CHAPTER - III
REVIEWS OF LITERATURE

DIABETICS MELLITUS

Diabetics mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia associated with abnormalities in carbohydrate, fat and protein metabolism, and resulting in chronic complication including microvascular, macrovascular and neuropathic (Julie et al., 2002). Another important factor is the currently ongoing change in lifestyle towards a more western-oriented way of living. The expected numbers in total individuals worldwide for diabetes mellitus are estimated to be 221 millions for year 2010 compared to about 124 millions in year 1997. The dominant part of diabetic persons was in 1997 represented by Type 2 diabetes (97%). WHO expects the numbers of adults (20 years and older) with diabetes (i.e. a mix of Type 1 and 2 diabetes) to rise to 300 millions by year 2025 from 135 millions in year 1995. This means that the increase is about 120% over 30 years. Moreover, 80% of those individuals will be found in developing countries by year 2025. The top three counties in the world with diabetes are in the following order: India, China and the USA (Bjork et al., 2001). DM is the leading cause of blindness in adult’s ages 20 to 74 years, and the leading contributor to development of end-stage renal disease (ESRD). Type 1 DM usually develops in childhood or early adulthood, although some latent forms do occur. Type 1 DM accounts for up to 10% of all cases of DM and results from an autoimmune destruction of the pancreatic β-cell. The process is likely initiated by the exposure of a genetically susceptible individual to an environmental agent. Candidate genes and environmental factor are reportedly prevent in the general population but development of β-cell autoimmunity occurs in less than 10% of the population and progresses to diabetes mellitus in less than 1% of the population. Obesity
may also be a confounder as overlapping insulin resistance with β-cell dysfunction may results in the clinical manifestation of DM. The marker of autoimmunity has been detected in 14% to 33% of persons with type 2 DM in some populations and manifest with early failure of oral agents and insulin dependence. This type of DM has also been referred as to latent autoimmune diabetics in adults or autoimmune DM in adults (AIDA). Type 2 DM account for as much as 90% of all case of DM, and usually results from defects insulin sensitivity and a relative defect in insulin secretion. The overall prevalence of type 2 DM in the United State is about 6.6% in person ages 20 to 74 years. However, there is likely one person undiagnosed with the disease. One subset of type 2 DM maturity-onset diabetics of youth (MODY), has an identification genetic defect in the glucokinase gene. Multiple risk factor for the developments of type 2 DM have been identified, including family history (i.e. parent or sibling with diabetes) obesity (i.e., ≥20% over ideal body weight, or body mass index (BMI) ≥7kg/m² habitual physical inactivity race or ethnicity, impaired glucose tolerance or impaired fasting glucose hypertension (≥140/90 mm Hg in adults) HDL cholesterol ≤5mg/dl and/or a triglyceride levels ≥250mg/dl. The prevalence of type 2 DM increases with ages is more common in women than in men in the United States, while the prevalence of type 2 DM increase with ages, the disorder is increasingly being recognized in adolescence. Much of the rise in adolescent type 2 DM is related to an increased in adiposity and sedentary lifestyle, in addition to an inheritable predisposition. Gestational diabetes mellitus (GDM) complicate roughly 4% of all pregnancies in the United States (Julie et al., 2002; Bjork et al., 2000).
PATHOGENESIS, DIAGNOSIS, AND CLASSIFICATION

The American Diabetes Association Expert Committee on the Diagnosis and Classification of Diabetes Mellitus recommends the uses of the terminology type 1 (Formerly insulin-dependent diabetes mellitus IDDM, or juvenile onset) and type 2 (Formerly non insulin-dependent diabetes mellitus, NIDDM or adult onset).

Classification of Diabetes Mellitus

Type 1

- Immune mediated
- Idiopathic

Type 2

- May range from predominately insulin resistance to predominately Insulin deficient

Other specific

- Genetic defect of β-cell function
- Genetic defect in insulin action
- Disease of the endocrine pancreas
- Endocrinopathies
- Drugs or chemical induced
- Infections
- Uncommon forms of immune-mediated diabetes
- Other genetic syndromes sometime associated with diabetes

In 1997, the Committee also redefined the diagnostic criteria for DM. In general, the criteria required that any one test consistent with diagnosis of DM be confirmed with a second test, most often fasting plasma glucose. Oral glucose tolerance tests (OGTT) are not routinely recommended but
may be considered in an individual with history of abnormal glucose or impaired fasting glucose in whom you highly suspect the presence of DM (Julie et al., 2002).

Symptom of diabetes plus a random plasma glucose $\geq 2000$mg/dl
Or
Fasting plasma glucose $\geq 126$mg/dl
Or
Two-hour plasma glucose $\geq 200$mg dl during an oral glucose tolerance test (OGTT)

PATHOGENESIS

TYPE 1 DIABETES MELLITUS

Type DM is characterized by an absolute deficiency of insulin. Most often this is the results of antoimmune-mediated destruction of pancreatic $\beta$-cells, but is rare unknown or idiopathic processes may contribute. What are evident are four main features

- a long preclinical may period marked by the presence of immune marks when $\beta$-cell destruction is thought to occur
- hyperglycemia when 80% to 90 of $\beta$-cell are destroyed
- established disease with associated risks for complications and deaths.

The autoimmune process is mediated by macrophages and T lymphocytes with circulating autoantibodies to various $\beta$-cell antigens. The most common detected for islet cell antibody, however, is difficult to standard across laboratories other more readily measured circulating antibodies including insulin autoantibodies, antibodies directed against glutamate acid decarboxylase, antibodies against islet tyrosine phosphatase (IA2 AND IA2$\beta$), and several others. Environmental factors such as infections agents, chemical agents, and dietary agents are likely contributing factors in the expression of the disease.
TYPE 2 DIABETES MELLITUS

Type 2DM is heterogeneous disorder characterized by the presence of both insulin resistance and relative insulin deficiency or β-cell dysfunction. Insulin resistance manifests by an increase in lipolysis and free fatty acid production, increase in hepatic glucose production and decrease in skeletal muscle uptake of glucose. Free fatty acids indirectly lead to hyperglycemia by stimulating hepatic glucose production β-cell dysfunction is progressive and contributes to worsening blood glucose control with time. Most patients have both insulin resistance and some degree of insulin deficiency. Type 2 DM occurs when a diabetogenic lifestyle is superimposed upon a susceptible genotype. Weight gain associated with ethnic groups.

In type 2 DM, increased cardiovascular risk appears to begin prior to the development of frank hyperglycemia, presumably because of the effect of insulin resistance. Insulin resistance is associated with a plethora of metabolic small, dense low-density lipoprotein [LDL] levels elevated remnant lipoprotein and thrombosis (elevated type-1 plasminogen activator inhibitor [PAL-1], elevated fibrinogen) abnormalities.

GESTATIONAL DIABETES MELLITUS

Gestational Diabetes Mellitus refers to the onset or initial recognition of glucose intolerance during pregnancy, usually in the second or third trimester. It occurs in about 4% of all pregnancies. Patient with gestational diabetes have a 30% to 50% chance of ultimately developing DM.

OTHER TYPES

Genetics defects of the β-cell or insulin action pathway (insulin receptor mutations or post receptor defects) as well as disease of the exocrine pancreas (e.g. Endocrinopathies producing insulin counterregulatory hormones excess (e.g. Cushing’s syndrome-cortisol;
acromegaly-growth hormone) may results in DM. Certain medication, such as glucocorticoids, pentamide niacin, and α-interferon, may also lead to DM.

**IMPAIRED GLUCOSE TOLERANCE**

Impaired glucose tolerance and impaired fasting glucose are terms that are used to describe patient whose plasma glucose levels are higher than normal but tolerance and impaired fasting glucose are risk factor for DM and cardiovascular disease, and are associated with the insulin-resistance syndrome.

**TREATMENTS**

**DESIRERD OUT COME**

- The primary goals of DM managements are to reduced risk for microvascular and macrovascular disease complications
- To ameliorate symptom, to reduced mortality, and to improve quality of life.
- Near normal glycemia will reduce the risk for development of microvascular disease complications.
- Improve white blood function that reduced polyuria, polydipsia polyphagia, and weight loss.

**GENERAL APPROACH TO TREATMENT**

- Appropriate care required goal setting for glycemia, blood pressure, and lipid levels, regular complication monitoring, dietary and exercise modifications, medications appropriates self-monitoring of blood glucose (SMBG), and laboratory assessment of the above mentioned parameter.
NONPHARMACOLOGICAL THERAPY

DIET

- Medical nutrition therapy is recommended for all persons.
- Higher-carbohydrate, low fat, low-cholesterol diet is appropriate.
- Calori restriction

Classification of lipids and lipoproteins levels

<table>
<thead>
<tr>
<th>LDL Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal &lt; 100</td>
<td>Normal &lt; 150</td>
</tr>
<tr>
<td>Near optimal 100-129</td>
<td>Borderline 150-199</td>
</tr>
<tr>
<td>Borderline High 130-159</td>
<td>High 200-499</td>
</tr>
<tr>
<td>High 160-189</td>
<td>Very High ≥ 500</td>
</tr>
<tr>
<td>Very High ≥ 90</td>
<td>Total Cholesterol (mg/dl)</td>
</tr>
<tr>
<td>HDL Cholesterol (mg/dl)</td>
<td>Desired &lt; 200</td>
</tr>
<tr>
<td>Low &lt; 40</td>
<td>Borderline High 200-239</td>
</tr>
<tr>
<td>High ≥ 60</td>
<td>High ≥ 240</td>
</tr>
</tbody>
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PHYSICAL ACTIVITY

