the generation of reactive oxygen, species and endogenous antioxidant systems. ROS exist in various forms of activated oxygen, which include free radicals such as superoxide anion radicals (O\textsubscript{2}•) and hydroxyl radicals (OH•) as well as non – free radical (H\textsubscript{2}O\textsubscript{2}) and the singlet oxygen (\textsuperscript{1}O\textsubscript{2}). Oxygen derived free radicals such as superoxide anions, hydroxyl radicals and hydrogen peroxide are cytotoxic and cause tissue injuries (Jainu and Shyamala Devi, 2005). Excessive production of free radicals results in oxidative stress, which leads to damage of macromolecules such as nucleic acid, proteins and lipids (Sinclair et al., 1990; Dreher and Junod, 1996).

Oxidative stress is characterized by an increased concentration of oxygen-derived products that stimulate critical, even irreversible cell injury. Oxidative stress occurs when
there is a serious imbalance in any cell compartment between the production of ROS and antioxidant defence, leading to significant physiological challenges (Ahmad et al., 2009). Oxidative stress is caused by an insufficient capacity of biological systems to neutralize excessive free radical production, which can contribute to human disease and aging, including cardiovascular disease, obesity and insulin resistance, neurodegenerative diseases, age-related cognitive decline and immune system dysfunction. Oxidative stress also contributes to the accumulation of damaged macro molecules and organelles including mitochondria (Jensen et al., 2008).

Oxidative stress derived from excessive superoxide production and imbalance in antioxidant enzymes has been related to many other pathologies such as diabetes, hepatitis, rheumatoid arthritis, influenza, ulcer, pneumonia, HIV infection, cataract and glaucoma (Quejeq and Rezvani, 2007).

2.20. Oxidative stress and diabetes

The increased oxidative stress and accompanying decrease in antioxidants may be related to the causation of diabetes (Mohamed et al., 1999). In diabetes mellitus, alterations in the endogenous free radical scavenging defence mechanisms may lead to ineffective scavenging of ROS, resulting in oxidative damage and tissue injury (Oberley, 1988). Oxidative stress in diabetes mellitus could cause disturbances at the level of sub cellular organelles, especially in the liver, which is the metabolic ‘powerhouse’ of the body. Evidence of mitochondrial alterations in diabetic rats has been noticed for a long time (Gerbitz et al., 1996). Mitochondrial damage can in turn generate a further oxidative stress inside the cell, therefore liver mitochondria from streptozotocin treated rats are likely to generate increased levels of reactive oxygen species (Kristal et al., 1997).

Oxidative stress in diabetes mellitus has been shown to coexist with a reduction in the endogenous antioxidant status (Baynes, 1991; Surtis and Ashwood, 1996). Diabetic individuals and experimental animals exhibit high oxidative stress due to persistent and chronic hyperglycemia, thereby depleting the activity of oxidative defence system and thus promoting de novo free radical generation (Hammers et al., 1991). Oxidative stress has recently been shown to be responsible for pancreatic β-cell dysfunction caused by glucose toxicity. Under hyperglycemia, production of various reducing sugars, such as glucose-6-phosphate and
fructose, increase through glycolysis and polyol pathways. During this process, ROS are produced and cause tissue damage (Hunt et al., 1990; Matsuoka et al., 1998; Sakurai and Tsuchiya, 1998).

Oxidative stress, implicated in the pathogenesis of a wide range of clinical disorders like diabetes refers to the cytological consequences of a mismatch between the production of free radicals and the ability of the cells to defend against them. Oxidative stress can thus occur, when the generation of free radicals and repair of oxidative modified macro molecules decreases or both (Sies, 1997). This imbalance leads to accumulation of oxidative modified molecules, predominantly end-products of superoxide O$_2^-$ and hydroxyl (OH) anions, hydrogen peroxide (H$_2$O$_2$) and peroxynitrate (ONOO$^-$), although not free radicals themselves, contribute to the cellular redox state. These molecules, collectively referred to as ROS, produce significant functional alterations in lipid, protein and DNA molecules (Jacob and Burri, 1996).

Oxidative stress induced by hyperglycemia in diabetes is a major cause for development and progression of diabetic micro vascular complications such as diabetic nephropathy, a state in which oxidative stress increases and anti-oxidant status reduces (Kikkawa et al., 2003). Oxidative stress plays a major role in generation of free radicals in the pathogenesis of diabetes and its complications. Auto oxidation of glucose and non-enzymatic protein glycation occurs during persistent hyperglycemia and this may cause disruption of cellular function and oxidative damage of cell membranes, due to increased level of free radicals (Bukan et al., 2003). Oxidative stress and oxidative damage to the tissue are common end points of chronic diseases such as atherosclerosis, diabetes and rheumatoid arthritis (Baynes, 1991).

Earlier reports have demonstrated that oxidative processes result in the loss of key antioxidant enzymes. The damage brought about by oxidative stress is expected to be exacerbated, if the antioxidant enzymes themselves are damaged and inactivated by glycation-induced oxidative stress ultimately resulting in the perturbation of cellular redox status (Shin et al., 2006). Oxidative stress is currently suggested as an important mechanism underlying diabetes and diabetic complications (Moussa, 2008).
2.21. STREPTOZOTOCIN AS A DIABETOGEN

Streptozotocin (STZ) is an antibiotic produced by Streptomyces achromogenes, which possesses pancreatic β-cell cytotoxic effect (Weiss, 1982). In the mid-1960s, Streptozotocin was found to be selectively toxic to the β-cells of the pancreatic islets, the cells that normally regulate blood glucose levels by producing the hormone insulin. This suggested that the drug may be used as an animal model of diabetes (Mansford and Opie, 1968; Rerup, 1970). Due to its high toxicity to β-cells, streptozotocin has also been used for inducing insulitis and diabetes on experimental animals in scientific researches (Rossini et al., 1997).

Streptozotocin is approved by the U.S. Food and Drug Administration (FDA) for treating metastatic cancer of the pancreatic islet cells. Since it carries a substantial risk of toxicity and rarely cures the cancer, its use is generally limited to patients whose cancer cannot be removed by surgery. In these patients, streptozotocin can reduce the tumour size and reduce symptoms, especially hypoglycemia due to excessive insulin secretion by insulinomas (Brentjens and Saltz, 2001).

