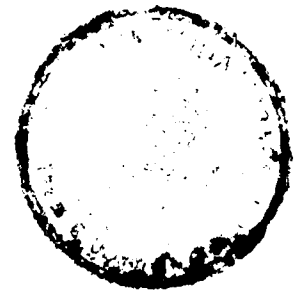
INTRODUCTION:



Diabetes mellitus is a chronic metabolic disorder caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or due to the ineffectiveness of insulin and /or its receptors, which is characterised by hyperglycaemia and disturbances in carbohydrate, protein and fat metabolisms (Alberti and Zimmet, 1998). Due to either deficiency or ineffectiveness of insulin and/or its receptors there is an increased concentration of glucose in the blood, which in turn damages many of the body's systems in particular, the blood vessels and nerves. A world wide survey has reported that, diabetes is affecting nearly 10% of the population (WHO, 2002) and is likely to remain a significant threat to public health in the years to come. The World Health Organisation has estimated that, by the year 2010 diabetes will affect 221 million people worldwide (Amos *et al.*, 1997). As the number of people with diabetes multiplies worldwide, the disease takes an ever-increasing toll on of national and international health care budgets. It is projected to become one of the world's main disablers and killers within the next 25 years. Regions with greatest potential are Asia and Africa, where DM rates could rise to two to three-folds at the present rate. The metabolic disturbances result in acute and long-term diabetic complications, which are responsible for premature death and disability (De Leiva *et al.*, 1995). Several studies have proposed a role for free radicals in the pathogenesis of various diseases including diabetes mellitus (Paolisso *et al.*, 1993). However, an array of non-enzymatic antioxidants [vitamin E, vitamin C and reduced glutathione] and enzymatic antioxidants [superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSHPx)] serve as endogenous defense mechanism that are involved in the protection of free radical induced oxidative

damage. Oxygen free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins and the subsequent oxidative degradation of glycated proteins (Maritim *et al.,* 2003). Recent reports indicate that diabetic complications are associated with overproduction of free radicals and accumulation of lipid peroxidation by-products (Palunduz *et al.,* 2001). Enhanced oxidative stress has been well documented in both experimental and human diabetes mellitus (Baynes, 1991).

Types of Diabetes mellitus:-

Diabetes can be classified as type 1 diabetes (insulin-dependent diabetes mellitus or IDDM) and type 2 diabetes (non-insulin dependent diabetes mellitus or NIDDM). Over 90% of patients have type 2 diabetes and the remainder have type 1 diabetes. Diabetes mellitus type 1 (Type 1 diabetes, IDDM, or juvenile diabetes) is a form of diabetes mellitus that results from autoimmune destruction of insulin-producing beta cells of the pancreas (Cihakova, 2008). The subsequent lack of insulin leads to increased blood and urine glucose. The classical symptoms are polyuria (frequent urination), polydipsia (increased thirst), polyphagia (increased hunger), and weight loss result (Cooke and Plotnic, 2008). Type 1 diabetes was previously known as juvenile diabetes because as it constitutes one of the most frequent chronic diseases in children; however, the majority of new-onset type 1 diabetes is seen in adults. Scientific studies that use antibody testing (glutamic acid decarboxylase antibodies (GADA), islet cell antibodies (ICA), and insulinoma-associated autoantibodies (IA-2)) to distinguish between type 1 and type 2 diabetes, demonstrate that, most new-onset type 1 diabetes is seen in adults. A subtype of type 1 (identifiable by the presence of antibodies against beta cells) typically develops slowly and so is often

confused with type 2. In addition, a small proportion of type 2 cases manifest a genetic form of the disease called maturity onset diabetes of the young (MODY).

Diabetes mellitus type 2 – formerly non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes – is a metabolic disorder that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency (Robbins and Cotran, 7th ed.). Diabetes is often initially managed on exercise regimes and dietary modification. As the condition progresses, medications may be needed. Unlike type 1 diabetes, there is very little tendency toward ketoacidosis though it is not unknown (Brian *et al.,* 2004). One effect that can occur is, nonketonic hyperglycemia. Long-term complication from high blood sugar include an increased risk of heart attacks, strokes, amputation, and kidney failure. There is a strong inheritable genetic connection in type 2 diabetes: having relatives (especially first degree) with type 2 increases risks of developing type 2 diabetes very substantially. In addition, there is also a mutation of the Islet Amyloid Polypeptide gene that results in an earlier onset, more severe, form of diabetes (Sakagashira *et al.,* 1996; Cho *et al.,* 2003). About 55 percent of type 2 diabetic patients are obese at diagnosis (Ebarhart *et al.,* 2004) —chronic obesity leads to increased insulin resistance that can develop into Type 2 diabetes, most likely because adipose tissue (especially that in the abdomen around internal organs) is a (recently identified) source of several chemical signals to other tissues (hormones and cytokines). Other research shows that type 2 diabetes causes obesity as an effect of the changes in metabolism and other deranged cell behavior attendant on insulin resistance (Camastra *et al.,* 1999). However, environmental factors (almost certainly diet and weight) play a large part in the development of Type 2 diabetes in addition to any genetic component. This can be seen from the adoption of the Type 2 epidemiological pattern in those who have

moved to a different environment as compared to the same genetic pool who have not. Immigrants to Western developed countries, for instance, as compared to lower incidence countries of origins (Cotran *et al.,* 1999). There is a stronger inheritance pattern for type 2 diabetes. Those with first-degree relatives with type 2 have a much higher risk of developing type 2, increasing with the number of those relatives. Genes significantly associated with developing type 2 diabetes, include *TCF7L2*, *PPARG*, *FTO*, *KCNJ11*, *NOTCH2*, *WFS1*, *CDKAL1*, *IGF2BP2*, *SLC30A8*, *JAZF1*, and *HHEX* (Lyssenko *et al.,* 2008). *KCNJ11* (potassium inwardly rectifying channel, subfamily J, member 11), encodes the islet ATP-sensitive potassium channel Kir6.2, and *TCF7L2* (transcription factor 7–like 2) regulates proglucagon gene expression and thus the production of glucagon-like peptide-1 (Rother, 2007). Moreover, obesity (which is an independent risk factor for type 2 diabetes) is strongly inherited (Walley *et al.,* 2006).

Although the two types of diabetes have distinct pathogeneses, hyperglycemia and various life-threatening complications resulting from long term hyperglycemia are the most common features. Epidemiological studies and clinical trials strongly support the notion that hyperglycemia is the principal cause of complications. Effective blood glucose control is the key to preventing or reversing diabetic complications and improving quality of life in patients with diabetes. Thus, sustained reductions in hyperglycemia will decrease the risk of developing microvascular complications and most likely reduce the risk of macrovascular complications. The ability of insulin to mediate tissue glucose uptake is a critical step in maintaining glucose homeostasis and in clearing the postprandial glucose load. Patients with type 2 diabetes exhibit a marked reduction in insulin mediated glucose disposal. Although insulin resistance is independently associated with obesity, which exists in 80% of type 2 diabetic patients in the West, it is more severe in obese patients.

Treatment modalities:-

There are several drugs available for type 2 diabetics—most are unsuitable or even dangerous for use by type 1 diabetics. They fall into several classes and are not equivalent, nor can they be simply substituted one for another. One of the now most widely used drugs now used for type 2 diabetes is the biguanide metformin; it works primarily by reducing liver release of blood glucose from glycogen stores and secondarily by provoking some increase in cellular uptake of glucose in body tissues. Both historically, and currently, the most commonly used drugs are in the Sulfonylurea group, of which several members (including glibenclamide and gliclazide) are widely used; these increase glucose stimulated insulin secretion by the pancreas and so lower blood glucose even in the face of insulin resistance. The newer drug classes include: Thiazolidinediones (TZDs) (rosiglitazone, pioglitazone, and troglitazone -- the last, as Rezulin, was withdrawn from the US market because of an increased risk of systemic acidosis). These increase tissue insulin sensitivity by affecting gene expression. α-glucosidase inhibitors (acarbose and miglitol) which interfere with absorption of some glucose containing nutrients, reducing (or at least slowing) the amount of glucose absorbed. Meglitinides which stimulate insulin release (nateglinide, repaglinide, and their analogs) quickly can be taken with food, unlike the sulfonylureas which must be taken prior to food (sometimes some hours before, depending on the drug). Peptide analogs which work in a variety of ways which increase insulin output from the beta cells among other effects. These include the Glucagon-like peptide (GLP) analog exenatide, sometimes referred to as *lizard spit* as it was first identified in Gila monster saliva. Dipeptidyl peptidase-4 (DPP-4) inhibitors increase incretin levels (sitagliptin) by decreasing their deactivation rates. Amylin agonist analog, which slows gastric emptying and suppresses glucagon

(pramlintide). In rare cases, if antidiabetic drugs fail (i.e., the clinical benefit stops), insulin therapy may be necessary – usually in addition to oral medication therapy – to maintain normal or near normal glucose levels (Diabetes report, 2010; Daibetes and Medication report, 2010). Typical total daily dosage of insulin is 0.6 U/kg (Jarvinen *et al.,* 1999). But, of course, best timing and indeed total amounts depend on diet (composition, amount, and timing) as well the degree of insulin resistance. Type 1 is treated with insulin replacement therapy—usually by insulin injection or insulin pump, along with attention to dietary management, typically including carbohydrate tracking, and careful monitoring of blood glucose levels using glucose meters. Today, the most common insulins are biosynthetic products produced using genetic recombination techniques; formerly, cattle or pig insulin were used, and even sometimes insulin from fish (Rother, 2007). Pancreas transplantation and islet cell transplantation are also the recent approaches in treating type 1 diabetes.