- Physical activity or exercise, have positive benefits should be practiced.
- Exercise improves insulin sensitivity or to ability to drive glucose into cells.
- Exercise lower blood glucose by allowing glucose to penetrate the muscles cell and be metabolized without the assistance of insulin.
- Helps maintain normal body weight (Julie et al., 2002)
**DRUGS**

**Insulin**

**Repaglinide:** Nateglinide, Repaglinide,

**Biguanides:** Metformin, Buformin, Phenformin

**Thiazolidinediones:** Troglitazone, Rosiglitazone, and Pioglitazone

**α-Glucosidase Inhibitors:** Acarbose, Miglitol

**Glucagon-Like Peptide 1:** Hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide (GLP-1)

**GLUCAGON**

**SOMATOSTATIN**

**DIABETIC COMPLICATION**

Diabetic patients pose serious problems in the care of neuropathy. Recent clinical and basic studies revealed characteristic pathophysiology of diabetic. The pathology of diabetic neuropathy is characterized by progressive nerve fiber loss that gives rise to positive and negative clinical signs and symptoms such as pain, paresthesia and loss of sensation. Endoneurial microangiopathic change is also a constant feature of peripheral nerve pathology and negatively correlates with nerve fiber density. Pathogenetic mechanisms underlying the progressive nerve fiber loss seem to be multifactorial, including polyol pathway, glycation, reactive oxygen species, and altered protein kinase C activity. Pathogenesis of neuropathy as alluded earlier, it is essential to prevent or halt the development of diabetic neuropathy based on the information about the pathogenesis (Yagihashi et al., 2007).
Multifactorial etiology of diabetic neuropathy. Hyperglycemia exerts increased polyol pathway, enhanced AGE formation, increased oxidative stress as well as cytokine release. These factors are complicatedly interactive or independently operate for the cause and development of diabetic neuropathy directly affecting nerve tissues or through nutrient vascular tissues.

Hyperglycemia

Hyperglycemia of diabetes, induces alterations in several signaling pathways that culminate in the clinical manifestations of diabetic vasculopathy (DV). Ultimately, cellular behavior is regulated by gene transcription.
Cellular effects and functional consequences of diabetes. Diabetes alters the homeostasis of the cellular components of the vasculature (including endothelial cells, monocytes and vascular smooth muscle cells) in predictable ways. This leads to dysregulation of the normal anti-thrombotic, anti-adhesive and vasoreactive state of vascular beds and eventually to the macrovascular and microvascular complications of diabetes. DM, diabetes mellitus; EC, endothelial cell; VSMC, vascular smooth muscle cells; phenotypic modulation, change from a quiescent state to one which includes elaboration of extracellular matrix, cell proliferation and migration; TF, tissue factor; PAI-1, plasminogen activator inhibitor-1; eNOS, endothelial nitric oxide synthase; VCAM-1, vascular cell adhesion molecule-1; AT-II, angiotensin II; ET-1, endothelin-1; COX-2, cyclooxygenase-2; TNFa, tumor necrosis factor a; MCP-1, monocyte chemo attractant protein-1; TGFb, transforming growth factor b; CTGF, connective tissue growth factor; CAD, coronary atherosclerotic disease; PAD, peripheral atherosclerotic disease

Diabetic Vasculopathy
Hyperglycemia insulin resistance and dyslipidemia. In turn, diabetes leads to the pathological activation of at least four interacting cellular pathways. The net effect is an accelerated vasculopathy-the vasculopathy of diabetes. AGE/RAGE, advanced glycation endproducts/receptor of AGE pathway; PKC, protein kinase C pathway.

**Major Signaling Pathways**

- **Oxidative stress pathway**

  The major pathways implicated in Diabetics vasculopathy. The Hyperglycemia induces oxidative stress in vascular cells by enhancing the production of reactive oxygen species (ROS). This results in damage to cellular proteins, reduced nitric oxide (NO) levels and activation of transcription factors such as activated protein-1 (AP-1) and nuclear factor-kB (NF-kB) antioxidants such as Vitamin E to prevent diabetic vascular complications has largely been ineffective all been
Diabetic Retinopathy

Diabetic retinopathy (DR) is the leading cause of blindness in the working-age group. It is one of the most serious long-term complications of type 2 diabetes mellitus. The prevalence of DR varies from 17% to 98%, depending on the duration of the diabetes. Ischaemia, macular oedema, and rapid progression of DR may cause vision loss. The earliest manifestations of DR are venous tortuosity and dot and blot haemorrhages with microaneurysm formation. Visual loss in DR has two primary causes: progression from non-proliferative DR (NPDR) to the stage of microvascular proliferation called proliferative DR (PDR), in which new, pathological vessels are formed in the retina and fibrotic scarring of the retina which is the culmination of PDR.

Severe non-proliferative diabetic retinopathy

Cataract
prevent diabetic vascular complications has largely been ineffective all been shown to reduce the burden of cardiovascular disease out of proportion to their respective glucose lowering, lipid lowering, or blood pressure lowering effects.

- **Advanced glycation end products pathway**

  Advanced glycation end products pathway (AGEs) are a heterogenous group of compounds produced as a consequence of the irreversible nonenzymatic glycation of proteins. AGEs can confer deleterious effects to the vessel wall in two main ways. First, cross linking of long-lived proteins such as collagen or elastin in the vessel wall can alter structural integrity. Second, binding of AGEs to their receptor on vascular cells can activate multiple signaling pathways (e.g. protein kinase C (PKC) and MAP kinase pathways) and activate nuclear factors (e.g. NF-kB and cyclic AMP-responsive element-binding protein resulting in an increase in ROS production and elaboration of inflammatory factors As such, ways to limit cross-linking of proteins and inhibit (receptors AGE) RAGE receptor activation have been the subject of intense study in both animal models and, more recently, human trials. Treatment of diabetic mice with inhibitors of AGE formation (e.g. aminoguanidine) or AGE cross link breakers such as alagebrum attenuated atherosclerosis and reduced vascular stiffness.

  **Polyol/aldose reductase pathway**- Elevation in glucose can activate the enzyme aldose reductase that converts glucose to sorbitol and ultimately to fructose. This pathway alters NAD(P)H/NAD(P) balance in a manner that leads to augmentation of oxidative stress and ultimately vascular disease redox stress can activate both the PKC and AGE pathways.
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Cataract

Diabetic cataract is characterized by opacification of the lens, eventual loss of vision and occurs at a much earlier age than senile cataract. It has been suggested that glycation of lens crystallins may cause conformational changes resulting in exposure of thiol groups to oxidation and cross-link formation. Furthermore, the lens crystallins have virtually no turnover and readily accumulate AGEs which in turn cause aggregation of the lens crystallins producing the high molecular weight material responsible for opacification. As demonstrated by animal studies, recently, evidence has even been presented suggesting that fluorescent AGEs isolated from human cataractous lenses are derived from glycation by ascorbate. Increased glycation of Na–K ATPase in vitro reduces its activity, altering intracellular ion concentration and subsequent water movement via osmosis. Such an effect *in vivo* may contribute towards cataract formation in diabetes (Ahmed *et al.*, 2005).

Diabetic Nephropathy

(DN) is a major cause of morbidity in patients with type 2 diabetes. One of the earliest clinical signs of DN is microalbuminuria, which often progresses toward proteinuria. Characteristic features associated with DN include hyperfiltration, followed by a decrease in glomerular filtration rate (GFR), glomerular hypertrophy, progressive expansion of the mesangial matrix, and thickening of the glomerular and tubular basement membranes. The onset of end-stage renal disease (ESRD). Little is known about the molecular mechanisms leading to ESRD in DN. Although the role of many genes in progressive renal diseases has been described, their interrelationship remains largely unclear (Baelde *et al.*, 2004).
Diabetic Atherosclerosis

Atherosclerosis is the most serious consequence of long-term diabetes and the major cause of death in these patients. It is characterized by deposition of atherosclerotic plaques on the insides of arterial walls, occlusion of blood flow and eventual myocardial infarction. Increased glycation of low-density lipoprotein (LDL) occurs in diabetes. Glycated LDL is not recognised by the LDL receptor but its uptake by macrophages is enhanced and this may account, at least in part, for the hyperlipidaemia and accelerated foam cell formation observed in diabetic patients. LDL is also modified by AGE and this LDL-AGE increases in diabetic patients and has reduced serum clearance Glycation and AGE formation are also accompanied by increased oxidation of LDL and an increase in this atherogenic oxidized LDL occurs in diabetes (Ahmed et al., 2005).

Diabetic Embryopathy

**Diabetic embryopathy**: Diabetic mothers with poor glycaemic control are prone to embryopathy, where the newborn have an increased frequency of congenital malformations. The precise mechanism underlying embryopathy in diabetes is unknown, but a reduction in congenital malformations is seen in pregnancies where the hyperglycaemia is well controlled. Hyperglycaemia causes oxidative stress which has been implicated in the pathogenesis of embryopathy. Indeed increased free radical activity and lipid peroxidation products have been detected in rat embryos cultured in a high glucose medium and in embryos from diabetic rats The increased free radical activity may be due to 3-DG as rat embryos cultured in the presence of 3-DG show increased malformation rates which are decreased by inclusion of superoxide dismutase in the culture medium Embryopathy may arise because of glycation of DNA and histones by reactive intracellular sugars and indeed increased AGEs have been detected on histones isolated from diabetic rats.
Wound Healing in Diabetes

Normal wound healing is a complex co-ordinated sequence of events involving migration of cells into the wound, inflammation, the proliferation of different cell types, angiogenesis, formation of matrix components, remodelling and eventual closure of the wound. Impaired wound healing in diabetic patients is a major cause of concern as it leads to a large number of amputations. The inflammatory response following injury is important for rapid wound healing. In diabetes, there is delayed influx of inflammatory cells into a wound site initially, but when these cells become established, then a state of chronic inflammation occurs preventing deposition of matrix components, remodelling and eventual closure of the wound this sustained inflammatory response occurs following interaction of AGEs with RAGE and release of pro-inflammatory molecules such as TNF-a and production of destructive matrix metalloproteinases (MMPs) which limit wound (Ahmed et al., 2005).