Chemical structure of Streptozotocin

Systematic name

2-Deoxy-2-{{methyl(nitroso)carbamoyl]amino}-α-D-glucopyranose
Pharmacokinetic data

Bioavailability : 17-25%

Metabolism : Liver, kidney

Half – life : 35-40 minutes

Chemical data

Formula : C₈H₁₅N₃O₇

Mole mass : 265.221g/mol

Clinical data

Trade name : Zanosar

Routes : Intravenous

Mechanism of action of Streptozotocin

Streptozotocin (STZ), a β - cytotoxin, induces ‘chemical diabetes’ in a wide variety of animal species, including rat by selectively damaging the insulin secreting β-cells of the pancreas. Intra peritoneal injection of STZ produces fragmentation of DNA of β-cells of pancreas, which stimulates poly (ADP- ribose) and depletes NAD ultimately leading to destruction of β-cells and it is evidenced by clinical symptoms of hyperglycemia and hypoinsulinemia (Hofteizer and Carpenter, 1973; Rodrigues and Mc Neill, 1986).

Streptozotocin induced hyperglycemia has been described as a useful experimental model to study the effect of anti -diabetic agents against diabetes mellitus. Streptozotocin enters the β- cell via GLUT₂ transporter and causes alkylation of DNA, which further induces the activation of polyADP- ribosylation, a process that is more important for the diabetogenicity of streptozotocin than DNA damage. PolyADP-ribosylation may lead to depletion of cellular NAD⁺ and ATP. Enhanced ATP dephosphorylation after streptozotocin treatment supplies a substrate for xanthine oxidase, resulting in the formation of free radicals. Furthermore, streptozotocin liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage. As a result of the streptozotocin action, β-cells undergo the destruction by necrosis. Streptozotocin action
in β-cells is accompanied by characteristic alterations in blood insulin and glucose concentrations (Szkudelski, 2001).

Streptozotocin administration has been widely used to induce Type I diabetes in large dose as well as Type II diabetes with multiple low doses in experimental animal models. The considerable destruction of β-cells after STZ injection is reported to be due to the inhibition of free radical scavenging enzymes, thus encouraging the production of various free radicals (Shankar et al., 2005; Gandhi et al., 2012). This destruction of β-cells accounts for the marked decrease in the amount of insulin produced by the β-cells of the pancreas, which in turn affects glucose metabolism. This leads to significant increase in blood glucose level in diabetic rats, which could be due to the destruction of pancreatic β-cells by streptozotocin, strengthening the hypothesis that STZ induces diabetes via the generation of free radicals. Because the liver performs most of the reactions involved in the synthesis and utilization of glucose, it is possible that the elevation of the glucose level in STZ-treated rats can be attributed by the oxidative stress, produced in the pancreas due to single-strand breaks in DNA of the pancreatic islets (Newsholme et al., 2012).

Although the mechanism of the β-cell cytotoxic action of STZ is not fully understood, experimental evidence has demonstrated that some of its deleterious effects are attributable to induction of metabolic processes, which lead to an increase in the generation of Reactive Oxygen Species (ROS) (Chen et al., 1990). Streptozotocin is not only damaging the pancreatic β-cells, but it is also harmful to hepatocytes, nephrons and cardiomyocytes (Frode and Medeiros, 2008).

2.22. Antioxidants and their free radical scavenging activity

Antioxidants are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals or their actions (Vadlapudi and Naidu, 2010). Antioxidants, also known as free radical scavengers by offering easy electron targets to the free radicals. In absorbing a free radical, antioxidants stabilize the lone free radical electron and make it stable enough to be transported to an enzyme, which combines two stabilized free radicals together to neutralize. Hence, compounds that inhibit or scavenge the ROS / RNS are of great interest as possible protective agent to help human body from the oxidative damage (Kuriakose and Kurup, 2010).

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Antioxidants have been detected in a number of agricultural and food products including cereals, fruits, vegetables and oil seeds. Antioxidants are increasingly being recommended because they act directly on oxidative processes and may be useful in preventing diseases and health problems related to ageing (Aguire and Borneo, 2010).

Nowadays a growing interest in searching natural antioxidants is for three main reasons:

i. Numerous clinical and epidemiological studies have demonstrated that consumption of fruits and vegetables rich in antioxidants is associated with reduced risk of developing chronic diseases such as cancer, cardiovascular disorders and diabetes.

ii. Safety consideration regarding the potential harmful effects of the chronic consumption of synthetic antioxidants such as butyl hydroxy anisole and butyl hydroxyl toluene in foods and beverages.

iii. The public’s perception that natural and dietary antioxidants are safer than synthetic analogues. Therefore, the food industry is making a great effort to find out new sources of safe and inexpensive antioxidants of plant origin (Rechc Cho et al., 2011).
Antioxidant means “against oxidation.” An antioxidant is any substance that retards or prevents deterioration, damage or destruction by oxidation (Dekkers et al., 1996). At a time one antioxidant molecule can react with single free radicals and are capable to neutralize free radicals by donating one of their own electrons ending the carbon stealing reaction. Antioxidants prevent cell and tissue damage against excessive free radicals by their repair mechanisms.

Fig. V. Neutralization of Free Radicals

A variety of components acts against free radicals to neutralize them from both endogenous and exogenous origin. They include

- Endogenous enzymatic antioxidants
- Non-enzymatic, metabolic and nutrient antioxidants
- Metal binding proteins like ferritin, lactoferrin, albumin and ceruloplasmin.
- Phytoconstituents and phytonutrients (Jacob, 1995).