Different Diabetopathies:-

Diabetes is preventable and so are its complications. One such micro-vascular complication of diabetes is diabetic nephropathy. Nearly 30% of chronic renal failures in India are due to diabetic nephropathy (Agarwal and Dash, 2000). Nonetheless attention towards diabetic nephropathy is not directed until the patient has progressed towards the stage of renal failure. Nephropathy due to diabetes can be diagnosed very easily and can be prevented. Diabetic nephropathy is clinically defined by the presence of persistent proteinuria of > 500 mg/day in a diabetic patient who has concomitant diabetic retinopathy and hypertension and in the absence of clinical or laboratory evidence of other kidney or renal tract disease. The pathogenesis of diabetic nephropathy is multifactorial and genetic susceptibility has been proposed to

be an important factor in the development and progression of diabetic nephropathy. Two major causative factors have been implicated in the development of diabetic nephropathy: metabolic and hemodynamic. Three major histologic changes occur in the glomeruli under diabetic nephropathy (McCarty and Zimmet, 1994) mesangial expansion directly induced by hyperglycemia, perhaps via increased matrix production or glycosylation of matrix proteins, (Agarwal and Dash, 2000) glomerular basement membrane thickening occurs, (Parving, 1992) glomerular sclerosis caused by intraglomerular hypertension. These different histologic patterns appear to have similar prognostic significance. Diabetes produces qualitative and quantitative changes in the composition of the capillary basement membrane and this altered material undergoes accelerated glycosylation and further rearrangement to form advanced glycosylation end-products (AGE), which stimulate protein synthesis (Doi *et al.,* 1992), further decrease degradability of the basement membrane (Brownlee *et al.*, 1988), increase its permeability (Esposito *et al.,* 1992) and causes endothelial dysfunction (Bucala *et al.,* 1991).

The exact cause of diabetic nephropathy is unknown, but various mechanisms postulated are hyperglycemia (causing hyperfiltration and renal injury) AGE and activation of cytokines. Hyperglycemia increases the expression of transforming growth factor beta (TGFß) in the glomeruli and of matrix proteins specifically stimulated by this cytokine. TGFß may contribute to both the cellular hypertrophy and enhanced collagen synthesis observed in diabetic nephropathy Hyperglycemia also may activate protein kinase C, which may contribute to renal disease and other vascular complications of diabetes. In addition to the renal hemodynamic alterations namely decreased glomerular filtration rate and renal plasma flow, patients with overt diabetic nephropathy (dip stick positive proteinuria and decreasing GFR) develop

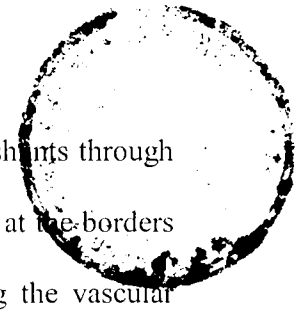
systemic hypertension. Hypertension is an adverse factor in all progressive renal diseases and seems especially so in diabetic nephropathy. The deleterious effects of hypertension are directed at the macro and microvasculature. Familial factors may play a role in the development of diabetic nephropathy. Certain ethnic groups, particularly American blacks, Hispanics, and Native Americans, maybe particularly pre disposed to renal involvement as a complication of diabetes. Some evidence has suggested that polymorphism in the gene for the angiotensin-converting enzyme contributes in either predisposing to nephropathy or accelerating its course. Diabetic neuropathies are a family of nerve disorders caused by diabetes. People with diabetes can, over time, have damage to nerves throughout the body. Neuropathies lead to numbness and sometimes pain and weakness in the hands, arms, feet, and legs. Problems may also occur in every organ system, including the digestive tract, heart, and sex organs. People with diabetes can develop nerve problems at any time, but the longer a person has diabetes, the greater the risk. An estimated 50 percent of those with diabetes have some form of neuropathy, but not all with neuropathy have symptoms. The highest rates of neuropathy are among people who have had the disease for at least 25 years. Diabetic neuropathy also appears to be more common in people who have had problems controlling their blood glucose levels, in those with high levels of blood fat and blood pressure, in overweight people, and in people over the age of 40. The most common type is peripheral neuropathy, also called distal symmetric neuropathy, which affects the arms and legs.

The causes are probably different for different varieties of diabetic neuropathy. Researchers are studying the effect of glucose on nerves to find out exactly how prolonged exposure to high glucose causes neuropathy. Nerve damage is likely due to a combination of factors: like metabolic factors, such as high blood glucose, long

duration of diabetes, possibly low levels of insulin, and abnormal blood fat levels, neurovascular factors, leading to damage to the blood vessels that carry oxygen and nutrients to the nerves, autoimmune factors that cause inflammation in nerves, mechanical injury to nerves, such as carpal tunnel syndrome, inherited traits that increase susceptibility to nerve disease, lifestyle factors such as smoking or alcohol use. Diabetic neuropathies can be classified as peripheral, autonomic, proximal, and focal. Each affects different parts of the body in different ways. Peripheral neuropathy causes either pain or loss of feeling in the toes, feet, legs, hands, and arms. Autonomic neuropathy causes changes in digestion, bowel and bladder function, sexual response, and perspiration. It can also affect the nerves that serve the heart and control blood pressure. Autonomic neuropathy can also cause hypoglycemia (low blood sugar) unawareness, a condition in which people no longer experience the warning signs of hypoglycemia. Proximal neuropathy causes pain in the thighs, hips, or buttocks and leads to weakness in the legs. Focal neuropathy results in the sudden weakness of one nerve, or a group of nerves, causing muscle weakness or pain. Any nerve in the body may be affected.

Ophthalmic complications of diabetes include corneal abnormalities, glaucoma, iris neovascularization, cataracts, and neuropathies. However, the most common and potentially most blinding of these complications is diabetic retinopathy. The exact mechanism by which diabetes causes retinopathy remains unclear, but several theories have been postulated to explain the typical course and history of the disease. Growth hormone appears to play a causative role in the development and progression of diabetic retinopathy. It was noted that diabetic retinopathy was reversed in women who had postpartum hemorrhagic necrosis of the pituitary gland (Sheehan syndrome). This led to the controversial practice of pituitary ablation to treat or prevent diabetic

retinopathy in the 1950s. The variety of hematologic abnormalities seen in diabetes, such as increased erythrocyte aggregation, decreased RBC deformability, increased platelet aggregation and adhesion predisposition to sluggish circulation, endothelial damage, and focal capillary occlusion. This leads to retinal ischemia, which, in turn, contributes to the development of diabetic retinopathy. Fundamentally, diabetes mellitus (DM) causes abnormal glucose metabolism as a result of decreased levels or activity of insulin. Increased levels of blood glucose are thought to have a structural and physiologic effect on retinal capillaries causing them to be both functionally and anatomically incompetent. A persistent increase in blood glucose levels shunts excess glucose into the aldose reductase pathway in certain tissues, which converts sugars into alcohol (eg, glucose into sorbitol, galactose to dulcitol). Intramural pericytes of retinal capillaries seem to be affected by this increased level of sorbitol, eventually leading to the loss of its primary function (ie, autoregulation of retinal capillaries). Loss of function of pericytes results in weakness and eventual saccular outpouching of capillary walls. These microaneurysms are the earliest detectable signs of DM retinopathy. Increased permeability of these vessels results in leakage of fluid and proteinaceous material, which clinically appears as retinal thickening and exudates. If the swelling and exudation would happen to involve the macula, a diminution in central vision may be experienced. Macular edema is the most common cause of vision loss in patients with nonproliferative diabetic retinopathy (NPDR). More extensive retinal hypoxia triggers compensatory mechanisms within the eye to provide enough oxygen to tissues. Venous caliber abnormalities, such as venous beading, loops, and dilation, signify increasing hypoxia and almost always are seen bordering the areas of capillary nonperfusion. Intraretinal microvascular abnormalities (IRMA) represent either new vessel growth or remodeling of preexisting vessels



through endothelial cell proliferation within the retinal tissues acts as shunts through areas of nonperfusion. Neovascularization most commonly is observed at the borders of perfused and nonperfused retina and most commonly occur along the vascular arcades and at the optic nerve head. The new vessels break through and grow along the surface of the retina and into the scaffold of the posterior hyaloid face. By themselves, these vessels rarely cause visual compromise. However, they are fragile and highly permeable. These delicate vessels are disrupted easily by vitreous traction, which leads to hemorrhage into the vitreous cavity or the preretinal space. These new blood vessels initially are associated with a small amount of fibroglial tissue formation. However, as the density of the neovascular frond increases, so does the degree of fibrous tissue formation. In later stages, the vessels may regress leaving only networks of avascular fibrous tissue adherent to both the retina and the posterior hyaloid face. As the vitreous contracts, it may exert tractional forces on the retina via these fibroglial connections. Traction may cause retinal edema, retinal heterotropia, and both tractional retinal detachments and retinal tear formation with subsequent detachment.