DIABETES AND OXIDATIVE STRESS

It is accepted that oxidative stress results from an imbalance between the generation of oxygen derived radicals and the organism’s antioxidant potential. Various studies have shown that diabetes mellitus is associated with increased formation of free radicals and decrease in antioxidant potential. Due to these events, the balance normally present in cells between radical formation and protection against them is disturbed. This leads to oxidative damage of cell components such as proteins, lipids, and nucleic acids. In both type 1 and type 2 there is increased oxidative stress in diabetes mellitus. Oxidative stress is increased in diabetes because of multiple factors. Dominant among these factors is glucose autoxidation leading to the production of free radicals. Other factors include cellular oxidation/reduction imbalances and reduction in antioxidant defenses (including decreased cellular antioxidant levels and a reduction in the activity of enzymes that
Another important factor is the interaction of advanced glycation end products (AGEs) with specific cellular receptors called AGE receptors RAGE. Elevated levels of AGE are formed under hyperglycemic conditions. Their formation is initiated when glucose interacts with specific amino acids on proteins forming a compound that then undergoes further chemical reactions. Glycation of protein alters protein and cellular function, and binding of AGEs to their receptors can lead to modification in cell signaling and further production of free radicals.

The history of oxygen free radicals began approximately $2 \times 10^9$ years ago, with the appearance of the first blue-green algae, the most primitive organisms capable of photosynthesis. By releasing molecular oxygen into the biosphere, they made possible the genesis of a multitude of aerobic beings that are in the biosphere today. However, in a number of physiological and pathological processes, oxygen can be transformed into a variety of potentially toxic reactive intermediates known as oxygen-free radicals or reactive oxygen species (ROS). Free radicals can be formed by hemolytic bond fission or by electron transfer reactions. In general, such processes proceed either through the absorption of radiation (ionizing, ultraviolet [UV], visible, or thermal) or by redox reactions. The precursors of free radicals include a variety of xenobiotic compounds (photochemical pollutants, cigarette smoke, chemicals, drugs, heavy metals, and the constituents of much foodstuffs) as well as endogenous compounds that can be converted to reactive radical species In vivo. Most often, however, a number of highly reactive free radicals are generated from the oxygen in living systems as an unavoidable consequence of aerobic respiration: $O_2^-$ (superoxide), HO (hydroxyl radical), and $H_2O_2$ (hydrogen peroxide). All these free radicals are partially reduced oxygen species that are involved not only in some of the useful physiological processes but also in the pathogenesis of many diseases (Djordjevic et al., 2004).
MOST FREQUENT REACTIVE OXYGEN SPECIES

Superoxide Anion Radical:-

Superoxide anion radical ($O_2^-$) can be formed by one-electron reduction of molecular oxygen or by one-electron oxidation of hydrogen peroxide. When oxygen accepts an electron from a reducing agent, the chemical nature of the molecule may change either into a superoxide anion or a protonated form $HO_2^-$. In the acidic environment, such as phagolysosome, $O_2^-$ is the conjugate base of the hydroxyperoxyl radical ($HO_2^-$), which is spontaneously dismutated to hydrogen peroxide. Although $O_2^-$ is less reactive than is a hydroxyl radical, it is potentially more damaging because of its ability to diffuse at a distance before encountering a possible target. In vivo $O_2^-$ is mostly removed by superoxide dismutase, which is abundant in the cell compartments with the highest production of $O_2^-$. However, low concentrations of $O_2^-$ and $H_2O_2$ are initially used by the cell exclusively for the mobilization of the antioxidant system (Djordjevic et al., 2004).

Hydrogen Peroxide

Hydrogen peroxide is considered a key oxygen-free radical because of its relatively high stability, diffusion, and involvement in cell signaling cascades. It may be formed by a direct two-electron reduction of molecular oxygen, by an electron reduction of super-oxide, or by its enzymatic dismutation by SOD. Hydrogen peroxide can be produced in peroxisomes, mitochondria, microsomes, and cell membranes. Direct production of $H_2O_2$ occurs following reduction of molecular oxygen during the reactions catalyzed by oxidases, autooxidation of δ-aminolevulinic acid, and electron transfer in mitochondria, in which about 2% of the existing oxygen is transformed into hydrogen peroxide when ADP concentration is low. The greatest amount of $H_2O_2$ is produced in
peroxisomes rich in catalase, which effectively protects these organelles from oxidative
damage. H$_2$O$_2$ is, indeed, a potent oxidant, and in sufficient concentrations, it could kill
any cell. However, a high concentration of H$_2$O$_2$ can never occur intracellularly. It is
suggested that H$_2$O$_2$ concentration that is four times normal could express the toxic effect.
In lesser amounts, H$_2$O$_2$ may serve as a messenger or cofactor in cell metabolism. H$_2$O$_2$
stimulates cell growth and division, instructs the cells (infected by viruses) and their
neighbors to commit suicide, and increases the pentose shunt activity necessary for an
enhanced formation of phosphoribosyl pyrophosphate, a cofactor in de novo nucleotide
synthesis. These facts could explain why catalase efficiently catabolizes high
concentrations of H$_2$O$_2$ but allows GSH-dependent metabolism of H$_2$O$_2$ trace amounts
(Djordjevic et al., 2004)

Hydroxyl Radical

The hydroxyl radical (HO') is highly unstable; that is, the most reactive radical
known to chemistry. It is formed by successive monovalent reduction of molecular
oxygen in the cell metabolism. First, it can be generated in the course of ionizing
radiation on water. The second source of HO' is the reduction of H$_2$O$_2$ by metal cations
such as Fe$^{2+}$ or Cu$^{1+}$ or by the iron-catalyzed Haber-Weiss reaction during enzymatic
reactions, producing both O$_2^-$ and H$_2$O$_2$ (e.g., during XO or NADPH oxidase activity).
The reaction proceeds as follows:

$$\text{Me}(n) + O_2^- \leftrightarrow \text{Me}(n-1) + O_2$$

$$\text{Me}(n-1) + H_2O_2 \leftrightarrow \text{Me}(n) + HO^- + HO'$$

Provided the metal ion in the second reaction is iron, some other intermediates can be
formed, such as (FeO)$^{2+}$ or (FeOH)$^{3+}$. These two, together with HO$^-$ and Fe$^{3+}$, can be
damaging to selective biomolecule targets. The short-living HO$^-$ molecule In vivo does
not endure for even a microsecond, and thus unspecifically attacks biomolecules; DNA,
proteins, polysaccharides, and lipids, in a diffusion-limited reaction. Also, \( \text{HO}^- \) can oxidize the [4Fe–4S] clusters of dehydratases, such as aconitase, triggering the release of Fe\(^{2+}\), which can further react with the available H\(_2\)O\(_2\). There are indications that HO\(^-\) generation may occur in the reaction of O\(_2^-\) with hydroperoxides formed by lipid peroxidation. This is an amplification mechanism for the continued production of HO\(^-\) after initiation of lipid peroxidation. Uncontrolled HO\(^-\) production is involved in numerous cellular disorders such as inflammation, embryo teratogenesis, cell death, phagocytosis, and herbicide effects (Djordjevic et al., 2004).

**Nitric Oxide**

Nitric oxide NO with MW 30 is certainly the smallest ubiquitous diffusible cell signaling molecule. It was first identified in ECs in 1987. Later, it was documented that many other cells (neutrophils, activated macrophages, Kupfer cells, vascular smooth muscle cells, adrenal glands, and the central nervous system) can produce this molecule. Nitric oxide is produced from guanidino nitrogen of the semiessential amino acid L-arginine by one of the three distinct forms of NO synthase (NOS) encoded by three separate genes located at 7, 12, and 17 human chromosomes. L-arginine is both the substrate and the regulator of NOS. Neuronal NOS (nNOS, NOS-I) primarily exists in the central and peripheral nervous system, but it is also present in the skeletal muscle and some epithelial tissues. Endothelial NOS (eNOS, NOS-3) is expressed in the ECs of blood vessels. Both neuronal and endothelial NOSs are constitutive cytoplasmatic proteins that can be induced during pregnancy and treatment with estradiol, but it is only eNOS that can be induced by shear stress and chronic exercise. Under normal settings, iNOS occurs at low levels. All three isoforms are heme-containing enzymes, have a sequence similar to cytochrome P-450 reductase, and are the only mammalian proteins
known to catalyze both a hydroxylation reaction and NADPH reduction (Djordjevic et al., 2004).