Table III. Various ROS and corresponding neutralizing antioxidants

<table>
<thead>
<tr>
<th>S. No</th>
<th>ROS</th>
<th>Neutralizing antioxidants</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hydroxyl radical</td>
<td>Vitamin C, Glutathione, Flavonoids, Lipoic acid</td>
</tr>
<tr>
<td>2</td>
<td>Superoxide radical</td>
<td>Vitamin C, Glutathione, Flavonoids, SOD</td>
</tr>
<tr>
<td>3</td>
<td>Hydrogen peroxide</td>
<td>Vitamin C, Glutathione, β-Carotene, Vitamin E, CoQ10, Flavonoids, Lipoid acid</td>
</tr>
<tr>
<td>4</td>
<td>Lipid peroxides</td>
<td>β-carotene, Vitamin E, Ubiquinone, Flavonoids, Glutathione peroxidase</td>
</tr>
</tbody>
</table>
2.23. Natural antioxidants

Plants are the potential source of natural antioxidants. Natural antioxidants or phytochemical antioxidants are the secondary metabolites of plants. Flavonoids, tocopherols, folic acid, carotenoids, ascorbic acid, cinnamic acid, benzoic acid, tocotrienols etc., are some of the antioxidants produced by the plant for their sustenance. β-carotene, ascorbic acid and alpha tocopherol are the widely used antioxidants (Mc Call and Frei, 1999). Plant antioxidants have been reported to prevent the occurrence of diabetes, cancer and ageing. It can interfere with the oxidation process by reacting with free radicals, catalytic metals and also by acting as oxygen scavengers (Buyukokuroglu et al., 2001).

Many synthetic antioxidants such as Butylated Hydroxyl Anisole (BHA), Butylated Hydroxy Toluene (BHT) and Propyl Gallate (PG) have been used to retard the oxidation process. However the use of synthetic antioxidants must be under strict regulation due to their potential health hazards (Park et al., 2001). The main disadvantage of the synthetic antioxidants include their side effects like carcinogenicity when taken in vivo (Chen et al., 1992).

Hence the search for natural antioxidants is an alternative source which is of great interest among researchers. Plants are endowed with free radical scavenging molecules such as vitamins, terpenoids, phenolics, lignins, tannins, flavonoids, quinones, coumarins, alkaloids, amines and other metabolites, which are rich in antioxidant activity (Zheng and Wang, 2001; Cai et al., 2003). Studies have shown that many of these antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, anti-tumour, anti-mutagenic, anti-carcinogenic, anti-diabetic, anti-bacterial and anti-viral activities (Rice–Evans et al., 1995; Sala et al., 2002).

Polar flavonoids (Anthocyanidins and flavonols) are generally extracted with alcohol or alcohol-water mixtures in the presence of small amount of (0.1-1%) hydrochloric acid, where as tannins may be extracted with alcohol and acetone. Less polar flavonoids (Isoflavones, flavones, methylated flavones and flavonols) are generally extracted by solvents (Chloroform, Dichloromethane, Diethyl ether or Ethylacetate). All phenolic compounds (flavonoids and non-flavonoid phenolics) react with ferric chloride to give a characteristic colour (Harbone, 1998).
Flavonoids, the most common group of polyphenolic compounds that are found ubiquitously in plants. These are widely distributed in plants fulfilling many functions. Flavonoids and other plant phenolics are common in leaves, flowering tissues and woody parts such as stems and barks (Kahkonen et al., 1999).

In recent years, there has been a worldwide trend towards the use of the natural photochemicals present in herbs, berry crops, tea, oilseeds, beans, fruits and vegetables (Kitts et al., 2000; Wang and Jiao 2000; Muselik et al., 2007).

Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing free radical induced tissue damages. Besides, well known and traditionally used natural antioxidants from tea, wine fruits, vegetables, spices and many other plant species have been investigated in the search for novel antioxidants (Koleva et al., 2002). Twenty two Nigerian medicinal plants were extracted and screened for antioxidant activity using the 2, 2-diphenyl picryl hydrazyl radical (Oke and Hamburger, 2002). There is still demand to find information concerning the antioxidant potential of more plant species. Current research is now directed towards finding naturally occurring antioxidants of plant origin. Plant and its products are being used as a source of medicine since long time. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities with economic viability and no side effects (Auudy et al., 2003).

Medicinal plants are the important source of antioxidants. Flavones, flavonols and proanthocyanidins are well known compounds associated with antioxidant activity in plants (Skerget et al., 2005). Phenolics can be classified into two groups namely polyphenolics and simple phenols which contain phenolic acids. Most of the antioxidant properties in plant are due to polyphenol, phenolic acid, flavonoid and vitamin C (Marinova et al., 2005). Naturally occurring antioxidants differ in their composition, physical and chemical properties, mechanism and site of action. Natural products such as herbs, fruits and vegetables become popular in recent years due to public conferences and increasing interest among consumers and scientific community (Thaipong et al., 2006).

Flavonoids have pigmentsary functions. They are responsible for the colour of flowers, fruits and sometimes leaves. They play an important role in pollination and
dispersion by attracting animals by their colours. Other functions of flavonoids include antioxidant and antimitogenic activities, role on plant growth regulation and on resistance to plant diseases. Flavonoids protect the plants from UV-damaging effects too (Gurib-Fakim, 2006).

Flavonoids have gained recent attention because of their broad biological and pharmacological activities. Flavonoids have been reported to exert multiple biological properties including anti-microbial, cytotoxicity, anti-inflammatory as well as anti-tumour activities but the best described property of flavonoids is their capacity to act as powerful antioxidant which can protect the human body from free radicals and reactive oxygen species. The capacity of flavonoids to act as an antioxidant depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. On the other hand, flavonoids such as luteolin and catechins are better antioxidants than the nutrient antioxidants such as vitamin C, vitamin E and β-carotene (Tapas et al., 2008).

The consumption of a diet rich in fresh fruits and vegetables have been associated with a number of health benefits including the prevention of chronic diseases. This beneficial effect is believed to be due to the action of antioxidant compounds which reduce oxidative damage in the body (Lana and Tijiskers, 2006). The ingestion of natural antioxidants has been associated with reduced risk of cancer, cardiovascular disease, diabetes and other diseases associated with ageing (Ashok Kumar et al., 2008; Veerapur et al., 2009).