Various animal models of diabetes:-

Different *in vivo* animal models of type 1 and type 2 diabetes mellitus have been developed either by pharmacologic, surgical or genetic manipulations in several animal species.

The most common animal models for type 1 diabetes are developed by

(i) **Pharmacologic induction:** By administration of certain the diabetogenic drugs / agents like Alloxan and Streptozotocin for type 1 diabetes. Streptozotocin (STZ, 69%) and alloxan (31%) have been useful for the study of multiple aspects/ manifestations of type 1 diabetes mellitus when administered intravenous,

intraperitoneally or sub-cutaneously. The cytotoxic action of these diabetogenic agents is mediated by generation of reactive oxygen species, but both drugs differ in their mechanism of action (Federiuk *et al.,* 2004; Lei *et al.,* 2005). Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide with a simultaneous massive increase in cytosolic calcium concentration, which causes rapid destruction of pancreatic β-cells (Szudelski, 2001). The range of dosage of alloxan is quite narrow and even light overdosing is generally toxic and may cause lethality to many animals. This lethality is likely to stem from kidney tubular cell necrotic toxicity (Lenzen *et al.,* 1996). The most frequently used intravenous dose of alloxan in rats is 65 mg/kg, but when it is administered intraperitoneally (i.p.) or subcutaneously, its effective dose is higher (Federiuk *et al.,* 2004). Streptozotocin is a nitrosourea derivative isolated from *Streptomyces achromogenes* with broad-spectrum antibiotic and anti-neoplastic activity. Streptozotocin enters the pancreatic β-cell via a glucose transporter-GLUT2 and causes alkylation of deoxyribonucleic acid (DNA). Furthermore, STZ induces activation of poly adenosine diphosphate ribosylation and nitric oxide release. As a result of STZ action, pancreatic β-cells are destroyed by necrosis (Mythili *et al.,* 2004). In adult rats, 60 mg/kg is the most common dose of STZ to induce insulin dependent diabetes (Patel *et al.*, 2006). Vacor, dithizone (diphenylthiocarbazone), and 8- hydroxyquinolone may also cause experimental diabetes, but their use in research is restricted due to their level of toxicity (Clark *et al.,* 1994). In general, by using these models of diabetes induced by chemical drugs, the majority of published studies report the amount of reduction of blood glucose that is always evaluated after a period of fasting following acute or chronic treatment with a specific natural product.

(ii) **Spontaneous animal models**: The non-obese diabetic (NOD) mouse and bio breeding (BB) rat are the two most commonly used animals that spontaneously develop diseases with similarities to human Type 1 diabetes. These animals are inbred in laboratories for many generations, by selecting for hyperglycaemia. The NOD mouse was developed by selectively breeding offspring from a laboratory strain that was first used in the study of cataract development (the JcI-ICR mouse) (Makino *et al.,* 1980). Insulitis is present when the mice are 4–5 weeks old, followed by subclinical β-cell destruction and decreasing circulating insulin concentrations. Inbred animals such as the NOD mouse also have benefits when studying other features of diabetes as, genetic heterogeneity need not be considered as a confounding factor. The BB rat was first recognized in the Bio Breeding Laboratories, a commercial breeding company based in Ottawa, in 1974 (Nakhooda *et al.,* 1977). In diabetes prone strains, weight loss, polyuria, polydipsia, hyperglycaemia and insulinopenia develop at around 12 week of age, often at the time of puberty. In common with the human disease, ketoacidosis is severe and fatal unless exogenous insulin is administered (Mordes *et al.,* 2001). As with the NOD mouse, the pancreatic islets are subjected to an immune attack with T cells, B cells, macrophages and natural killer cells being recruited to the insulitis (Bone *et al.,* 1999; Yoon *et al.,* 1998; Rabinovicz *et al.,* 1996; Lohr *et al.,* 1989; Lally *et al.,* 2001). A variety of auto-antibodies, including GAD, have been reported in both BB rats and the NOD mouse, although it remains far from clear which, if any, of these are primary auto-antigens (Mackay *et al.,* 1996; Yoon *et al.,* 1999). Other prone strains to type 1 diabetes mellitus include New Zealand white rabbit, Kreesbond dog, Chinese hamster and Celebes black ape. However, they have not been employed in studies to evaluate natural products to treat diabetes, except in preclinical trials of exenatide (incretin analog) (Rees and Alcolado, 2005).

Different animal models for type 2 diabetes:

Animal models of Type 2 diabetes are likely to be as complex and heterogeneous as the human condition. In some animals, insulin resistance predominates, whilst in others β -cell failure is pre-eminent. Models where glucose intolerance is part of a wider phenotype of obesity, dyslipidaemia and hypertension may also provide valuable insights into human Type 2 diabetes.

Spontaneously diabetic animals This model of type 2 diabetes may be obtained from the animals with one or several genetic mutations transmitted from generation to generation. (*e.g.*, *ob/ob*, *db/db* mice) or by selected from non-diabetic outbred animals by repeated breeding over several generation [*e.g.*, (GK) rat, Tsumara Suzuki Obese Diabetes (TSOD) mouse]. These animals generally have inherited diabetes either as single or multigene defects.

(i) ob/ob mouse: *ob/ob* mouse (obese mouse) (now relabeled as Lep^{ob}) is inherited as (monogenic) autosomal recessive mutation on chromosome 6 (obese) in C57BL/6J mouse strain, originating from the Bar Harbor, Jackson laboratory (Shafrir, 2003). The mutation in *ob/ob* mice is now identified in leptin gene, which encodes for leptin. It is characterized by diabetes like syndrome of hyperglycemia, mild impaired glucose tolerance, severe hyperinsulinemia, sub fertility and impaired wound healing.

(ii) db/db mouse: The *db/db* (diabetic) mouse (now relabeled as $lepr^{db}$) is originally derived from an autosomal recessive mutation on chromosome 4 in mice of C57BL/KsJ strain originating from Bar Harbor, Maine (Shafrir, 2003). The mutation in this diabetic animal was traced to *db* gene, which encodes for the leptin receptors. These mice are spontaneously hyperphagic insulin over-secretors becoming obese, hyperglycaemic, hyperinsulinemic and insulin resistant within the first month of age

and develop hypoinsulinemia and hyperglycaemia later with a peak between 3-4 months of age. Animals then exhibit ketosis and progressive body weight loss.

(iii) KK mouse: KK (Kuo Kondo) mouse is polygenic model of obesity and type 2 diabetes produced by selective inbreeding for large body size in Japan, also named as Japanese KK mouse (Mcintosh and Pederson, 1999; Velasquez *et al.,* 1990). These animals are hyperphagic, hyperinsulinaemic, insulin resistant and show moderate obesity by 2 months of age, and maximal by 4-5 months. Insulin resistance precedes the onset of obesity. The increase in pancreatic insulin content is associated with increase in number and size of pancreatic islets but histologically degranulation of beta cells and hypertrophy of islets are found [24, 25].

(iv) KK/Ay mouse: KK/Ay mouse (also known as Yellow KK obese mouse) carries both lethal yellow obese (Ay) and diabetic gene unlike KK mouse, which carries only diabetic gene. Mice homozygous for the yellow spontaneous mutation (Ay) die before implantation or shortly thereafter. KK/Ay mouse is heterozygous which shows severe obesity, hyperglycaemia, hyperinsulinemia and glucose intolerance by 8 wk of age.

(v) New Zealand Obese (NZO) mouse: The NZO strain is a polygenic model of obesity and diabetes obtained by selective inbreeding over several generation with the parents selected for their agouti coat color. It exhibits a polygenic syndrome of hyperphagia, obesity, mild hyperglycaemia, hyperinsulinemia, impaired glucose tolerance and insulin resistance. Body weight rises rapidly during first 2 months of life, due to hyperphagia. Hyperleptinemia and leptin resistance which may account for hyperphagia, have been reported in NZO mice (McNeill, 1999).

(vi) NONcNZO10 mouse: It is a recombinant congenic new mouse strain model of type 2 diabetes developed by introgressing 5 genomic intervals containing NZO/H1Lt (NZO) diabetogenic quantitative trait loci onto the non obese non diabetic (NON/Lt or

NON) genetic background at Jackson laboratory, Maine (Haskell *et al.,* 2002). Whereas parental NZO males exhibit the unwanted phenotypes of hyperphagia, morbid obesity, poor fertility, and a variable frequency of hyperglycaemia, NONcNZO10 males are not hyperphagic, develop more moderate level of obesity, and reproduce normally.