Catalase

Catalases (CAT) are ubiquitously present in aerobic organisms, including almost all mammalian tissues (with the exception of ECss), in which catalases show the highest enzyme activity in the liver and erythrocytes. Within cells, catalases are mostly located in peroxisomes (a strategic location because of the presence of many H₂O₂-producing enzymes) and mitochondria as both soluble and membrane-bound forms. The mammalian catalases are homotetrameric ferriheme-containing enzymes whose molecular mass is approximately 240 kDa. Enzymes from different sources differ among one another with respect to their structure and properties. In erythrocytes, catalase is the first line of defense against H₂O₂. Beyond the normal concentration range of H₂O₂, ‘catalatic’ types of reaction proceed according to the catalytic cycle:

\[
\text{Catalase} + \text{H}_2\text{O}_2 \leftrightarrow \text{Compound I} + \text{H}_2\text{O}
\]

\[
\text{Compound I} + \text{H}_2\text{O}_2 \leftrightarrow \text{Catalase} + \text{H}_2\text{O} + \text{O}_2
\]

The second molecule of H₂O₂ functions as a hydrogen donor in the “catalatic” reaction. At low H₂O₂ concentrations and in the presence of small molecular electron donors (alcohols, nitrites, and formate), catalase can also act as a peroxidase. Compound I is then converted to a catalytically inactive compound II, and the process is termed “suicide inactivation”. A number of disorders are associated with the altered catalase activity. There is evidence that a moderate oxidative stress induces catalase expression in vascular cells and, thereby, could be beneficial in prevention of further oxidative stress. Superoxide anions can inhibit catalase. In the leukocytes of subjects with Swiss type acatalasemia, the rate of dehydroascorbate reduction is four times that of normal, indicating that the catalase protective function is supported by dehydroascorbate
reductase. An increase in catalase activity is observed in experimental halothane hepatotoxicity, endotoxemia, and hepatitis, as well as in patients with hemolytic diseases, liver disorders, acute pancreatitis, muscular dystrophies, and so forth. However, decreased catalase activity was found in patients with malignant diseases, diabetes (Djordjevic et al., 2004)

**Glutathione Peroxidases**

Unlike catalases, Glutathione Peroxidases (GPx) use a variety of electron donors to reduce H₂O₂ to 2H₂O and are widely distributed in yeast (cytochrome c peroxidase), plants (ascorbate peroxidase), bacteria (Escherichia coli contains alkyl hydroperoxide reductase), as well as in mammals who contain at least three peroxidases. The first peroxidase type, “classical” glutathione peroxidase, is present in both the cytosol and mitochondria of various mammalian tissues. It is a homotetramer with a molecular mass of approximately 80 kDa. The enzyme also contains four atoms of selenium ion and eight moles of free sulphhydryl groups per mole of protein. Peroxidases are the only human enzymes known to require selenium for their activity. GPx exerts a general specificity for hydroperoxides and a high specificity for reduced glutathione (GSH). It reduces H₂O₂ and organic alkyl hydroperoxides to water and corresponding alcohols, and GSH is oxidized to the corresponding disulfide (GSSG).

2GSH + H₂O₂ (ROOH) ↔ GSSG + ROH + H₂O

These reactions are coupled with the glutathione cycle, so GSSG converts back to GSH by glutathione reductase, which uses NADPH as a reductant (Djordjevic et al., 2004)

**Superoxide Dismutases (SOD)**

Superoxide dismutases include several metalloproteins (CuZn SOD, Mn SOD, Fe SOD, and Ni SOD) that contain corresponding metal ions at the active sites. Fe SOD is found in prokaryotes and plants, and CuZn SOD, Mn SOD, and Fe SOD have been
described in mammals. The intracellular CuZn SOD is present in the cytoplasm, nucleus, and peroxisomes of all mammalian cells. Its molecular mass is approximately 32 kDa, and it is composed of two identical subunits, each of which contains one Cu(II) and one Zn(II). Cu(II) is responsible for the catalytic activity of SOD and alternates between the cupric and cuprous state during the catalytic cycle, whereas Zn(II) stabilizes the enzyme conformation. Cytosolic CuZn SOD may be inactivated by hydrogen peroxide, leading to the generation of either Cu(II)-OH or its ionized form Cu(II)-O\(^{2-}\). This enzyme can further catalyze the peroxidation of a wide variety of compounds. Because the human gene encoding CuZn SOD is located on chromosome 21, a significant enhancement of this enzyme level was observed in patients with Down’s syndrome. On the other side, mutations in the cytosolic CuZn SOD, noted in patients with amyotrophic lateral sclerosis, are associated with the enzyme deficiency of different degree. In addition, endotoxemia induces a rapid decline in the expression of CuZn SOD, as well as an increase in the expression of Mn SOD (Djordjevic et al., 2004).

**Lipid Peroxidation (LPO)**

Oxidative stress occurs as a consequence of imbalance between prooxidants and antioxidants. Many of the prooxidants are free radicals capable of modifying distinct biomolecules including lipids, proteins, carbohydrates, and nucleotides. Oxidative stress and the oxidative modification of biomolecules are involved in a number of physiological and pathophysiological processes such as aging, atherosclerosis, inflammation, carcinogenesis, and drug toxicity. Lipid peroxidation is both a free radical-mediated process and a source of secondary free radicals, some of which can be second messengers and others can directly react with surrounding molecules or diffuse before further reaction, thereby spreading the biochemical lesion.
Lipid peroxidation itself is associated with decreases in membrane fluidity resulting in its augmented permeability for one- and two-valence ions and in the inactivation of membrane enzymes and receptors. Peroxidation can lead to the destruction of all lipid membranes. Lysosomes contain several proteolytic enzymes that are released during membrane disruption and that can, after activation, augment the cellular injury. Thus, the uncontrolled peroxidation of biomembranes can lead to profound effects on the membrane structure and function and may be sufficient to cause cell death (Djordjevic et al., 2004)

Antioxidants counter the action of free radicals by several mechanisms. These mechanisms include:

1. enzymes that degrade free radicals
2. proteins such as transferrin that can bind metals which stimulate the production of free radicals, and
3. antioxidants such as vitamins C and E that act as free radical scavengers.

In a study, the total antioxidant capacity in plasma of type 1 diabetics was shown to be 16% lower than that of normal subjects. Decreased activities of antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) in the kidney of streptozocin (STZ)-induced diabetic rats has been reported (Rahimi et al., 2005).

Plants have been considered as sources of medicinal agents for the treatment of many diseases. Before the advent of insulin injections and other pharmaceutical preparations, healers relied heavily upon medicinal plants and herbs to treat diabetes. Actually, more than 1200 plants have been described to be experimentally or ethnopharmacologically used in the treatment of diabetes mellitus. To date, a few of these medicinal plants have received scientific or medical scrutiny, despite the fact that the World Health Organization has encouraged and recommended that traditional treatment for diabetes
warrant further (Lemhadri, et al., 2006), evaluation (WHO, 1980). Currently, there is
great interest in finding antioxidants from natural sources to minimize oxidative damage
to cells. Oxidative damage is caused by free radicals and reactive oxygen species, mostly
generated endogeneously. They are recognized to be involved in the pathogenesis of
various diseases such as atherosclerosis, cancer, diabetes mellitus and reperfusion
disorder. Researchers have demonstrated that appropriate consumption of foods
containing antioxidants such as herbs and vegetables can prevent such deleterious effect.
There are many types of antioxidants in the vegetables and medicinal plants (Abraham et
al., 2008).

A wide array of plant derived active principles representing numerous chemical
compounds have demonstrated activity consistent with their possible use in the treatment
of NIDDM. Among these are alkaloids, glycosides, galactomannan gum, polysaccharides,
peptidoglycans, hypoglycans, guanidine, steroids, carbohydrates, glycopeptides,
terpenoids, amino acids and inorganic ions. Even the discovery of widely used
hypoglycemic drug, metformin came from the traditional approach of using Galega
officials. Thus, plants are a potential source of anti-diabetic drugs (and others too) but this
fact has not gained enough momentum in the scientific community. The reasons may be
many including lack of belief among the practitioners of conventional medicine over
alternative medicine, alternative forms of medicine are not very well-defined, possibility
of quacks practicing such medicine providing alluring and magical cures and natural
drugs may vary tremendously in content, quality and safety. Although, oral hypoglycemic
agents/insulin is the mainstay of treatment of diabetes and are effective in controlling
hyperglycemia, they have prominent side effects and fail to significantly alter the course
of diabetic complications. As the knowledge of heterogeneity of this disorder increases,
there is needed to look for more efficacious agents with lesser side effects. Though
development of modern medicine resulted in the advent of modern pharmacotherapeutics including insulin, biguanides, sulfonylureas and thiazolidinediones, there is still a need to look for new drugs as no drug (except strict glycemic control with insulin) has been shown to modify the course of diabetic complications. In relation to plants also, barring a few studies (Grover et al., 2002). Diabetes mellitus also results in severe metabolic imbalances and non-physiologic changes in many tissues especially the pancreas which is the organ that secretes insulin. Streptozotocin, a monofunctional nitrosourea derivative, is one of the most commonly used substances to induce diabetes in experimental animals. Evidences suggest that the diabetogenic capacity of streptozotocin may depend on its ability to damage β-cell and induce oxidative stress. Increases in oxidative stress markers in pancreatic islets in experimental diabetic rats have been reported (Lei Jin et al., 2008). Several author suggested that increased fructose ingestion my be responsible for the epidemic of obesity and the increased incidence of metabolic syndrome and diabetic. Diets rich particularly fructose, have been shown to be associated with hypertriglyceridemia both in human and rodents (Davide et al., 2005; Mehdi, et al., 2003). Fructose-fed rats were shown to have an impaired ability to suppress hepatic glucose production and to eliminate peripheral glucose. The Increased in the gluconeogenic enzymes glucose-6-phosphatase and phosphoenolpyruvate, carboxy-kinase in liver fructose fed rats. The fructose-induced insulin resistance was associated with a slight decrease in insulin receptor substrate-1/Phosphorylation and Insulin receptor Substrate-1/Phosphoinositol 3-kinase associated with the liver and muscles of intact rats (Bezerra et al., 2001), Shown significantly increased in fasting serum glucose, insulin and serum concentration in rats that consumed 15% of energy of fructose (Sharon et al., 2002). Recently, there has been increasing interest in the use of medicinal plants. The plants kingdom has become a target for the search by multinational drugs and biological active
lead compound. Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes (Eidi et al., 2005) Hence, the present study was under taken in fructose-induced Insulin resistance by *N. arbortristis* and *C. gigantea* in rats.