The following plants were reported to have antioxidant properties viz., *Andrographis paniculata* (Kapil et al., 1993); *Curcuma domestica* (Selvam et al., 1995); *Trigonella foenum-graeum* (Hettiarachchy et al., 1996); *Camellia sinensis* (Lee et al., 1997); *Centenella asiatica* (Shukla et al., 1999); *Mangifera indica* (Martinez et al., 2000); *Garcinia cowa* (Mahabusarakam et al., 2005); *Vitex negundo* (Tandon and Gupta, 2005); *Catharanthus roseus* (Jaleel et al., 2006); *Oxalis corniculata* (Karthiriya et al., 2010); *Bauhinia pupurea* (Shajiselvin and Kottaimuthu, 2011); *Withania somnifera* (Sharma et al., 2011); *Hypericum perforatum* (Franchi et al., 2011); *Alternathera sessilis* (Borah et al., 2011); *Minusops elengi* (Karmakar et al., 2011) and *Tylophora asthmaica* (Malathi et al., 2012).
Cook and Samman (1996) reported that phenolic compounds are very important plant constituents because their hydroxyl groups confer scavenging ability. Triterpenoids from *Boswellia regia* have been shown to have anti-elastase activity (Melzig *et al*., 2001). Sang *et al.* (2001) stated that lipid peroxide level is decreased in *Morinda citrifolia* leaf extract treated animals, because of the presence of β-carotene, flavol glycosides and iridoid glycosides, which have the antioxidant activity. Phenolic acids are hydroxylated derivatives of benzoic acid and cinnamic acid. The most common caffeic and ferulic acids, which frequently occur in food as simple esters with quinic acid or glucose (Mattila and Kumpulainen, 2002). Kumaraguruparan *et al.* (2002) reported that elevated level of serum lipid peroxide in breast carcinoma may be due to defective antioxidant system. The balance between the formation and removal of lipid peroxides determine the peroxide level in cells.

Joharapurkar *et al.* (2003) reported that ethanol extract of *Rubia cardifolia* has antioxidant activity. Flavonoids show antioxidant activity and their effects on human nutrition and health are considerable.

The mechanism of action of flavonoids are through scavenging or chelating process. *Gallium aparine*, a Rubiaceae member contains tannins, phenolic acids, flavonoids and iridoid glycosides in leaves and stems (Vanwyk and Wink, 2004). Flavonoids, phenols, tannins and terpenoids in the plants exhibit antioxidant activity (Rice Evans, 2004; Aderogba *et al*., 2005).

Phenolic constituents found in vegetables have antioxidant activity and play an important role in the adsorption or neutralization of free radicals (Basile *et al*., 2005). Polyphenols isolated from *Diospyros* sps. leaf showed anti-collagenolytic and anti-elastase activity. This activity was thought to be due to the flavonoids present in the plant extract (An *et al*., 2005). Plant polyphenols, a large group of natural antioxidants present in vegetables and fruits have protective effect against cancer and cardiovascular diseases. Epidemiologic studies are useful for evaluation of human health effects of long term exposure to physiologic concentrations of polyphenols, but reliable data on polyphenol contents of foods are still scarce. The epidemiologic data on the health effects of polyphenols, focusing on the flavonoid subclasses of flavonols, flavones and catechins and on lignans, showed that both flavonoids and lignans have beneficial effects on
cardiovascular diseases but not on cancer with the possible exception of lung cancer. Other functions of polyphenolics are regulating nitric oxide, decreasing leukocyte immobilization, inducing apoptosis, inhibiting cell proliferation and angiogenesis and exhibit phytoestrogenic activity (Arts and Hollman, 2005). Flavonoids constitute a group of natural compounds that occur in fruits, vegetables, wine, tea, chocolate and other cocoa products (Sies et al., 2005).

Marinova et al. (2005) determined the total phenolics and flavonoids content in 42 food products, 20 fruits and 22 vegetable species. The results of fruits showed the highest phenolic content in blue berries (670.9 mg gallic acid equivalents (GAE / 100 g), dogwood berries (432.0 mg GAE/ 100 g) and sour cherry (429.5 mg GAE /100 g). The greatest total flavonoid content was revealed in blue berries (190.3 mg catechin equivalents (CE) /100 g). The lowest total phenolics and total flavonoids were established in peaches (50.9 mg GAE/100 g and 15.0 mg CE /100 g, respectively). The result of vegetables showed the greatest values of phenolics in green peppers (246.7 mg GAE / 100 g) and red peppers (173.2 mg GAE / 100 g). Significant difference was found between total phenolic content in red and spring onions at almost equal total flavonoid values.

According to Li et al. (2006), polyphenols is one of the most numerous groups of substances in plant kingdom ranging from simple molecules, such as phenolic acids to complex compounds such as tannins. In addition, polyphenols function in trapping and scavenging free radicals due to their antioxidant properties.

According to Hernandez et al. (2006), Vitamin C is the most important vitamin for human nutrition. L-Ascorbic acid is the main biologically active form of vitamin C which reversibly oxidized to form L-dehydroascorbic acid (DHA).

Thangaraj et al. (2007) investigated the antioxidant property of Emblica officinalis during restrain—stress in albino rats. For this the experimental rats were grouped as control, restrain-stress (4 h / day for 15 days) and E. officinalis + restrain stress. The oxidative stress was assessed by measuring the lipid peroxidation (LPO) and enzymatic antioxidant status. Superoxide dismutase (SOD), catalase (CAT) and Glutathione peroxidase (GPx) in the lymphoid organs of thymus, spleen and plasma corticosterone level. Following restrain-stress, enzymatic antioxidant status was
significantly reduced with concomitant increase in LPO and corticosterone levels. Administration of *E. officinalis* (500 mg kg\(^{-1}\) body weight for 30 days) significantly prevents the restrain – stress induced oxidative stress and elevation in LPO and corticosterone levels which may be due to its strong antioxidant property.

There are claims that phenolic compounds and their derivatives are strongly correlated with antioxidant activities (Maisuthisakul *et al.*, 2007) and plants with high antioxidant activities also have high total phenolics and flavonoid contents. Vaijanathappa *et al.* (2008) reported the *in vitro* antioxidant activity using nine different methods in *Enicostemma axillare*. All the four extracts of *E. axillare* showed potent antioxidant activity with IC\(_{50}\) values ranging from 13.26 to 24.36 \(\mu\)g / ml. The chloroform extract has shown potent antioxidant activity in H\(_2\)O\(_2\), nitric oxide and hydroxyl radical using the deoxyribose and lipid peroxidation methods, with IC\(_{50}\) values of 16.99 \(\pm\)0.38, 60.66 \(\pm\)0.30, 25.06 \(\pm\)0.12 and 94.66 \(\pm\)2.40 \(\mu\)g/ml, respectively. Potent activity was also observed for the petroleum ether extract with the deoxyribose, p-nitroso dimethyl aniline (p-DNA), and H\(_2\)O\(_2\) methods and for the ethyl acetate extract with the H\(_2\)O\(_2\) and nitric oxide method.