(vii) TSOD mouse: By selective breeding of obese male mice of ddY strain, Tsumara and Suzuki described the two inbred strains, one with obesity with increase in urinary glucose named TSOD (Tsumara Suzuki Obese Diabetes) and other without them (TSNO, Tsumara Suzuki Non Obese). TSOD mouse is of polygenic origin and characterized by polydipsia and polyuria at about 2 months old only in male mice followed by hyperglycaemia and hyperinsulinemia.

(viii) M16 mouse: M16 mouse is a new model for obesity and type 2 diabetes which results from long-term selection for 3 to 6 wk weight gain from an Institute of Cancer Research, London, UK (ICR) base population. M16 mice exhibit early onset of obesity and are larger at all ages characterized by increased body fat percentage, fat cell size, fat cell numbers, and organ weights. These mice also exhibit hyperphagia, accompanied by moderate obesity, and are hyperinsulinemic, hyperleptinemic and hypercholesterolemic relative to ICR.

(ix) Zucker fatty rat: The spontaneous mutation 'obese' (fatty) was found in the rat stock of Sherman and Merck, by Zucker, Harriet Bird Memorial Laboratory, Stow, Manssachusetts, USA in 1961. The Zucker (*fa/fa*) fatty or obese rat (now labeled as $Lepr^{fa}$) results from the simple autosomal recessive (*fa*) gene on chromosome 5. It is characterized by hyperphagia and early onset of obesity which appear at 4 wk of age along with increased growth of subcutaneous fat depot. It is also associated with mild hyperglycaemia, insulin resistance, mild glucose intolerance, hyperlipidemia,

hyperinsulinemia and moderate hypertension (Shafrir, 2003; Durham and Truett, 2006). The hyperphagia seen in this rat has been attributed to hypothalamic defect in leptin receptor signalling (McNeill, 1999).

Dietary or nutrition induced type 2 diabetic models:

C57BL/6J mouse: Type 2 diabetic model by simply feeding high fat feed to non obese, non diabetic C57BL/6J mouse strain was initially developed in Japan and is now available at Jackson laboratory, Bar Harbor. It is characterized by marked obesity, hyperinsulinemia, insulin resistance and glucose intolerance (Surwit *et al.,* 1998). In addition, they exhibit marked fasting as well as basal hyperglycaemia in contrast to normal basal glucose level seen in C57BL/6J (ob/ob) mice. These mice are demonstrated to develop peripheral leptin resistance. They manifest most of the characteristic features of the patients with genetic predisposition to develop type 2 diabetes when they become obese. This animal model represents both genetic and environmental risk factors in contrast to C57BL/6J (*ob/ob*) mouse in which onset of symptoms is highly genetically determined.

Surgical type 2 diabetic models:

This method consists of complete or partial pancreatectomy in animals used for the induction of type 1 or type 2 diabetes, respectively. *Non obese partial pancreatectomized diabetic animals*: Partial pancreatectomy in animals performed as 70 or 90 per cent (usually 90%) dissection of pancreas has been reported in various animal species mostly in dogs, pigs, rabbit and also rats (McNeill, 1999, Camacho *et al.,* 1983; Sasaki *et al.*, 2000). It does not cause severe form of diabetes and is characterized by moderate hyperglycaemia with neither reduction in body weight nor reduction in plasma insulin levels. The 90 per cent partially pancreatectomized rats also show defect or selective impairment to glucose stimulated insulin release but

remain intact to other insulin secretogogues. Recently, yet another model on stable form of type 2 diabetes has been produced by combination of 50 per cent partial pancreatectomy along with NAD (350-mg/kg) and STZ (200 mg/ kg) treatment in BALB/c mice (Kurup and Bhonde, 2000).

***In-vitro* studies on insulin secretion:**

(i) Studies using isolated pancreatic islet cell lines:

Several *in vitro* assays are available to study different steps of insulin secretion. It is known that insulin secretion occurs when pancreatic β-cells utilize glucose to generate adenosine triphosphate (ATP) from adenosine diphosphate (ADP) (Affourtit and Brand, 2006). The resulting increase in cytoplasmic ATP/ADP ratio closes ATP-sensitive potassium channels, causing depolarization of the plasma membrane, which activates voltage-dependent Ca2+ channels. This results in elevation of the intracellular Ca2+ concentration which triggers insulin secretion (Ashcroft and Rorsman, 2004). In type 2 diabetes, pancreatic β-cells exhibit atypical ion channel activity and an abnormal pattern of insulin secretion (Ashcroft and Rorsman, 2004). These pathways can be studied with isolated pancreatic β-cells from either control or diabetic rat or mouse that can be obtained by collagenase digestion technique, followed by adequate separation and transference to appropriated culture medium (Zhao *et al.,* 2005a; Storling *et al.,* 2005). Afterwards, the experimental protocol is assayed.

(ii) Studies using insulin-secreting cell lines:

Bioengineered technologies have provided new opportunities to improve and establish more appropriate cultured cell lines to help to facilitate studies of mechanisms of both insulin secretion and β-cell dysfunction, being also the target for the study of natural products. The most widely used insulin-secreting cell lines are RIN, HIT, beta-TC,

MIN6 and INS-1 cells (Poitout *et al.,* 1996). These cell lines release mainly insulin and small amounts of glucagon and somatostatin. Although the behaviour of none of these cell lines perfectly mimics primary β-cell physiology, they are extremely valuable tools for the study of molecular events underlying β-cell function (Poitout *et al.,* 1996).

(iii) *In vitro* studies on glucose uptake:

Adipose tissue is considered a key link between obesity and type 2 diabetes by promoting the development of lipotoxicity, i.e. cell damage as a consequence of elevated intracellular lipid concentrations and insulin resistance (Lelliott and Vidal-Puig, 2004). Insulin resistance either at the adipocyte or skeletal muscle levels contribute to hyperglycemia. However, adipocytes from different sites of the body may have different biological or pathological effects. Pathways related to insulin resistance may be studied in cell lines of adipocytes such as murine 3T3-L1 cells (Karalee *et al.,* 2001) and rat L6 muscle engineered to over-express GLUT4 (Maddux *et al.,* 2001) and may be employed as tools to evaluate the effects of natural products on glucose uptake.

In spite of the worldwide use of herbs and medicinal plants, the effective treatment of diabetes with phytochemicals has not been validated with scientific criteria which may support their substitution for the current therapy. Although some studies have been published with raw natural products they have neither shed light on the mechanisms of action of these products nor have they shown a potential to be employed as new therapeutic drugs. This implies that several models are necessary to called for, in addition to the demonstration that a putative natural product exerts antihyperglycemic activity. Thus, by focusing on other targets of pancreatic islet cell dysfunction, new models may help to elucidate effects of medicinal plants employed in the treatment of diabetes mellitus.

Insulin Signalling:-

Insulin is the most potent anabolic hormone known and is essential for appropriate tissue development, growth, and maintenance of whole-body glucose homeostasis. This hormone is secreted by the β cells of the pancreatic islets of Langerhans in response to increased circulating levels of glucose and amino acids after a meal. Insulin regulates glucose homeostasis at many sites, reducing hepatic glucose output (via decreased gluconeogenesis and glycogenolysis) and increasing the rate of glucose uptake, primarily into striated muscle and adipose tissue. In muscle and fat cells, the clearance of circulating glucose depends on the insulin-stimulated translocation of the glucose transporter GLUT4 isoform to the cell surface (see Shulman, 2000). Insulin also profoundly affects lipid metabolism, increasing lipid synthesis in liver and fat cells, and attenuating fatty acid release from triglycerides in fat and muscle. Insulin resistance occurs when normal circulating concentrations of the hormone are insufficient to regulate these processes appropriately. Thus, by definition, insulin resistance is a defect in signal transduction. The signalling mechanisms involved in the various biologic responses to insulin remain somewhat elusive, but recent progress has shed light on a few pathways that are critical for its regulation of glucose and lipid metabolism. Although insulin affects such diverse processes as cellular growth, differentiation, apoptosis, and lipid, protein, and glucose synthesis and breakdown, we focus here on the regulation of glucose transport as the rate-limiting step in glucose utilization and storage.

The insulin receptor

Insulin action is initiated through the binding to and activation of its cell-surface receptor, which consists of two α subunits and two β subunits that are disulfide linked into an $\alpha_2\beta_2$ heterotetrameric complex. Insulin binds to the extracellular α subunits, transmitting a signal across the plasma membrane that activates the intracellular tyrosine kinase domain of the β subunit. The receptor then undergoes a series of intra-molecular transphosphorylation reactions in which one β subunit phosphorylates its adjacent partner on specific tyrosine residues. Some evidence suggests that different tyrosine residues account for distinct functions. For example, phosphorylation of COOH-terminal tyrosines mediates the mitogenic actions of insulin. The phosphorylated tyrosines in the juxtamembrane domain may participate in substrate binding, whereas those found within the kinase domain regulate the catalytic activity of the insulin receptor β subunit.