Hence, the present study was under taken to explore antidiabetic activity of *N. arbortristis* and *C. gigantea* of different extracts on normal, streptozotocin induced diabetic and high fructose induced Insulin resistance in rats. *N. arbortristis Linn* is widely used as a decoction of leaves by Ayurvedic physicians for the treatment of diabetes (Chetty, et al., 2008), arthritis, obstinate, sciatica, malaria, intestinal worms and as tonic, cholagogue and laxative. Phytochemical investigations have shown that *N. arbortristis* and *C. gigantea*. Commonly Iridoid reported from the seeds are nyctanthoside, 6β-hydroxyloganin, and its 6-p-coumaroyl ester. Earlier works reported the isolated of mannitol, β-amyrin, β-sistosterol hentriacontane, Benzoic acids astragalin, nicotiflorin, oleanolic acids nyctantheic acids, friedelin and lupeol from the leaves, two new ester of 6β-hydroxyloganin 1 and 2 in *N. arbortristis* leaves (Mathuram et al., 1991), due to presence of nyctanthocide or iridoid glucosides (arbortistosides A, B and C) or 6β-hydroxyloganin which has been identified as active constituents against *Plasmodium* ssp. Two new iridoid glycosides, 6,7-di-O-benzoynyctanthoside (1) and 6-O-transcinnamoyl-6β-hydroxyloganin (2) along with the previously reported iridoid 7-O-trans-cinnamoyl-6β-hyrdoxyloganin (3) (Stuppner, et al., 1992). Arbortristoside-A, (Das et al., 2008 ). Iridoid glucosides (arbortistosides A [I], B [2], C [3], and 6beta-hydroxyloganin [4] isolated from the traditional plant *N. arbortristis*.

*C. gigantea*:- from ancient the times, has been used in several in loss of memory, defective eyesight, infertility, overall body weakness and incidence of early aging. *Swarnabhasma* has been used by Ayurvedic physicians to treat different diseases like
bronchial asthma, rheumatoid arthritis, diabetes mellitus, nervous disorders (Mitra, et al., 2002). The roots of C. gigantea have been used in leprosy, eczema, syphilis, elephantiasis, ulceration, anti-diarrhoeal activity and cough in the Indian system of traditional medicine. (Chitme, et al., 2005), C. gigantea two new triterpene esters, viz. 3'-methylbutanoates of α-amyrin and ψ-taraxasterol, besides the known 3'-methylbutanoates of three triterpene alcohols. alkane fraction, total triterpene alcohol fraction, and free, acetyl and 3'-methylbutanoyl triterpene alcohol (Thakur, et al., 1984). Two proteinase containing carbohydrate, called calotropain-FI and calotropain-FII, C. gigantea reported (Abraham et al., 1979). Isorhamnetin-3-O-rutinoside, isorhamnetin-3-O-glucopyranoside and taraxasteryl acetate, a new flavonol trisaccharide was isolated from the aerial parts of C. gigantea, and its structure was established as isorhamnetin-3-O-[2-O-β-D-galactopyranosyl-6-O-α-L-rhamnopyranosyl]-β-D-glucopyranoside. (Sen et al., 1992) Flavonol glycosides four new chemical constituents, one naphthalene derivative, named calotropnaphthalene, two terpene derivatives, calotropisesquiterpenol and calotropisesterterpenol and an aromatic product designated as calotrophenzofuranone along with a known compound, sucrose, have been isolated from the roots of the C. gigantea. The structures of these chemical constituents have been established as 1-methoxy-4-ethyl naphthalene, 6-(2-methyl-2,3-dihydroxypentyl)-11,11-dimethyl cyclohex-8-ene-10-one-7-oic isopentenyl ester,14-(15,15-dimethyl cyclohexanyl-14,19,25-tricyclo)-3,7,11-trihydroxymethylene-tridecane and 8,15-dihydro benzofuranyl-18-hepta-7,15-dione-16-oic acid, respectively, (Gupta et al., 2000). Autodigestion of the cystiene protienases, calotropis D1 and D2 (Sengupta et al., 1984). The ionisation of the phenolic hydroxy groups in calotropin D1 and DII isolated from the latex of C. gigantea (Bhattachary et al., 1985). The cardenolides from the latex and leaves triterpenoids, anthocyanins from flowers and hydrocarbons. The leaves and latex of Calotropis gigatea
found to have cardiac glycosides various glycosides were isolated and studied. An active principle mudarine was isolated from leaves of Calotropis gigantea. Besides this, a yellow bitter acid and resin were also found. The cardiac glycosides were identified as calotropigenin(1) calotropin(2) Uscharin(3) and Calotoxin(4), Calactin(5) Three cardenolide glycosides, Coroglaucigenin(6), frugoside(7) 4-O-Beta-D-Glycopyranosyfrugoside (roots of Calotropis gigantea.L). Two new oxypregnane-oligoglycosides named Calotroposides A and B have been isolated from the roots of C. gigantea. The 12-obenzoyllineolon 3-O-beta-D-cymaropyranosyl(1-4)-beta-D-oleandropyranosyl (1-4)beta-D-oleandropyranosyl(1-4)-beta-D-cymaropyranosyl (1-4)-beta-D-cymaropyranoside and 12-O-benzoyl deacetylmplexigenin 3-O-beta-D-cymaropyranosyl(1-4)-beta-Dcymaropyranoside, (Ahmed Mueen et al., 2005).

The N. arborretisis commonly containing iridoid glucoside isolated antioxidant and pancreas-protective effect of on rats (Lei et al., 2008). has been known to have diverse biological activities such as inhibiting the synthesis of RNA and proteins in the liver of mice, protecting against liver damage induced by carbon tetrachloride or in mice and rats, and antimicrobial activity. (Lei et al., 2008)

The plants contain a large variety of substances that possess antioxidant activity. Phytochemicals with antioxidant effects include, diterpenes, flavonoids, monoterpenes, and triterpenes reported (Rahimi et al., 2005). And other phenolic compound content of the plant are well corrected with their antioxidant activity and well documented Natural antioxidants. The C. gigantea containing diterpenes, flavonoids, monoterpenes, and triterpenes, anthocyanins and flavonol glycosides one naphthelene derivative, named calotropnaphthalene, two terpene derivatives, calotropisesquiterpenol and calotropisesesterterpenol. Therefore it seems that plants particularly those with high levels and strong antioxidant compounds have an important role in improvement of disorders
involving oxidative stress such as diabetes mellitus. There are many investigations which have studied the effects of these plants and their antioxidant ingredients on diabetes and its complications and achieved good results (Yesilyurt et al., 2008).

**Streptozotocin**

Streptozotocin is a naturally occurring nitrosourea derived from *Streptomyces achromogenes* variety 128. It is related structurally to lomustine, another nitrosourea. Streptozotocin is used as a chemotherapeutic agent, specifically for the treatment of pancreatic tumors. It also displays antibacterial properties.

Chemical Names: 2-Deoxy-2-[(methylnitrosoamino)-carbonyl]amino]-D-glucopyranose

Chemical Formula: \( \text{C}_8 \text{H}_{15} \text{N}_3 \text{O}_7 \)

**Chemical structure**

![Chemical structure of Streptozotocin](image)

**Chemistry**

STZ consists of 1-methyl-1-nitrosourea linked to position \( \text{C}_2 \) of D-glucose. The solid is usually a mixture of \( \alpha \)- and \( \beta \)-isomers with regard to \( \text{C}_1 \) in the glucose moiety of the molecules. The \( N \)-nitrosourease to which STZ belongs, are chemically unstable, and it has been suggested that the carcinogenic and the other biological action of the nitrosoureas are due to alkylation of cellular constitutes caused by reactive intermediates formed during the degradation of the substance \( N \)-nitrosomethyurea which constitutes the
side-chain of STZ has been shown to methylate nucleic acids in several organs of rat. Upon solution in saline or distilled water at room temperature and neutral pH it decomposes within a few minutes visible formation of gas. Its stability in solution is optimal at pH 4-4.5 and low temperature.

**Histopathological change in the pancreatic islets**

The action of STZ on the cellular elements of the islet parenchyma on its β-cells in particular appears to be well understood, both by light microscopy and the ultrastructures levels STZ acts as a β-cytotoxic substance in many laboratory mammals caused necrosis or marked degenerative lesions in the β-cells with nuclear pyknosis and cytoplasmic vacuolization. Degenerative change and necrosis were also observed in the cells in rabbits and hamsters. Further an increased in the mean nuclear diameter of β-cells and α₂ cells of STZ diabetic rats in β-cells and α of STZ-treated guinea pigs and, β α and α₂-cells of Chinese hamsters has been reported repeatedly. An increased nuclear size is suggestive of an augmented protein synthetic activity in general although the mechanism underlying an increased α-cell activity in STZ diabetes remains an enigma.

The phenomenon of persistent marked atrophy observed in STZ-diabetics rats an evident decrease in islet cell volume and its β-cells mass as compared to normal, its also observed on rats are in according to finding reported in hamsters.

STZ administration in animals consistely causes a triphase blood sugar response induced in diabetic, an initial increases thought to be secondary to glycogenolysis or to a rise an free fatty acid which in turn is caused by a reduction in circulating insulin if followed by a profound hypoglycemia, finally an irreversible diabetic state and produced severe hyperktonemic diabetic state developed by 24 hrs post-injection
Properties: Streptozotocin is an ivory colored crystalline powder with a melting point of 115°C. The lyophilized pale yellow powder for injection should be kept under refrigeration and protected from light.