Concentrations of the plant extracts required for 50% inhibition of DPPH radical scavenging effect (IC\(_{50}\)) were recorded as 0.04 mg ml\(^{-1}\), 0.313 mg ml\(^{-1}\), 0.58 mg ml\(^{-1}\), 2.30 mg ml\(^{-1}\) and 0.054 mg ml\(^{-1}\) for *Psidium guajava*, *Mangifera indica*, *Carica papaya*, *Vernonia amygdalina* and vitamin C, respectively (Ayoola *et al.*, 2008). Kumar *et al.* (2008) reported the antioxidant activity of selected medicinal plants namely *Albizia amara*, *Achyranthes aspera*, *Cassia fistula*, *Cassia auriculata* and *Datura stramonium* determined by inhibition of lipid peroxidation technique revealed that the highest inhibition of lipid peroxidation activity was observed in *A. amara* (96%) followed by *C. fistula* (89%) and *C. auriculata* (89%). The potency of protective effect of *A. amara* was about 4 times greater than the synthetic antioxidant butylated hydroxy toluene (BHT). The total alkaloid content varied from 24.6 \(\pm\) 0.18 to 72.6 \(\pm\) 2 mg g\(^{-1}\) in the extracts. The Flavonoid contents were between 23.15 \(\pm\) 0.2 and 63.3 \(\pm\) 0.6 mg g\(^{-1}\) in the methanolic extracts of these plants which indicated that the antioxidant activity of *A. amara* could be harnessed as a drug formulation.
Ethanol and water extracts of *Ficus racemosa* was subjected to free radical scavenging by both steady state and time resolved methods such as nano second pulse radiolysis and stopped- flow spectrophotometric analyses. Ethanolic extract exhibited significantly higher steady state antioxidant activity than the water extract of *F. racemosa*. Ethanolic extract of *F. racemosa* exhibited concentration dependent DPPH, ABTS⁺, hydroxyl radical and superoxide radical scavenging and inhibition of lipid peroxidation with IC₅₀ comparable with tested standard compounds (Veerapur *et al.*, 2009).

The radical scavenging activity of methanolic extract of four Indian medicinal plants viz. *Plumbago zeylanica* (Root), *Acorus calamus* (Rhizome), *Hemidesmus indicus* (Stem) and *Holarrhena antidysenterica* (Bark) was measured as decolorizing activity followed by the trapping of the unpaired electron of DPPH. The percentage decrease of 1, 1-diphenyl -2-picryl hydrazyl radical (DPPH) standard solution was recorded maximum for *H. indicus* (77.0%) followed by *P. zeylanica* (73.41%), *A. calamus* (20.88%) and *H. antidysenterica* (20.06%) extracts at a concentration of 100 µg ml⁻¹. Moreover total phenolics concentration equivalent to gallic acid was found in the range of 59.50 to 109.0 mg g⁻¹ of plant extracts, which correlated with antioxidant activity (Zahin *et al.*, 2009).

The antioxidant activity of methanolic extract of stem bark of *Gmelina arbora* was studied by Patil *et al.* (2009) and found that it possesses significant free radical scavenging properties and a clear correlation was observed between the antioxidant activity and phenolic content.

Antioxidant activity was evaluated by DPPH method and the leaves of *V. negundo* showed 23.21 mg /100 g of Ascorbic acid Equivalent antioxidant Capacity (AEAC) (Praveenkumar *et al.*, 2010).

In a comparative study undertaken by Patel *et al.* (2010) showed that major amount of phenols was found in *Gemelia* leaf followed by stem of *Kigelia* and *Hibiscus*. Whereas maximum flavonoid content was found to be present in the leaf of *Hibiscus* followed by *Gemelia* and *Parthenium* and high radical scavenging activity was observed with the stem extract of *Kigelia* followed by leaf of *Hibiscus, Gemelia* and *Kigelia*. These observations clearly indicated a cross linkage between phenolics and antioxidant activity.
Ethanolic extract of *Cassia auriculata* leaves found to possesses IC$_{50}$ value for DPPH, nitric oxide, superoxide and hydroxyl radical scavenging activity of 49.45, 125.31, 247.52 and 142.04, respectively (Senthilkumar and Vijayakumari, 2012c).

Alcoholic extract of stem bark of *Tamarindus indica* and *Cassia fistula* showed significant antioxidant activity in DPPH, nitric oxide and hydroxyl radical induced *in vitro* assay methods (Agnihotri and Singh, 2013). The antioxidant activity of methanolic extract of dried leaves of four medicinally important herbs namely *Ocimum sanctum*, *Mentha spicata*, *Trigonella foenum-graecum* and *Spinacia oleracea* studied by Soni and Sosa (2013). The study stated that the IC$_{50}$ value obtained by DPPH activity for *Mentha spicata* crude extract was found to be 170 µg ml$^{-1}$ and reducing power was found to be maximum (1.92) at 1 mg ml$^{-1}$ concentration. The results suggested that *Mentha spicata* had promising antioxidant activity and could serve as a potential source of natural antioxidants.

### 2.24. Endogenous antioxidants

The body produces different antioxidants (endogenous antioxidants) to neutralize free radicals and protect the body from different diseases. The endogenous antioxidant defence system can be classified into two groups namely enzymatic and non-enzymatic antioxidants. The enzymatic defence system includes different endogenous enzymes like Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxides (GP$_X$), Glutathione Reductase (GR) and non-enzymatic defence system which includes Vitamin E, Vitamin C and Reduced Glutathione (GSH) (Harris, 1992).