Some forms of insulin resistance may involve the receptor itself. Alterations in insulin receptor expression, binding, phosphorylation state, and/or kinase activity could account for many insulin- resistance phenotypes. In addition, it is possible that the selected blockade of distinct phosphorylation sites selectively inhibits certain actions of insulin. In this regard, individuals have been identified with rare genetic defects in the insulin receptor that influence expression, ligand binding, and tyrosine kinase activity. These patients demonstrate severe insulin resistance; manifest as clinically diverse syndromes including the type A syndrome, leprechaunism, Rabson-Mendenhall syndrome, and lipoatropic diabetes (Taylor and Arioglu, 1998; Krook and O'Rahilly, 1996).

The mode of inheritance found in families afflicted with insulin receptor mutations offers insight into insulin receptor function. Most individuals with severe familial

insulin resistance carry lesions in both insulin receptor (*INSR*) alleles, either as homozygotes or compound heterozygotes. In these individuals, the entire cellular complement of the insulin receptor is defective. However, in several reported cases of the type A syndrome of insulin resistance (characterized by polycystic ovarian disease, signs of virilization, acanthosis nigricans, and enhanced growth rate), affected individuals were apparently simple heterozygotes with only one defective allele. The substantial loss of insulin receptor function in these patients cannot be explained by a 50% decrease in insulin receptor levels, as such a reduction in the level of wild type receptor would not be expected to adversely influence insulin action.

Several mechanisms could account for the greater than expected degree of insulin resistance in these individuals. First, because the insulin receptor precursor can form hybrids, the mutant receptor might function in a dominant-interfering manner, inhibiting the function of the normal allele. However, an interesting alternative model has emerged from the study of *Insr* knockout mice. The developmental characteristics of homozygous insulin receptor null mice are different from those of the compound receptor mutations in humans, and these mice die shortly after birth owing to extreme insulin resistance (Accili *et al.*, 1996; Joshi *et al.*, 1996). Heterozygous mice, carrying only one disrupted *Insr* allele are phenotypically normal, with no apparent defects in insulin signaling. Similarly, heterozygous knockout mice lacking a single allele of the gene for the insulin receptor substrate protein IRS1 lack any significant phenotype, whereas homozygous disruption of the *IRS1* gene results in a mild form of insulin resistance (Tamemoto *et al.*, 1994; Araki *et al.*, 1994). $IRS1^{-/-}$ mice do not become diabetic, presumably owing to pancreatic β–cell compensation. Nevertheless, mice that are doubly heterozygous for these null alleles ($Insr^{-/+}$ $IRS1^{-/+}$) develop both insulin resistance and diabetes (Bruning *et al.*, 1997), indicating that development of

diabetes can be a polygenic, multihit process. At least in mice, then, mild defects in several genes can generate insulin resistance and diabetes. Although defects in the *INSR* gene are too rare in the general population to account for garden-variety insulin resistance, the possibility remains that a reduction in insulin receptor levels, which by itself has no effect, can interact with other downstream alterations to generate insulin resistance. In either case, these data strongly argue for a postinsulin receptor defect(s) as a primary cause leading to peripheral insulin resistance.

In addition to tyrosine autophosphorylation, the insulin receptor is also subjected to β-subunit serine/threonine phosphorylation. Data from some experimental models suggest that this modification allows receptor function to be attenuated. Thus, in vitro studies show that the tyrosine kinase activity of the insulin receptor decreases as a consequence of serine/threonine phosphorylation. The chronic elevation in insulin levels that occurs as a result of insulin resistance might stimulate the relevant serine kinases, perhaps through the IGF-1 receptor, which can also be stimulated by high concentrations of insulin. Such an interaction could provide a mechanism for a vicious cycle of insulin-induced insulin resistance. Similarly, counter-regulatory hormones and cytokines can activate serine kinases, particularly protein kinase C (PKC), which has been implicated in the development of peripheral insulin resistance. Several PKC isoforms are chronically activated in human and rodent models of insulin resistance (Ishizuka *et al.,* 1998; Considine *et al.,* 1995; Avignon *et al.*, 1996). These kinases can catalyze the serine or threonine phosphorylation of the insulin receptor or its substrates. Pharmacologic inhibition of PKC activity or reduction in PKC expression enhances insulin sensitivity and insulin receptor tyrosine kinase activity (Donnelly and Qu, 1998).

A number of protein tyrosine phosphatases (PTPases) have also been described that can dephosphorylate the insulin receptor, reducing its kinase activity and thereby attenuating insulin action. Two PTPases have been implicated in the negative regulation of the insulin receptor, PTP1B and LAR. Elevated expression of each these phosphatases has been reported in insulin-resistant patients (Goldstein *et al.,* 1998; Drake and Posner, 1998). In cultured systems, increased expression of these enzymes prevents insulin receptor kinase activation and insulin signaling. More recently, a *PTP1B* mouse knockout resulted in enhanced insulin sensitivity, suggesting that the regulation of PTP1B function could represent an important target for insulin-sensitizing agents (Elchebly *et al.,* 1999).

Proximal insulin receptor signalling events

Once activated, the insulin receptor phosphorylates a number of important proximal substrates on tyrosine, including members of the insulin receptor substrate family (IRS1/2/3/4), the Shc adapter protein isoforms, SIRP family members, Gab-1, Cbl, and APS. Tyrosine phosphorylation of the IRS proteins creates recognition sites for additional effector molecules containing Src homology 2 (SH2) domains. These include the small adapter proteins Grb2 and Nck, the SHP2 protein tyrosine phosphatase and, most importantly, the regulatory subunit of the type 1A phosphatidylinositol 3–kinase (PI 3-kinase).

Critical physiologic functions for both IRS1 and IRS2 have recently been established. As already described here, homozygous *IRS1* knockout mice develop a mild state of insulin resistance (Tamemoto *et al.,* 1994; Araki *et al.,* 1994) but do not become diabetic, presumably owing to β-cell compensation. On the other hand, homozygous disruption of the *IRS2* gene results in impaired insulin secretion, in addition to

peripheral insulin resistance and diabetes (Withers *et al.,* 1998). Given that skeletal muscle IRS2 does not appear to be necessary for insulin- or exercise-stimulated glucose transport, the insulin resistance observed in the *IRS2* knockout animals most likely reflects secondary events occurring as a consequence of alterations in β-cell function or survival (Higaki *et al.,* 1999). This finding is consistent with recent studies on β cell–specific insulin receptor knockout mice. These animals develop both peripheral insulin resistance and diabetes, presumably due to alterations in the normal pattern of insulin secretion (Kulkarni *et al.,* 1999).

Downstream signalling events

At present, only one downstream signalling molecule is unequivocally essential for insulin-stimulated GLUT4 translocation, the type 1A PI 3-kinase. Multiple studies using various pharmacologic inhibitors, microinjection of blocking antibodies, and expression of dominant-interfering and constitutively active mutants are all consistent with a necessary role for PI 3-kinase activity in insulin-stimulated glucose uptake and GLUT4 translocation (Czech and Corvera, 1999). Several studies have suggested that, the interaction of IRS with PI 3-kinase is necessary for the appropriate activation and/or targeting of the enzyme to a critical intracellular site, perhaps including its association with GLUT4 vesicles. However, expression of the dominantly interfering IRS PH and PTB domains completely prevents insulin-stimulated IRS tyrosine phosphorylation and DNA synthesis but have no significant effect on GLUT4 translocation.

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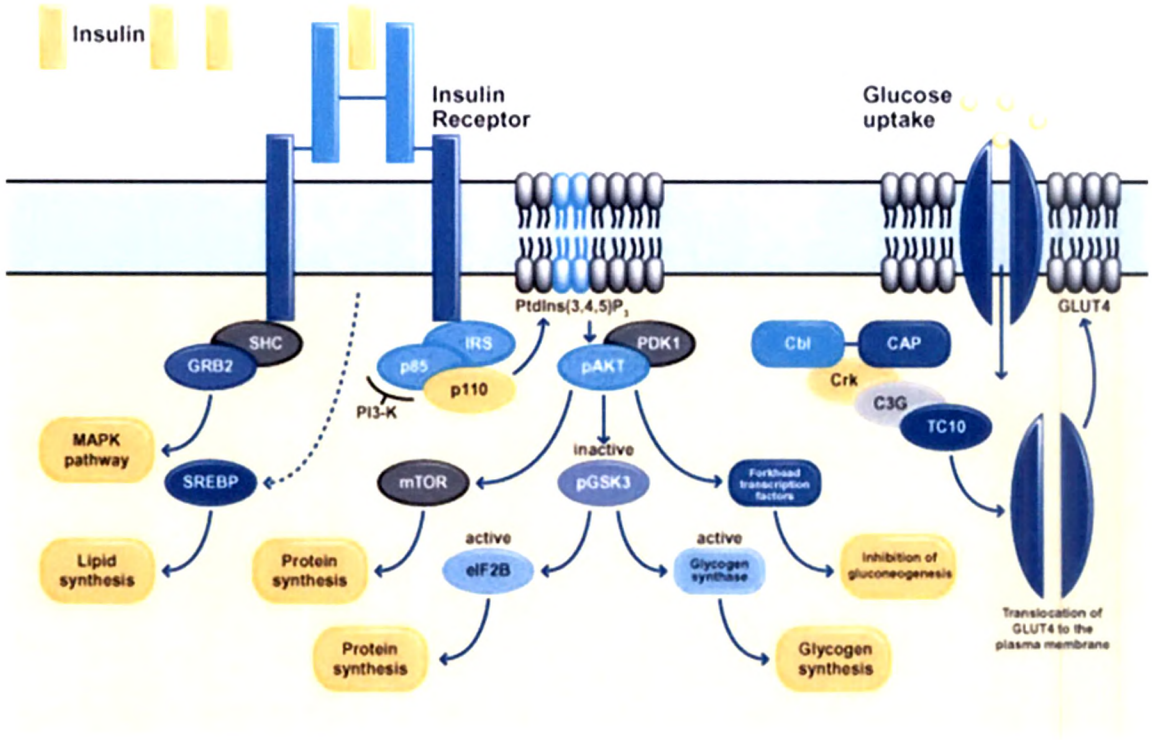