**Diabetogenic action of Streptozotocin**

The diabetogenic action of STZ produced experimentally in conventional laboratory mammals is attributable to the irreversible damage it exerts, specifically on the β-cells Langerhans islets. The mechanism of diabetogenic action of STZ is due to destruction of islet β-cells where before triggering its diabetogenic response, the drugs are bound in the cells inducing significant derangements in their insulin-releasing mechanism. STZ is known to possess carcinogenic. Antibacterial and antitumoral properties (Srivastava et al., 1982). STZ induced diabetics in experimental anima is evidence suggest that the diabetogenic capacity of STZ may depend on its ability to damage β-cell and induce oxidative stress (Lei Jin et al., 2008). Oxidative stress plays an important role in chronic complications of diabetes and is postulated to be associated with increased lipid peroxidation, Streptozotocin is frequently used to induce diabetes mellitus in experimental animals through its toxic effects on pancreatic-cells The cytotoxic action of STZ is associated with the generation of reactive oxygen species causing oxidative damage. Diabetes manifested by experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia, which thereby depletes the activity of antioxidative defense system Increased oxidative stress and changes in antioxidant capacity, observed in both clinical and experimental diabetes mellitus, are thought to be the etiology of diabetic complications In diabetes there are significant changes such as increased lipid peroxidation, dyslipidemia and irregularities in the metabolism of proteins, lipids and carbohydrates. Lipid is known to impair the exocrine pancreas by damaging the endothelium of blood vessels. People who develop diabetes usually pass through the
phases of excessive adipogenesis, nuclear peroxisome proliferator-activated receptor (PPAR) modulation, insulin resistance, hyperinsulinemia, pancreatic β-cell stress and damage leading to a progressive decrease in insulin secretion and impaired glucose postprandial and fasting levels. Disturbances of antioxidant defense systems in diabetes have been demonstrated, including alteration in the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and impaired glutathione (GSH) metabolism. Chemicals with antioxidant properties and free radical scavengers may help in the regeneration of β-cells and protect pancreatic islets against cytotoxic effects of streptozotocin (Anwer et al., 2007)

**High fructose diet**

**Insulin**

Insulin is polypeptide hormone secreted by the beta- islets of pancreas. insulin has widespread effects on carbohydrate, lipid, and protein metabolism. It accelerates the transport of glucose into cells, resulting in equilibrium of intra and extra cellular concentrations. In addition, Insulin affects the intracellular metabolism of glucose. Insulin facilitates the movements of amino acids into cells and their utilization in protein synthesis, and the transport of Na⁺ and K⁺ ions across cell walls. Decreased rats of protein synthesis resulting from inadequate insulin levels affects normal growth. Lipogenesis in adipose and hepatic cells is also amplified by insulin. Diabetes mellitus is the results of insulin deficiency brought about either by insufficient insulin secretion or by rapid insulin catabolism. Impairment of the metabolic process affected by insulin causes decreased glucose utilization, and increased glucogenesis, with much of the glucose wasted, leading to hyperlycemia. insulin levels vary in diabetics, but their determination is important for the differentiation and diagnosis of various types of diabetes.
The measurement of insulin useful for the diagnosis of insulinomas and other hypoglycemic condition not resulting from insulinomas. insulin determination have been recommended in the evaluation of patient with chronic pancreatitis. A number of test that alter the insulin levels have been suggested as a means of expanding the diagnostic utility of insulin measurements. These oral and intravenous glucose tolerance tests, Tolbutamide infusion.

Over the past decade, per capita consumption of high-fructose corn syrups has increased dramatically. Several author suggested that increased fructose ingestion my be responsible for the epidemic of obesity and the increased incidence of metabolic syndrome and diabetic. Diets rich particularly fructose, have been shown to be associated with hypertriglyceridemia both in human and rodents (Davide et al., 2005; Mehdì et al., 2003). Fructose-fed rats were shown to have an impaired ability to suppress hepatic glucose production and to eliminate peripheral glucose. The Increased in the gluconeogenic enzymes glucose-6-phosphatase and phosphoenolpyruvate, carboxy-kinase in liver fructose fed rats. The fructose-induced insulin resistance was associated with a slight decrease in insulin receptor substrate-1/Phosporylation and Insulin receptor Substrate-1/Phospoinositol 3-kinase associated with the liver and muscles of intact rats (Bezerra et al., 2001) Shown significantly increased in fasting serum glucose, insulin and serum concentration in rats that consumed 15% of energy of fructose (Sharon et al., 2002).

Insulin resistance may occur through different mechanisms, including defects in insulin binding and signal transduction, or defects at the level of effect or molecules such as glucose transporters and enzymes involved in carbohydrate metabolism. These molecular defects have been characterized in animal models of insulin resistance, including rat models of insulin resistance associated with hypertension. Initial studies
The exposure of the liver to such large quantities of fructose leads to rapid stimulation of lipogenesis and TG accumulation, which in turn contributes to reduced insulin sensitivity and hepatic insulin resistance/glucose intolerance. Fructose is a potent regulator of glycogen synthesis and liver glucose uptake. Therefore any catalytic improvements are due to hepatic glucokinase and glucose uptake facilitation. However, as mentioned, the beneficial effects do not continue with chronic fructose utilization. Because of its lipogenic properties, excess fructose in the diet can cause glucose and fructose mal absorption, and greater elevations in TG and cholesterol.

**Hepatic fructose metabolism:** A highly lipogenic pathway. Fructose is readily absorbed from the diet and rapidly metabolized principally in the liver. Fructose can provide carbon atoms for both the glycerol and the acyl portions of triglyceride. Fructose is thus a highly efficient inducer of *de novo* lipogenesis. High concentrations of fructose can serve as a relatively unregulated source of acetyl CoA. In contrast to glucose, dietary fructose does NOT stimulate insulin or leptin (which are both important regulators of energy intake and body adiposity). Stimulated triglyceride synthesis is likely to lead to hepatic accumulation of triglyceride, which has been shown to reduce hepatic insulin sensitivity, as well as
NOT stimulate insulin or leptin (which are both important regulators of energy intake and body adiposity). Stimulated triglyceride synthesis is likely to lead to hepatic accumulation of triglyceride, which has been shown to reduce hepatic insulin sensitivity, as well as increased formation of VLDL particles due to higher substrate availability, increased apoB stability, and higher MTP, the critical factor in VLDL assembly (Basciano et al., 2005).

The insulin receptor number was significantly lower in both skeletal muscle and liver of fructose-fed rats as compared to controls, whereas no difference was observed in the kidney. No significant differences were found in binding affinity. Insulin receptor mRNA levels were determined by slot-blot hybridization with a cRNA probe encoding the 5’end of the rat insulin receptor cDNA. Consistent with binding data, mRNA levels were significantly lower in skeletal muscle and liver of fructose-fed rats as compared to controls (Catena et al., 2003).

An addition to the above, hyperinsulinemia is a central pathophysiological feature of NIDDM and has been shown to play a key role in the disease evaluation and macrovascular complication (Md. Shalam et al., 2006).
NYCTANTHES ARBORTRISTIS

Sans: - Parijata
Eng: - Night Jasmine
Division: - Magnoliophyta
Class: - Magnoliopsida
Order: - Lamiales
Family: - Oleaceae
Scientific Name: - Nyctanthes arbortristis

It is a small tree with its fragrant flower found wild in the forests and distributed wild in sub-Himalayan regions and Karnataka. A well-documented plant. It is a native of India, It is also found in Indian gardens for ornamental purposes Nyctanthe means “Night flowering” and arbortritis means, in the sad tree it loses its brightness during daytime and flowers are highly fragrant, Flower contain an essential oil similar to that of jasmine and which is utilized in perfumery, leaves contain an alkaloidal principles named Nyctanthine; they contain an astringent principle, a resinous substance, colouring matter, suger and a trace of an oily substance

TRADITIONAL USES

The indigenous people of India used in Sidha and Unani systems of medicines to cure various ailments, the better leaves are used in Ayurveda for the treatment of diabetes, rheumatism, sciatica, intestinal worm infection, powered seed are recommended as acure
for scurvy affections of the scalp treatment (Chetty, et al., 2008; Stuppner, et al., 1992), has been reported to possessed hepatoprotective (Paul, et al., 1997), for chronic fever, liver disease, laxative, diaphoretic and diuretic (Saxena, et al., 1987), helminthocide, and a liver and nerve tonic used as antimalarial activities. The plant as whole is used by Santal tribes in central India in snake bite, bite of wild animals, cachexia, sores, cancer, ulcers, menorrhagia and dysentery (Chitrawanshi, et al., 1992).