SOD is an important endogenous antioxidant enzyme acts as the first line defence system against ROS, which scavenges superoxide radicals to H$_2$O$_2$. GPX present in the cytoplasm of the cells removes H$_2$O$_2$ by coupling its reduction to H$_2$O with oxidation of GSH. GR is a flavoprotein enzyme, which regenerates GSH from oxidized glutathione in the presence of NADH. GSH is a tripeptide and a powerful antioxidant present within the cytosol of cells and is the major intracellular non-protein thiol compound (NPSH). SH groups present in GSH react with H$_2$O$_2$ and the OH$^*$ radical and prevent tissue damage and GSH is also capable of scavenging ROS directly or enzymatically via GP$_X$. Vitamins C and Vitamin E are non-enzymatic endogenous antioxidants, exist within...
normal cells and react with free radicals to form radicals themselves. They break radical chain reaction by trapping peroxyl and other reactive radicals (Siems et al., 1995; Ali et al., 1996; Willcox et al., 2004).

Vitamin E and vitamin C are the non-enzymatic antioxidants exist within normal cells as well as they can be supplied through diet (Tiwari, 2001). Non-enzymatic antioxidants can also be divided into two types namely metabolic antioxidants and nutrient antioxidants. Metabolic antioxidants are the endogenous antioxidant produced by metabolism in the body like lipoic acid, glutathione, L-arginine, coenzyme Q_{10}, melatonin, uric acid, bilirubin, metal-chelating proteins, transferrin etc., (Droge, 2002). Antioxidants may exert their activity by several mechanisms like by suppressing the production of active species by reducing hydro peroxides and H_2O_2 by sequestering metal ions, termination of chain reaction by scavenging and / or clearing cell damage. Biosynthesis of other antioxidants or defence enzymes are also induced by some antioxidants (Tiwari, 2004). The nutrient antioxidants belonging to exogenous antioxidants cannot be produced in the body but provided through diet supplements viz., trace metals (selenium, manganese, zinc), flavonoids, omega-3 and omega-6 fatty acids etc. (Pham-Huy et al., 2008).

2.25. Structure and Importance of Liver, kidney and Pancreas

2.25 A: Liver

Liver is roughly a triangular organ consists of 4 distinct lobes namely the right, left, caudate and quadrate lobes. Each lobe is made up of thousands of smaller units called lobules which are functional units of the liver. Every lobule is accompanied by a central vein surrounded by six portal arteries and veins and are found in bundles. Cavities namely sinusoids separate these bundles of cells which provide a spongy texture to the liver and thereby enabling the liver to absorb large quantities of blood along with essential nutrients.
The cytology of the liver reveals the presence of two distinguished cells viz kupffer cells and hepatocytes. The kupffer cells act as macrophage which catch and destroy the old red blood corpuscles, bacteria, fungi, etc. The hepatocytes play the major functions of the liver like metabolism, digestion and absorption activities of the liver.

Liver has a dual circulatory system of getting oxygenated blood from the heart through the hepatic artery and blood containing digested food particles with essential nutrients through the portal vein in addition to supplying oxygenated blood from the duodenum. Liver also consists of bile capillaries which join together and form bile duct through which the bile secreted by liver being transported. As the liver continuously producing the bile whether the intestine doing digestion or not, the excess amount of bile is being stored in gall bladder. When the food containing fats comes to the duodenum, the cells of duodenum secrete an enzyme called cholecystokinin which stimulates the gall bladder to release the stored bile juice. The bile juice acts on clumps of large fat bodies and breaks into smaller fat bodies and thereby increasing the surface area of digestion or in other words the bile juice just emulsifying the fat bodies which facilitates the easy digestion of fat bodies. The kupffer cells engulf the old red blood corpuscles and worn out into the hematocytes which metabolize the RBC in to heme and globin protein.
This globin can be used as source of providing energy to the body, whereas the heme cannot be recycled by the body and it is converted into bilirubin which mixtures with bile and being excreted out.

When we see the functional part of the liver it has been observed that when the blood enriched with glucose enters the liver through portal vein the excess amount of glucose being stored by the hepatocytes in the form of glycogen through the process of glycogenesis under the influence of insulin hormone. When the blood comes with fatty acids to the liver that can be immediately used as the source of energy for the body. However glycerol, lipoproteins, phospholipids and other fatty components require metabolic activities. In such cases the liver influences the gluconeogenesis which is a process of producing glucose from non-carbohydrate substances. In protein metabolism, this organ converts the amino acids in to amines which in turn converted into urea and ammonia. Urea seems to be lesser toxic than ammonia and being eliminated through urine.

Liver is the second largest organ of the body next to skin having a rapid regeneration capacity for a while 25% of the liver part can produce 100% of the organ in a short span of time (Haussinger, 2011). This is predominantly due to the hepatocytes re-entering the cell cycle. That is the hepatocytes go from the quiescent G0 phase to the G1 phase and undergo mitosis. This process is activated by the p75 receptors (Suzuki et al., 2008).

It also serves as a storage organ for essential vitamins and minerals such as Vitamin A, D, E, K, B12 and Iron, copper respectively apart from playing an important role in the production of blood plasma components viz prothrombin, fibrinogen and albumin. The prothrombin and fibrinogen play a vital role in blood clotting process while the albumin helps to maintain the isotonic environment of the blood and thereby the body cannot lose or gain of water in the presence of body fluids.

2.25 B : Kidney

Kidney is a bean shaped organ having two surfaces namely convex and concave regions. The convex part of the kidney is called as the renal hilum where the renal artery enters and the renal vein as well as ureter leave. The histology of the kidney reveals the occurrence of two different regions viz the superficial zone which is termed as renal cortex and the inner deep medulla region.
The renal cortex and medulla together take up 8 to 18 cone shaped renal lobes and each lobule containing renal cortex surrounding a portion of medulla called a renal pyramid. The renal pyramid is also named as malpighian’s tubules. The projections of cortex in between the renal pyramids constitute the renal columns (of Bertin). Glomerulus is a bundled network of capillaries that increases the surface area of blood in contact the blood vessel walls.