The targets of PI 3-kinase action are likewise controversial. Two classes of serine/threonine kinases are known to act downstream of PI 3-kinase, namely the serine/threonine kinase Akt, also known as protein kinase B (PKB), and the atypical protein kinase C isoforms ζ and λ (PKCζ/λ). Stable expression of a constitutively active, membrane-bound form of Akt in 3T3L1 adipocytes results in increased glucose transport and persistent localization of GLUT4 to the plasma membrane (Kohn *et al.,* 1996; 1998). Conversely, expression of a dominant-interfering Akt mutant inhibits insulin-stimulated GLUT4 translocation (Cong *et al.,* 1997; Wang *et al.,* 1999). Similarly, PKCζ is also activated by the formation polyphosphoinositides, which accumulate in insulin-treated cells; PKCζ is therefore also sensitive to pharmacologic PI 3-kinase inhibitors, such as wortmannin (Bandyopadhyay *et al.,*

(Kotani *et al.,* 1998; Kitamura *et al.,* 1998). Thus, although PI 3-kinase activation is essential, the protein kinase targets that mediate the effects of this pathway remain uncertain.

Several investigators have examined the role of Akt and PI 3-kinase in the regulation of peripheral insulin sensitivity. There appears to be a relative decrease in insulin-stimulated association of IRS proteins with PI 3-kinase and activation of Akt in insulin-resistant skeletal muscle (Krook *et al.,* 1998; Cusi *et al.,* 2000). Surprisingly however, patients with reduced insulin-stimulated PI 3-kinase maintain normal activation of Akt (Kim *et al.,* 1999). Even though these studies involved a small number of patients, the data suggest that PI 3-kinase is in substantial excess, with only a relatively small activation necessary for the full expression of downstream signaling. These data further imply that, defects in the pathway leading from IRS tyrosine phosphorylation to Akt activation may not be responsible for insulin resistance in patients with type II diabetes. Clearly, additional studies of Akt and/or PKCζ/λ activation and localization are required in both animal models and more insulin-resistant patient populations. Although PI 3-kinase activity is clearly necessary for insulin-stimulated glucose uptake, additional signals are also required for the stimulation of GLUT4 translocation. Thus, activation of PI 3-kinase by stimulation with IL-4 or by engagement of certain integrins does not induce GLUT4 translocation (Isakoff *et al.,* 1995; Guilherme *et al.,* 1998). Furthermore, two natural insulin receptor mutations that were fully capable of activating PI 3-kinase nevertheless failed to induce GLUT4 translocation and glucose uptake (Krook *et al.,* 1997). The stimulation of endogenous PI 3-kinase activity must be distinguished from the overexpression of a constitutively active PI 3-kinase. Under the latter conditions, there are massive increases in polyphosphoinositide formation and serine/threonine

protein phosphorylation, as well as marked changes in cellular morphology. Although this treatment can also induce GLUT4 translocation, it is questionable whether it does so through the normal insulin regulatory pathways or through a less-specific stress response. The most compelling evidence for a required additional PI 3-kinase–independent pathway makes use of a cell-permeable analog of PI (3, 4, 5) P3 (Jiang *et al.,* 1998). In these experiments, addition of the PI (3, 4, 5) P3 analog had no effect on GLUT4 translocation. As expected, treatment of cells with wortmannin prevented insulin-stimulated translocation of GLUT4. However, treatment of adipocytes with wortmannin, insulin plus the PI (3, 4, 5) P3 analog, resulted in enhanced glucose uptake. These data suggest that although the PI 3-kinase pathway is necessary, there is at least one additional pathway that is independent of PI 3-kinase activation.

Recent studies have shown that insulin can also rapidly induce tyrosine phosphorylation of the Cbl proto-oncoprotein, but only in insulin-responsive cells (Ribon and Saltiel, 1997). This phosphorylation requires the presence of the adapter protein CAP, which associates with a proline-rich domain in Cbl through its COOH-terminal SH3 domain. CAP appears to be important in insulin signaling, as it is markedly induced during adipocyte differentiation and is transcriptionally regulated by the thiazolidinedione family of insulin-sensitizing PPARγ agonists (Ribon *et al.,* 1998). In support of this hypothesis, it has been recently observed that expression of a dominant-interfering CAP mutant (CAPΔSH3) completely inhibited insulin-stimulated glucose uptake and GLUT4 translocation. This occurred through a marked reduction in the localization of tyrosine-phosphorylated Cbl in the plasma membrane lipid raft subdomain that is enriched in caveolin. Together, these data suggest that the insulin-dependent tyrosine phosphorylation and/or compartmentalization of CAP/Cbl

complex may provide a necessary second signal that functions in parallel with the activation of the PI 3-kinase–dependent signaling pathway.

GLUT4 vesicle trafficking, docking, and fusion

The mechanisms by which upstream signaling pathways converge on the intracellular GLUT4-containing vesicles to translocate this protein to the cell surface remain obscure. In the basal state, GLUT4 continuously recycles between the cell-surface membrane and various intracellular compartments. After insulin stimulation, there is marked increase in the rate of GLUT4 vesicle exocytosis, with a small decrease in the rate of internalization. The insulin-stimulated exocytosis of GLUT4 resembles the regulated exocytosis of synaptic vesicles (Pessin *et al.,* 1999; Rea and James, 1999). In particular, GLUT4 vesicles contain the v-SNARE proteins VAMP2 and VAMP3, which physically interact with their t-SNARE counterparts (syntaxin 4 and SNAP23) in the plasma membrane during GLUT4 vesicle translocation. Several lines of evidence have suggested that insulin specifically stimulates the translocation of the GLUT4 from VAMP2-containing compartments (Millar *et al.,* 1999). Although these SNARE interactions are essential, none of these core proteins appear to be direct targets of insulin action. Similarly, although several important SNARE accessory proteins, such as Munc18c, Synip, and NSF, also appear required for the control of GLUT4 docking and fusion events, the molecular mechanism by which insulin regulates their function has yet to be elucidated. It is tempting to speculate that, specific lesions in the SNARE protein complexes and/or the poorly defined signaling pathways that function in parallel with the PI 3-kinase pathway, may also contribute to insulin resistance.

Fructose and Metabolic syndrome:-

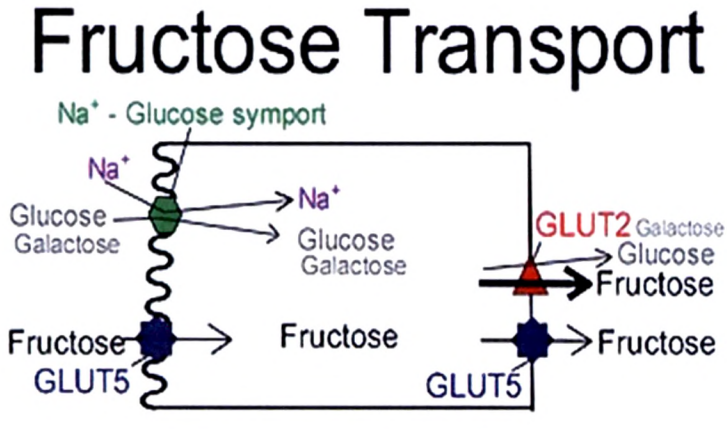
Insulin resistance is a metabolic disorder that is increasing worldwide and plays a role in the pathophysiology of the most common human diseases including type 2 diabetes mellitus, hypertension, obesity, dyslipidemia and coronary heart disease (Adeli *et al.,* 2001). The insulin resistance state is commonly associated with lipoprotein abnormalities that are risk factors for atherosclerosis, including hypertriglyceredemia, high levels of very low density lipoproteins (VLDL), low levels of high density lipoprotein cholesterol (HDL-C) (Ruotolo & Howard 2002) and small, dense LDL (Friedlandre *et al.,* 2000). These metabolic abnormalities together with hypertension and type 2 diabetes mellitus may cluster together constituting a syndrome referred to as the metabolic syndrome X (Nadig and Kotchen, 1997). The development of insulin resistance and hypertension are linked to both genetic and non-genetic environmental factors and the interaction between these two is strong and combined effects are multiplicative. A key environmental element is diet composition (Thresher *et al.,* 2000) and changes in diet in recent past have been studied as contributing factors to the development of both the diseases. Varying the type of carbohydrate in the diet can influence blood glucose control and insulin action (Storlien *et al.,* 1998; Crapo *et al.,* 1986; Bornet *et al.,* 1987; Jenkins *et al.,* 1984; Reiser *et al.,* 1987). Different sugars are produced when starch and sucrose are digested. Starch is digested to glucose whereas sucrose enters the portal vasculature as fructose and glucose moieties. A moderate amount of fructose from fresh fruits and vegetables has long been a component of human diets. However, abundant production of refined sweeteners has dramatically increased fructose intake. Fructose consumption has largely increased because of an increased consumption of soft drinks and other beverages high in fructose and consumption of foods such as processed food, sauces, and ready to serve