SCIENTIFICALLY SCREENED PHARMACOLOGICAL ACTION

Antioxidant activity of carotenoids from N. arbortristis in the methanolic extract of flower which are enriched with carotenoids (Dasgupta, et al., 2007). Antioxidant activity N. arbortristis leaf extracts the acetone-soluble fraction of ethyl acetate showed impressive antioxidant activity In vivo (Rathee et al., 2007) Antitrypanosomal potential of 50% ethanolic extract of Nyctanthes arboritis leaves In vitro, vivo studies, significantly prolonged the survival period of the Trypanosoma evansi infected mice (Talakal, et al., 2000). The water soluble portion of the alcoholic extract of the leaves on CNS activities (viz Hypnotic, tranquilizing, local anaesthetics, hypothermic, anticonvulsant) and the Antihistamine and purgative activities (Saxena, et al., 2002). The water soluble portion of the alcoholic extract of N. arbortristis leaves, anti-inflammatory activity in acute inflammatory edema, produced by different Phlogistic agents, viz. Carrageenin, formalin, histamine, 5-Hydroxytryptamine and hyaluronidase in the hindpaw of rats and the acute inflammatory swelling in the knee joint of rats, induced by turpentine oil, acute and chronic phases of formaldehyde induced arthritis were significantly inhibited also Freud’s adjuvant arthritis (Saxena, et al., 1984). The N. arbortristis possesses anti-inflammatory and analgesic activity of ethanolic extract of seeds, arbortristoside-A was found to possess significant and dose-dependent anti-inflammatory and antinociceptive activity. Its seems arbortristoside-A inhibited the
histamine, serotonin and carrageenan-induced edema suggesting its inhibiting effect on carrageenan, arachidonic acid, histamine and serotonin-induced edema suggesting, its anti-inflammatory activity may be due to the inhibiting effect of prostaglandin, histamine and serotonin. The analgesic activity of arboristoside-A may be due to the inhibition of the action of prostaglandin. (Das et al., 2008). Nyctanthes arboristis has strong stimulation of antigen specific and non specific immunity with 50% ethanolic extract of seeds, flowers and leaves (Puri, et al., 1994). The ethanolic extract and fractions of the various parts of the plants Nyctanthes arboristis was shown caecal amoebiasis. The extract from the leaves, seeds, roots, flower and stem of the plants were effective in clearing E histolytica infections. The leaf hexane fraction possessed therapeutic efficacy against caecal amoebiasis of rats (Chitravanshi, et al., 1992). The N. arboristis analgesic, anti-inflammatory and ulcerogenic activity of water soluble portion of an ethanol extract of the leaves was possess antipyretic activity against brewer’s yeast-induced Pyrexia in rats, also produced gastric ulcers (Saxena, et al., 1986). N. arboristis were tested for antileishmanial activity against Leishmania donovani in golden, (Singha, et al., 1992). The plants of alcoholic extract of Nyctanthes arboristis inhibit passive cutaneous anaphylaxis (Gupta, et al., 1993) N. arboristis on tumor necrosis factor alpha (TNF-α) levels in plasma of arthritic and soluble A (SpA)-treated Balb/c mice, shown a consistent depletion of TNF-α from the host plasma (Paul, et al., 1997). The anti-inflammatory activity of the ethanolic of the orange tubular calya of N. arboristis inhibited carrageene induced rat paw edema. (Omkar et al., 2006). The water soluble ethanol extracts from different organs of N. arboristis leaf and fruit extracts in arthritic mice reduced joint homogenate levels of tumor necrosis factor-alpha, interleukin-1beta, and interleukin-6 (Rathore, et al., 2007).
Sedative potential of *N. arbortristis* flowers in rats, the infusion had a moderate dose-dependent conscious sedative activity in male but, surprisingly, not in female rats. Sedation appears to result mainly by antioxidant membrane stabilizing, (Ratnasooriya *et al.*, 2005)

The ethanolic extracts, various fractions of two pure compounds isolated arbortristoside A and arbortristoside C from the *N. arbortristis* plant were tested against Encephalomyocarditis Virus (EMCV) and Semliki Forest Virus (SFV). In addition, ethanolic extracts and n-butanol fraction protected EMCV infected mice SFV (Gupta *et al.*, 2005). Iridoid glucosides shown antileishmanial activity in both *In vitro* (against amastigotes in macrophage cultures) and *In vivo* (in hamsters) (Tandon, *et al.*, 1991). *N. arbortristis* leaf extract in the prevention of lung injury induced by silica particles. Inhalation of silica increased the level of tumor necrosis factor-alpha (TNF-alpha), and of the 66 and 63 kDa peptides in the bronchoalveolar lavage (BAL) fluid in comparison to sham-treated control. Pre-treatment of silica exposed mice with *N. arbortristis* leaf extract significantly prevented the accumulation of TNF-alpha in the BAL fluid, but the 66 and 63 kDa peptides remained unchanged. The extract was also effective in the prevention of silica-induced early fibrogenic reactions like congestion, edema and infiltration of nucleated cells in the interstitial alveolar spaces, and thickening of alveolar septa in mouse lung (Paul, *et al.*, 2002). The methanolic extract of *N. arbortristis* Antistress in open arm in plus maze test, increased exploratory behavior in open field test and increased number of crossings in light dark model. It improved cognitive function with respect to spatial and working memory processes. The treatment with extract ameliorated the stress-induced variations in the biochemical levels of corticosterone, glucose, triglycerides; dopamine, 5-HT and nor epinephrine, the extract exhibited anxiolytic, antistress and nootropic activity. (Deshmukh *et al.*, 2006). *N. arbortristis* leaves extract
against hepato suppression induced by carbon tetrachloride (CCl₄), significantly restored all the serum and liver parameters near to the normal levels (Deshmukh, et al., 2007). Alcoholic and aqueous extracts of the leaves of N. arbortristis protect the liver from toxic effects of carbon tetrachloride showed significant hepatoprotective activity by reducing the elevated levels of biochemical (Hukkeri et al., 2006). An ethanolic extract of N. arbortristis in rats for humoral and cell-mediated immune responses significantly enhanced the circulating antibody titre, when challenged with sheep red blood cells (SRBC) and heat-killed Salmonella antigens confirms the strong immuno-bioactivities in extracts of N. arbortristis (Kannan et al., 2007). The antibacterial activity was evaluated on gram positive (Staphylococcus aureus) and gram-negative (Escherichia coli, Klebsiella Pneumoniae, Pseudomonas aeruginosa) bacteria. The dried leaf flowers fruits, and seeds extract prepared in ethyl acetate and chloroform were used to assess their antibacterial potential in term of zone of inhibition of bacterial growth. These activities of plants parts were due to the presence of various plant secondary metaboliste viz Glycosides and phenolic contents (Priya et al., 2007) Antibacterial activity and cytotoxicity of N. arbortristis flowers shows antibacterial activity against some grame-positive and gram-negative microorganism (Khatune et al., 2001).

ISOLATED ACTIVE CONSTITUENTS

The Seeds of N. arbortristis isolated of two new iridoids designated as arbortristoside A and B. Iridoids in general show a wide spectrum of biological activity.
Arbortristoside A (I), C_{27}H_{34}O_{13}, Mp 226-228^0 [\alpha]_D-90^0 (MeOH; c1.16) was identified as an iridoid glucoside on preliminary examination of its spectroscopic data and chemical reactions (Kozhiparambil et al., 1985)

1. Iridoids reported from the seeds are nycanthoside, 6\beta-hydroxyloganin, and its 6-p-coumaroyl ester. Earlier works reported the isolated of mannitol, \beta-amyrin, \beta-sistosterol hentriacontane, Benzoic acids astragalin, nicotiflorin, oleanolic acids nycanthic acids, friedelin and lupeol from the leaves.
6β-hydroxyloganin 1 and 2

The characterization of mixtures of two new ester of 6β-hydroxyloganin 1 and 2 in *N. arbortristis* leaves (Mathuram *et al.*, 1991)

2. *Nyctanthes arbortritis* leaves shown antitrypanosomal activity (Talakal, *et al.*, 2000) ether due to presence of nyctanthocide or iriodoid glucosides (arbotistosides A, B and C) or 6β-hydroxyloganin which has been identified as active constituents against *Plasmodium* ssp. (Badam *et al.*, 1987) and Leishmania spp (Tandon *et al.*, 1991).

3. Two new iriodoid glycosides, 6,7-di-0-benzoynyticanthoside (1) and 6-O-transcinnamoyl-6β-hydroxyloganin (2) along with the previously reported iriodid 7-O-trans-cinnamoyl-6β-hyrdoxyloganin (3) (Stuppner, *et al.*, 1992)
Two new iridoid glycosides

4. **Isolation of arbortristoside-A**

Arbortristoside-A, C_{27}H_{34}O_{13}, Mp 225–228°C, [α]_D^{25} -92° (MeOH) was identified as an iridoid glucoside on preliminary examination of its chemical reactions (Das, *et al.*, 2008)
A new iridoid glycoside along with the known compounds, nyctanthic acid, oleanolic acid, friedelin, β-sitosterol-glucoside, 6β-hydroxyloganin and arbortristoside A have been isolated from Nyctanthes from seeds.

5. The n-butanol soluble fraction of the seeds of N. arbortristis after column chromatography resulted in the isolation of a new iridoid glucoside (1), arbortristoside A (2) and 6β-hydroxyloganin (3). Compound 1. Was obtained as a white amorphous powder, C_{26}H_{32}O_{13}, Mp 200-202\degree, [α]_D -78\degree (MeOH). Compound 2. C_{27}H_{34}O_{13}, Mp 220-222\degree (Ethanol) [α]_D -92.5\degree (MeOH) was found to be identical to arbortristoside A, Compound 3. C_{17}H_{26}O_{11}, Mp 221-223\degree was obtained as white needles (EtOH-H_2O). (Rathore, et al., 1989).
1) $R^1 = \begin{array}{c}
\text{HO} \\
\text{CH}_2\text{OH}, R^2 = \text{H}
\end{array}$

1a) $R^1 = \begin{array}{c}
\text{HO} \\
\text{CH}_2\text{OAc}, R^2 = \text{Ac}
\end{array}$

1b) $R^1 = \text{H}, R^2 = \text{H}$
India is one of the richest countries in the world in natural resources. In particular the variety of plants species is enormous, so that Indian medicinal plants are very important from the point of view of findinging. Parts of the plant, which grows wild in most developing countries, are used for medicinal and other purposes. *C. gigantea* a stout, hairy tomentose shrub 4-10 ft high with milky juice leaves sessile, thick, glaucous green, 10-12 cm in length, elliptic or obovate oblong clothed beneath with fine cottony tomentum. Flowers 4-6 cm in diameter, not scented. Corolla purplish or white, lobes spreading, coronal scales hairy with two obtuse auricles just below the rounded apex. Follicles 7-10 cm long curved. Seeds with a tuft of silky hair. Three layers of closely packed palisade cells filled with chloroplasts follow the epidermis of the leaf lamina. Multicellular thin walled trichomes are distributed throughout the leaf. A rubiaceous type of stomata is found in the lower epidermis. Frequently met with throughout India as a weed on fallow land and in the waste grounds. All parts of the plants have Alternative...
properties when taken in small doses in the form of tincture (14-28) and power (0.5-1g)
The plant is considered to be crude of Bangladesh and medicinal plants of Indonesia. (Chitme et al., 2005)