The glomerulus is surrounded by the glomerular capsule which is a cup shaped structure formed by double layer of simple squamous epithelial cells with a hallow space in between the layers. Special epithelial cells namely podocytes form the layer of glomerular capsule surrounding the capillaries of glomerulus. The podocytes function with the endothelium of the capillaries to form a thin filter to separate urine from the blood passing through the glomerulus. The exterior layer the glomerular capsule holds the urine separated from the blood within the capsule.

**Fig. VII. Section of the Kidney**
The glomerular capsule ends with renal tubules. The curvy first section of the renal tubule is named as the proximal convoluted tubule which is followed by a long straight tube called Henle’s loop. The Henle’s loop consists of descending and ascending limbs of having different physiology. That is, the descending limb of loop of Henle is permeable to water while the ascending limb is not permeable to water. The Henle’s loop is followed by the distal convoluted tubule which ends in collecting ducts and finally in to the renal pelvis. The renal pelvis joins with ureter.

Nephrons are the functional units of kidneys which span the cortex and medulla portions of the kidney. The initial filtering portion of the nephron is termed as renal corpuscle which is located in cortex zone of the kidney. The renal corpuscle is followed by a renal tubule that passes from the cortex to deep medullary pyramids. The tip or the papilla of each pyramid empties urine into a minor calyx which ends with major calyces. The major calyces empty the urine in to the renal pelvis which becomes the ureter. The medullary interstitium is the functional space in the kidney beneath the individual filters namely glomeruli. The glomeruli are rich in blood vessels. The interstitium absorbs fluid recovered from urine. Congestion of this area leads to kidney dysfunction and failure.

Kidney plays an important role in whole body homeostasis, regulation of acid-base balance, electrolyte concentrations, extracellular fluid volume and regulation of blood pressure. The kidney accomplishes these homeostatic functions both independently and in concert with the endocrine system. Various endocrine hormones coordinate these endocrine functions are renin, angiotensin II, aldosterone, antidiuretic hormone and atrial natriuretic peptide.

The primary function of the kidney is to excrete the waste products resulting from protein metabolism and muscular contraction. The liver metabolizes the protein foodstuffs in to amino acids along with ammonia. This ammonia which is toxic to the body is converted in to urea and uric acid by the liver. When compared to urea and uric acid, ammonia is lesser toxic and 50% of the urea is reabsorbed by the liver. The urea found in the blood helps to maintain the isotonic balance between the urine and blood. For muscular activity creatine is used as a source of energy which results in the production of creatinine which is toxic to the body being to be eliminated.
Blood pressure forces the blood in to the capillaries of the glomerulus and the glomerular capsule which is also called as Bowman’s capsule. As the blood cells are larger in size which passes through the capillaries only the fine filtered liquid called tubular fluid gets in to the glomerular capsule. The remaining concentrated blood moves in to the efferent arterioles and on to the proximal convoluted tubule. The epithelial cells lining the tubule actively absorb the valuable molecules like glucose, amino acids and ions from the filtrate and deposit them back into the blood. From the proximal convoluted tubule the tubular fluid enters into the loop of Henle.

The tubular fluid comes to the descending limb of Henle’s loop is hypotonic while the tissues of medulla surrounding the capillaries are hypertonic. In addition to it, the wall of descending limb of Henle’s loop is permeable to water. Therefore the osmotic pressure between the hypotonic filtrate and hypertonic medullary cells pushes water out of the filtrate and into the cells and the cells of the medulla return this water to the blood through nearby capillaries.

As the filtrate which is highly concentrated comes to the ascending limb of Henle’s loop because of the impermeable nature of cells lining the descending limb the ions are easily diffused out of the filtrate which are returned to the blood stream through nearby capillaries. From the descending limb the tubular fluid goes to the distal convoluted tubule and the collecting duct of the nephron. These tubules continue to reabsorb small amounts of water and ions which are still left in the filtrate. The tissues surrounding the collecting duct actively absorb excess potassium and hydrogen ions from the nearby capillaries and secrete these ions as waste into the filtrate. When the filtrate reaches the end of the collecting duct, almost all of the valuable nutrients, ions and water have been returned to the blood supply and only a small amount of water with waste products are left to form urine. Finally urine from other collecting ducts jointly reach the renal pelvis from where to ureters.

When we see the water homeostasis maintained by the kidney, two hormones namely antidiuretic hormone (ADH) and aldosterone play a vital role in re absorption of 100% water. When there is too much of water in the blood, the heart secretes a hormone namely atrial natriuretic peptide (ANP) in order to increase the excretion of Na⁺ and Cl⁻
ions. The increased concentration of Na\(^+\) and Cl\(^-\) in urine draws water into the urine via osmosis and thereby increasing the volume of urine produced.

While seeing the acid/base homeostasis the kidney regulates the pH level of the blood by duly controlling the excretion of hydrogen ions and bicarbonate ions. Hydrogen ions accumulate in the body when the liver metabolizes the protein while the bicarbonate ions produced from the carbonic acid which is formed when carbon dioxide reacts with water in the blood. Both ions are filtered out of the blood in the glomerulus of the kidney. But the tubule cells lining the nephron selectively reabsorb bicarbonate ions and leaving hydrogen ions.

When focussing the electrolyte homeostasis by the kidney it is very clear that the kidneys play a unique role on the essential electrolytes such as Sodium, Potassium, Chloride, Calcium and Magnesium. The kidneys also help to control the blood pressure in the body by regulating the excretion of sodium ions and water by producing the enzyme renin.

The kidneys maintain a small but important endocrine function by producing the hormones namely calcitriol and erythropoietin. Out of which the calcitriol is the active form of vitamin D in the body. The tubule cells of proximal convoluted tubule produce calcitriol from inactive vitamin D molecules and this calcitriol travels from the kidney to the intestine where it increases the absorption of calcium from food in the intestinal lumen. Erythropoietin is a hormone which produced by the cells of peritubular capillaries in respond to hypoxia (a low level of oxygen in the blood). The erythropoietin stimulates the cells of red bone marrow to increase their output of red blood cells.