foods sweetened with sucrose and high-fructose corn syrup. Moreover, the sweetness intensity of fructose is higher than that of glucose or sucrose, which means that smaller quantities are required to obtain a sweeter taste in comparison with glucose or sucrose. It may be the fructose component of sucrose that is deleterious. Many studies have demonstrated that, normal rats fed a fructose enriched diet develop hypertension (Dai and McNeill, 1995; Katakam *et al.,* 1998) accompanied by metabolic abnormalities such as hyperinsulinemia, insulin resistance, and dyslipidemia, the cluster of risk factors which has been called syndrome X (also known as metabolic cardiovascular syndrome or insulin resistance syndrome). In humans, high fructose in diets reduces insulin sensitivity (Beck-Neilsen *et al.,* 1980) and elevates plasma triglycerides in both fed and fasting conditions (Bantle *et al.,* 2000). Fructose feeding and to a lesser extent glucose feeding were shown to produce elevations in plasma triglyceride (Zavaroni *et al.,* 1982; Sleder *et al.,* 1980; Hallfrisch *et al.,* 1983), insulin (Reiser *et al.,* 1987; Zavaroni *et al.,* 1982; Sleder *et al.,* 1980; Hallfrisch *et al.,* 1983), and sometimes blood glucose levels (Zavaroni *et al.,* 1980; Hallfrisch *et al.,* 1983). Elevated triglyceride levels were associated in a number of circumstances with impaired insulin action (Zavaroni *et al.,* 1982; Sleder *et al.,* 1980; Hallfrisch *et al.,* 1983).

Metabolism of dietary fructose, which occurs mainly in liver, differs from that of glucose. Hepatic glucose metabolism is acutely regulated by phosphofructokinase I (PFK I), a key regulatory step of glycolysis. In contrast, fructose enters the glycolytic pathway at the triose level, bypassing PFK I. This difference in initial metabolism of fructose not only acutely affects carbohydrate metabolism by changing supply of intermediate metabolites, but also induces metabolic adaptation including changes in gene expression. Previous studies reported increased mRNA expression of glycolytic

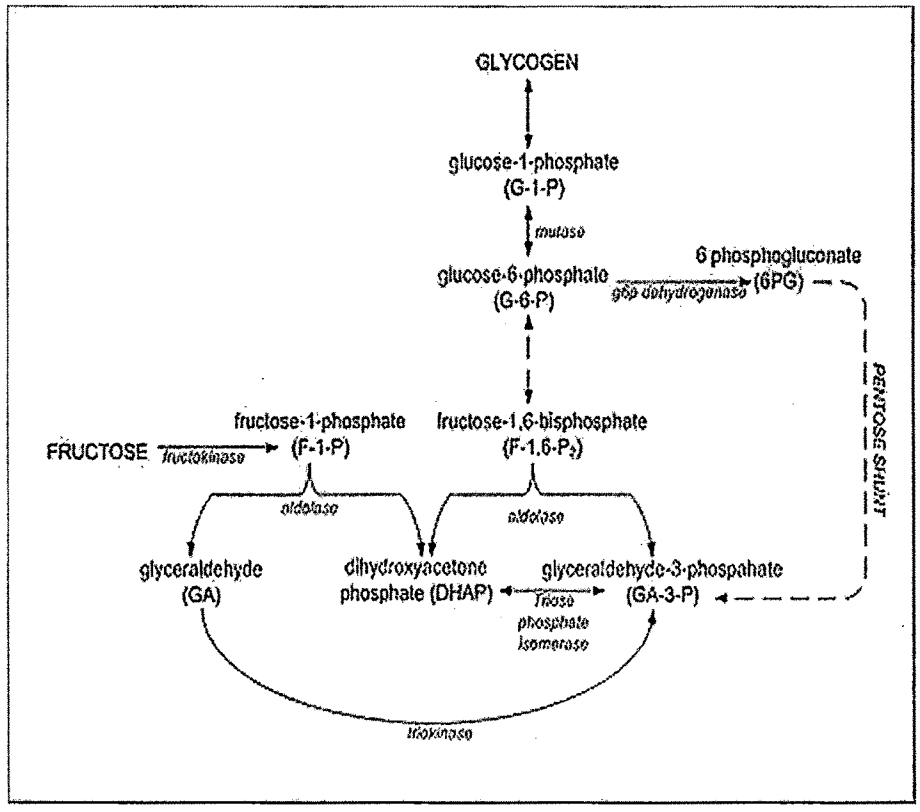
(liver-type pyruvate kinase PK) (Matsuda *et al.,* 1990), lipogenic (Fatty acid synthase, FAS) (Fiebig *et al.,* 1998), and gluconeogenic enzymes (glucose-6-phosphatase, G6Pase, Phosphoenol pyruvate kinase, PEPCK) (Commerford *et al.,* 2002). However, several confounding factors must be resolved to define the differential effect between fructose and glucose on gene expression and metabolic consequences. First, genes involved in carbohydrate metabolism are strongly regulated by hormones such as insulin, glucagon and glucocorticoids, and are sensitive to nutritional status (Goodridge, 1987). A small amount of fructose in the presence of high levels of glucose increased the net hepatic glucose uptake and glycogen synthesis (Shiota *et al.,* 2002a; Shiota *et al.,* 2002b; McGuinness and Cherrington, 2003). Rats fed a high fructose diet (>60% of total calories) provide a useful animal model of insulin resistance (Dai *et al.,* 1994; Thorburn *et al.,* 1989; Bezerra *et al.,* 2000). The sites of fructose induced insulin resistance are documented to be liver (Thorburn *et al.,* 1989), skeletal muscle (Zavaroni *et al.,* 1980) and adipose tissue (Vrana *et al.,* 1974). Adipose tissue mass (especially visceral adipose) enlargement has been identified as a crucial factor responsible for insulin resistance (Amer, 2003). A high fructose diet alters the activity of several enzymes regulating hepatic carbohydrate metabolism and lowers insulin stimulated glucose oxidation leading to hepatic insulin resistance (Blakely *et al.,* 1981; Nandhini and Anuradha, 2002). Fructose is readily absorbed and metabolised by human liver. Impairment in glucose production includes a reduced ability of insulin to suppress glucose production (insulin resistance) and accelerated/ inappropriate gluconeogenesis (McDonald 1966, Nestel *et al.,* 1970). Impairment in liver glucose metabolism can contribute to development of obesity and type 2 diabetes. The dyslipidemia observed in the metabolic syndrome is characterised in part by high plasma triglycerides and low high density lipoprotein (HDL) - cholesterol

diabetes. The dyslipidemia observed in the metabolic syndrome is characterised in part by high plasma triglycerides and low high density lipoprotein (HDL) - cholesterol concentration (Nikkila & Ojala 1965). The responsiveness of liver to changes in the composition and rate of nutrient delivery are predicted based on its anatomic position and regulatory features specific to this organ.