TRADITIONAL USES

From ancient times, has been used in several clinical manifestations including loss of memory, defective eyesight, infertility, overall body weakness and incidence of early aging. *Swarnabhasma* has been used by Ayurvedic physicians to treat different diseases like bronchial asthma, rheumatoid arthritis, diabetes mellitus, nervous disorders (Mitra, et al., 2002). The roots of *C. gigantea* have been used in leprosy, eczema, syphilis, elephantiasis, ulceration, anti-diarrhoeal activity and cough in the Indian system of traditional medicine. (Chitme, et al., 2005),

SCIENTIFICALLY SCREENED PHARMACOLOGICAL ACTION

*C. gigantea* an indigenous plant is known to have cardiac action as mentioned by Nadkami in indain Materia Medica. Antipyretic activity *C. gigantea* by using yeast-induced and TAB (Typhoid) vaccine-induced pyrexia in rats and rabbits. In both yeast-induced and TAB vaccine-induced fever, the fever was significantly reduced and the body temperature was normalized. (Chitme, et al., 2005). Anti-diarrhoeal effect of hydroalcoholic (50:50) extract of aerial part of *C. gigantea* was studied there were significant reductions in fecal out put and frequency of droppings when the plant extracts, compared with castor oil treated rats (Chitme et al., 2004). Analgesic activities of *C. gigantea* hydroalcoholic (50:50) extract of aerial part of *C. gigantea* by using Hot-plate test in mice, Tail- flick latent period in rats and Acetic acids-induced response in mice extract produced significantly increased in the latency to response of mice to hot plate thermal stimulation dose dependent (Chitme et al., 2005), the pregnancy interceptive activity *C. gigantea* of ethanolic extract of the roots and its hexane, chloroform, n-
butanol-soluble and n-butanol-insoluble fractions, the ethanolic extract of the roots of C. gigantea exhibited 100% pregnancy interceptive activity in rats, chloroform fraction showed 100% activity, whereas the hexane, n-butanol-soluble and n-butanol-insoluble fractions were found to be inactive at this dose. The active ethanolic extract and its chloroform fraction were devoid of any estrogen agonistic or antagonistic activity at their respective minimum effective contraceptive dose in the ovariectomized immature rat (Srivastava, et al., 2007). The alcoholic extract of the flowers of C. gigantea was shown for its analgesic activity in chemical and thermal models in mice. In acetic acid induced writhing test, in the hot plate method the paw licking time was delayed. (Pathak, et al., 2007) In an experimental animal model, chronic Swarnabhasma-treated animals showed significantly increased superoxide dismutase and catalase activity, two enzymes that reduce free radical concentrations in the body (Mitra, et al., 2002). Alcoholic extract of peeled roots of C. gigantea was tested for analgesic, anticonvulsant, anxiolytic and sedative effect, the prominent analgesic activity was observed in Eddy’s hot plate and acetic acid induced writhings having significant anticonvulsant, antianxiety activity. potentiation in the pentobarbitone-induced sleep due to the sedative (Argal, et al., 2006).

The procoagulant activity of Calotropis gigantea has been studied. The crude latex extract contained many protein, which are highly basic in nature and exhibited strongly proteolytic activity. The crude extract hydrolyses casein, human fibrinogen and crude fibrin clot (Rajesh et al., 2005), repellant activity of the C. gigantea leaf, flower, stem, root extracted by using petroleum ether solvent and repellency test was carried out using glass olfactometer (Arulprakash et al., 2005).

**ISOLATED ACTIVE CONSTITUENTS**

The hexane and methanol soluble extract of the latex coagulum of C. gigantea two new triterpene esters, viz. 3'-methylbutanoates of α-amyrin and ψ-taraxasterol, besides the
known 3'-methylbutanoates of three triterpene alcohols. alkane fraction, total triterpene alcohol fraction, and free, acetyl and 3'-methylbutanoyl triterpene alcohol (Thakur, et al., 1984). The molecular weights of purified calotropain-FI and FII were determined the results obtained from inhibition studies by various enzyme modifying reagents suggest the possible role of cysteine and histidine residues in the active site of both the enzymes. The free and total sulphhydryl contents of both the enzymes were determined by the use of 5,5'-dithio-bis-2-nitrobenzoic acid. Total amino acid compositions of both the enzymes were also determined (Abraham, et al., 1979b). Two proteinase containing carbohydrate, called calotropain-FI and calotropain-FII, were purified from C. gigantea some properties of these enzymes are reported (Abraham et al., 1979a). Isolation and characterization of isorhamnetin-3-O-rutinoside, isorhamnetin-3-O-glucopyranoside and taraxasteryl acetate, a new flavonol trisaccharide was isolated from the aerial parts of C. gigantea, and its structure was established as isorhamnetin-3-O-[2-O-β-D-galactopyranosyl-6-O-α-L-rhamnopyranosyl]-β-D-glucopyranoside by a combination of fast atom bombardment mass spectroscopy, $^1$H and $^{13}$C NMR spectra (Sen, et al., 1992)

\[
\begin{align*}
\text{R} & = \text{Glc} - \text{ORha} \\
1 & = \text{Glc} - \text{ORha} \\
2 & = \text{Glc} - \text{ORha} \\
3 & = \text{Glc} \\
4 & = \text{H}
\end{align*}
\]
Flavonol glycosides

Four new chemical constituents, one naphthalene derivative, named calotropnaphthalene, two terpene derivatives, calotropisesquiterpenol and calotropisesterterpenol and an aromatic product designated as calotropbenzofuran one along with a known compound, sucrose, have been isolated from the roots of the *C. gigantea*. The structures of these chemical constituents have been established as 1-methoxy-4-ethyl naphthalene, 6-(2-methyl-2,3dihydroxypentyl)-11,11-dimethyl cyclohex-8-ene-10-one-7-oic isopentenyl ester, 14-(15,15-dimethyl cyclohexanyl-14,19,25-tricyclo)-3,7,11-trihydroxymethylene-tridecane and 8,15-dihydro benzofuranyl-18-hepta-7,15-dione-16-oic acid, respectively, on the basis of the spectral data analyses and chemical reactions (Gupta *et al.*, 2000). Autodigestion of the cystiene protienases, calotropis D1 and D2 has been studied at pH 7.5 and 37°C in the presence of activating agents (Sengupta *et al.*, 1984). The ionisation of the phenolic hydroxy groups in calotropin DI and DII isolated from the latex of *C. gigantea* has been studied by spectrophotometric titration at 295nm in the pH rang 6-13.2 (Bhattachary *et al.*, 1985). The three-dimensional structure of the sulphydryl protease calotropin I from *C. gigantea* has been determined at 3.2 a resolution (heiseimass *et al.*, 1982)

The cardenolides from the latex and leaves, triterpenoids, anthocyanins from flower and hydrocarbons. The leaves and latex of *Calotropis* found to have cardiac glycosides various glycosides were isolated and studied. An active principle mudarine was isolated from leaves of *Calotropis gigantea*. Beside this, a yellow bitter acid and resin were also found. The cardiac glycosides were identified as calotropogenin (1) calotropin(2),Uscharin (3) and Calotoxin (4), Calactin (5). Three cardenolide glycosides, Coroglaucigenin (6), frugoside (7) 4-O-Beta-D- Glycopyranosyfrugoside were obtained as the cytotoxic Principles of “akond nul” ( roots of *Calotropis gigantea*.L). The
cytotoxic of these compounds against various cell lines of human and mouse origin was tested. They showed similar cell line selectivity to those of cardiac glycosides such as digoxin and ouabain. They are toxic to cell lines of human origin, but not those from mouse at 2 μg/ml. The isolation, crystallization, and properties of Calotropin DI and DII from *Calotropis gigantea*.

Two new oxypregnane-oligoglycosides named Calotroposides A and B have been isolated from the roots of *C. gigantea*, an Indonesian medicinal plant, and their chemical structures have been elucidated by chemical and spectroscopic methods. 12-obenzyllineolon 3-O-beta-D-cymaropyranosyl(1-4)-beta-D-oleandropyranosyl (1-4)beta-D-oleandropyranosyl(1-4)-beta-D-cymaropyranosyl (1-4)-beta-D-cymaropyranoside and 12-O-benzoyl deacetylmplexigenin 3-O-beta-D-cymaropyranosyl(1-4)-beta-Dcymaropyranoside (Ahmed Mueen et al., 2005).
6- Corolaugeogenin, 7-Frutoside, 8-4β-D-glucofrugoside

9-Caotroposide-A, 10-Caotroposide-B
Nomenclature | R
---|---
Isorhamnatin-3-O-rutinoside | Glu-Orha
 | O Gal
Isorhamnatin-3-O-glucoside | Glucose
Isorhamnatin rhimnaoglucoside | Glucose-O-Rhamnose
QUALITATIVE CHEMICAL ANALYSIS

Preliminary Phytochemical Screening

Qualitative chemical analysis of *N. arbortristis* and *C. gigantea* of Petroleum ether, Benzene, Chloroform Ethyl acetate and Methanol extract were carried out by described (Khadelwal *et al.*, 1995).

**Nyctanthes arbortristis**

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**Calotropis gigantea**

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