2.25 C : Pancreas

Pancreas is a dual functioning organ of having exocrine as well as endocrine activities. Being an exocrine gland it produces carbohydrate enzyme amylase, protease enzymes like trypsinogen, chymotrypsinogen, carboxypeptidase and lipid enzyme lipase. Functioning as an endocrine gland it produces hormones like glucagon, insulin, somatostatin and polypeptides.
Pancreas can be divided into three parts namely head which rests at second and first part of the duodenum, body which lies underneath to the stomach and the tail ends in spleen. The cytology of the pancreas represents the presence of two distinguished cells viz. Islets of Langherhans which constitute the endocrine part of the pancreas and acinar cells which comprise the exocrine segment of the organ.

The anatomy of the Islets of Langherhans illustrates the presence of four well distinguished cells namely alpha cells which produce glucagon hormone, Beta cells which produce the hormone insulin, Gamma cells which secrete polypeptides and the Delta cells which secrete somatostatin.

**Fig. VIII. Cytology of Pancreas and its Component Cells**
While seeing the embryonic development of pancreas, the exocrine part of pancreas involves the differentiation of progenitor cells through three different stages viz pre-differentiated, protodifferentiated and differentiated stages with the influence of important molecules like follistatin, fibroblast growth factors apart from the activation of Notch receptor system. Whereas the endocrine part of the pancreas arise from the cells of protodifferentiated stage under the influence of neurogenin – 3 and Isl – 1 but in the absence of notch receptor signalling and these cells differentiate to form two lines of committed endocrine precursor cells. The first line, under the control of Pax – 0 constitutes Alpha and Gamma cells which produce glucagon and pancreatic polypeptides respectively. While the second line under the direction of Pax -6, generates Beta and Delta cells which secrete insulin and somatostatin, respectively.

Pancreas is governed under the control of Autonomic Nervous System (ANS) as well as the endocrine system. The ANS comprises of sympathetic and parasympathetic nervous systems. The sympathetic nervous system is found in active during exciting, emergency and extreme stress situations that stimulate the alpha cells of the pancreas which in turn produces the hormone glucagon. The secreted glucagon influences the breakdown of glycogen into glucose (Glycogenolysis) in liver, muscles and also converts the triglycerides found in adipose tissue into glucose apart inhibiting the secretion (Insulin) of beta cells. The parasympathetic nervous system is active in normal condition which stimulates the beta cells to secrete the hormone insulin. Insulin facilitates the absorption as well as the storage of excess glucose in the form of glycogen in the liver through the process of glycogenesis.

Pancreatic juice consists of water, salts, bicarbonates and digestive enzymes. Due to the presence of bicarbonates the pancreatic juice is in alkaline nature which helps to neutralize the acidic food chyme comes from the stomach. The oxytentic cells of stomach produce hydrochloric acid which acts upon the foodstuffs and makes it acidic nature. The cells of intestine secrete the hormone secretin which stimulates the pancreas to produce pancreatic juice and it assists to neutralize acidic food chyme emerging from the stomach.
Among the various pancreatic digestive enzymes, enzymes like trypsinogen, chymotrypsin, carboxypeptidase are relatively major components and pancreatic lipase, amylase secreted to a lesser degree. It also secretes phospholipase A2, lysophospholipase and cholesterol esterase. The pancreatic amylase acts upon the polysaccharides and breaks down in to maltose and glucose which could be absorbed by the intestine. Maltase secreted by the intestine converts the maltose in to glucose. Trypsin, chymotrypsin and carboxypeptidase act upon the protein and break in to amino acids. Pancreatic lipase acts on large triglyceride molecules and breaks in to fatty acids and monoglycerides. With the help of bile juice which influences the emulsification of fat bodies from the gall bladder the lipase enzyme converts the fat food stuffs into fatty acids.

There are two types of diabetes mellitus namely Type I (also known as Juvenile Diabetes), a chronic autoimmune disorder in which the immune system attacks the insulin secreting cells in the pancreas and the Type II is commonly found in overweight adults along with uncommon occurrence in children.
2.26. Histopathological Investigation

In modern medicine no satisfactory effective therapy is still available to cure diabetes, which is a syndrome resulting from a variable interaction of hereditary and environmental factors and characterized by abnormal insulin secretion or insulin receptor or post-receptor events affecting metabolism involving carbohydrates, proteins and fats in addition to damaging beta cells of pancreas, liver and kidney in some cases (Ghosh and Surawanshi, 2001; Thakran et al., 2004; Bolkent et al., 2004; Huang et al., 2005; Gholamani et al., 2005).

Patients depend on insulin for management of IDDM. They develop degenerative complications such as microangiopathy, nephropathy and retinopathy without insulin. Diabetic nephropathy is the most important cause of death in Type I diabetic patients of whom, 30 - 40 % eventually develop end stage renal failure (Giorgino et al., 2004).

Liver disease is one of the leading cause of death in person with Type II diabetes. The standardized mortality rate for death from liver disease is greater than that of cardiovascular disease. The spectrum of liver disease in Type II diabetes ranges from non alcoholic fatty liver disease to cirrhosis and hepatocellular carcinoma (Keith et al., 2004).

Das et al. (1996) reported Cassia auriculata supplementation normalized the histopathological alterations in the pancreas, liver and the kidney tissues of diabetic
rats indicating the potential hypoglycemic nature of the plant extract. The methanolic leaf extract of *Cassia auriculata* was reported to be effective in alcoholic liver injury, used for hypoglycemic and antioxidant activity (Senthilkumar et al., 2003; Sabu and Kuttan, 2004; Upadhya et al., 2004) and the fruit extract improved functional state of beta cells and partially reversed the cell damage (Kamalakannan and Prince, 2005). Ragavan and Krishnakumari (2006) reported the regenerative effect of pancreatic islet cells by *Terminalia arjuna* may enlighten the positive effects of the plant extract on production of insulin. Nandakumar et al. (2007) observed increase in plasma insulin level in diabetic rats may be due to long lasting effect of beta cells of pancreatic islets or pancreatic beta cell regeneration. The effect of combined extracts of *Cassia auriculata* and *Aegle marmelos* revealed the restoration of beta cells in diabetic rats (Sivaraj et al., 2009). Antidiabetic activity of *Cassia sophera* in streptozotocin – induced diabetic rats and its effect on insulin secretion in isolated pancreatic islets studied by Sharma et al. (2013) indicated that AECS induced insulin secretion was independent of K+ATP channels of beta cells of pancreas.