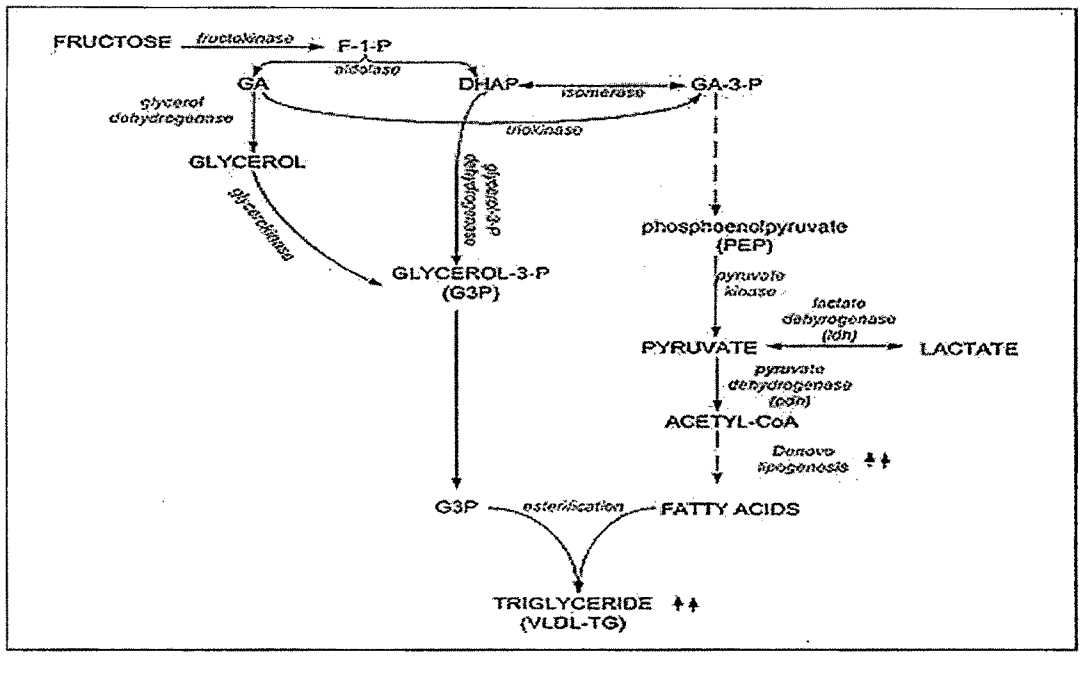


The portal vein receives the bulk of absorbed amino acids and simple sugars. Following the ingestion of meal containing complex carbohydrate/ glucose, the liver becomes a glucose consuming organ accounting for 20-30% of the total dietary carbohydrate disposal (Caro *et al.,* 1989, Weyer *et al.,* 1999). Most of this glucose is used to replenish glycogen stores with the remainder directed to glycolysis (Heinz *et al.,* 1968; Kahn 2003).

Metabolic conversion of fructose to glycogen in the liver



Metabolic conversion of fructose to triglyceride in the liver

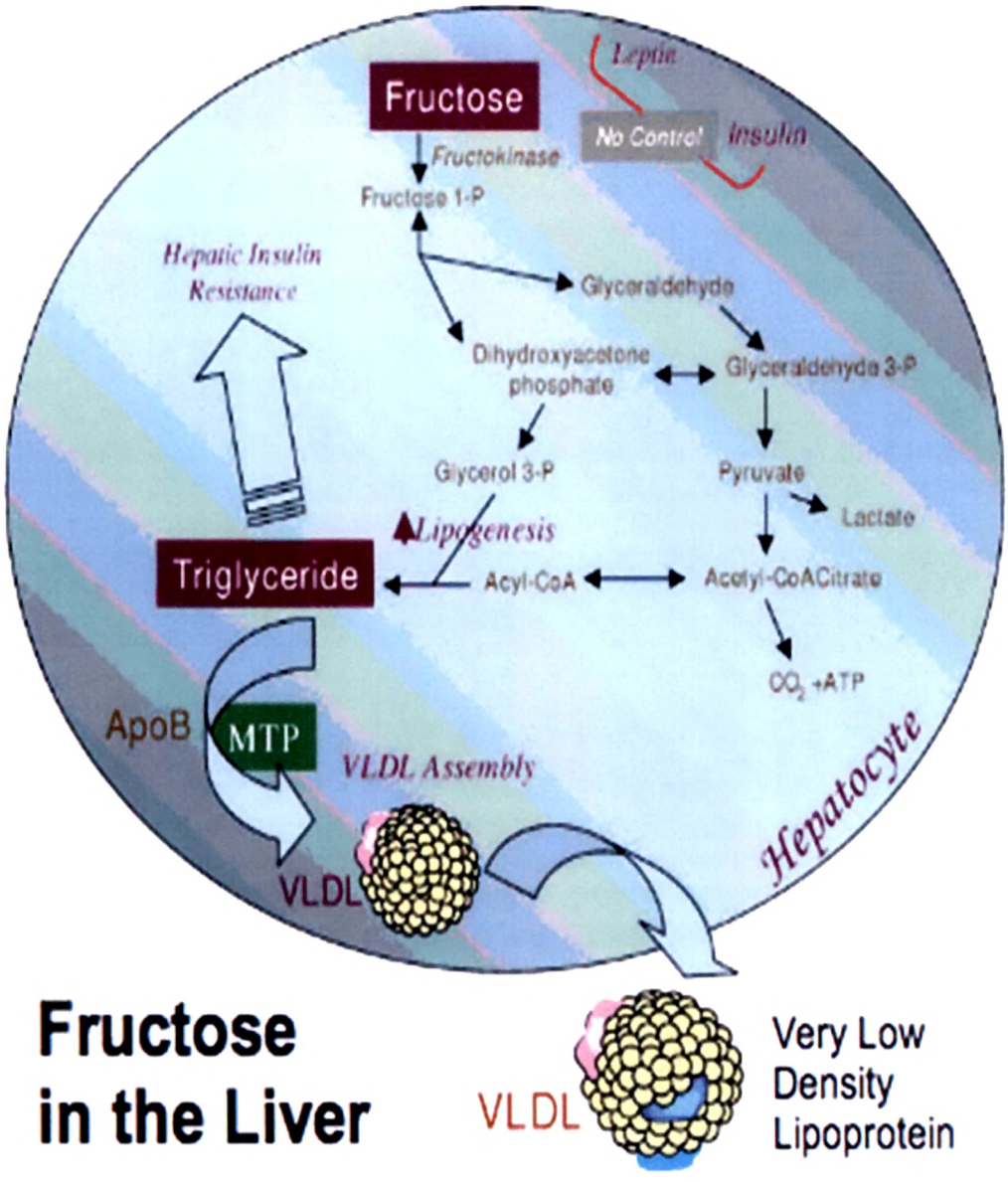


Sucrose, a disaccharide consisting of fructose and glucose are targeted for hepatic metabolism. Fructokinase, the protein responsible for phosphorylation of fructose to fructose-1-phosphate is expressed at highest concentration in the liver (Kohen-Avramoglu *et al.,* 2003, Liu *et al.,* 2005). In addition, fructose-1-phosphate stimulates glucose uptake in liver (Defronzo *et al.,* 1978; Pagliassoti & Cherrington 1992). Dietary fructose exerts a number of adverse metabolic effects in experimental animals and in humans including hypertriglyceridaemia (Heinz *et al.,* 1968; Selder *et al.,* 1980; Zavaroni *et al.,* 1982; Davies *et al.,* 1990; Mayes 1993; Pagliassoti *et al.,* 1996; Shiota *et al.,* 1998; Ferre *et al.,* 2003), hyperinsulinemia and hypertension (Reaven 1998). For hypertriglyceridaemia, studies have indicated that very low density lipoprotein triacylglycerol (VLDL-TG) overproduction rather than impaired

peripheral clearance is involved, although some have suggested a role for decreased VLDL clearance. The VLDL –TG overproduction is consistent with the initial metabolism of fructose being in liver, in contrast with the initial metabolism of glucose which is mainly extra hepatic (Hirano *et al.,* 1988, Waddell & Fallon 1973) owing to the exclusively hepatic location of fructokinase, the lack of fructose phosphorylation by extra hepatic hexokinase in the presence of competing glucose and the approximately 10 fold greater activity of hepatic fructokinase compared with combined hepatic glucokinase and hexokinase activities (Katz & McGarry 1984). Increased hepatic TG production might result from conversion of fructose carbon into new fat (lipogenesis *de novo*) or from increased esterification of circulating non-esterified fatty acids (NEFA) by the liver. Greater hepatic re-esterification of incoming NEFA is plausible mechanism in that fructose ingestion results in greater hepatic availability of pyruvate and α- glycerophosphate (Radziuk *et al.,* 1978). Exposure of the liver to 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. Interestingly, small catalytic quantities of fructose can have positive effects and actually decrease the glycemic response to glucose loads and improve glucose tolerance. These effects are also observed without any changes in insulin responses and NEFA and TG levels. 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. Because of its lipogenic properties, excess fructose in the diet can cause glucose and fructose malabsorption and greater elevations of TG and cholesterol compared with other carbohydrates. Upon gastric absorption both fructose and glucose are delivered via the portal vein to the liver. It is believed that the ability

of the liver to metabolise high doses of fructose is responsible for the disruption of energy stores and fuel metabolism observed (Zakin *et al.,* 1967, Chevalier *et al.,* 1972, Daly *et al.,* 1997, Dirlewanger *et al.,* 2000; Commerford *et al.,* 2002). In liver, fructose is metabolised to glyceraldehyde and dihydroxyacetone phosphate. This fructose end product can then readily converge with the glycolytic pathway. Of key importance is the ability of fructose to by pass the main regulatory step of glycolysis, the conversion of glucose-6-phosphate to fructose 1, 6-bisphosphate controlled by phosphofructokinase. Thus, while glucose metabolism is negatively regulated by phosphofructokinase, fructose can continuously enter the glycolytic pathway and can uncontrollably produce glucose, glycogen, lactate and pyruvate providing both the glycerol and acyl portion of acyl-glycerol molecule. These substrates and the resultant excess energy flux due to unregulated fructose metabolism, will promote the overproduction of TG (Pagliassotti *et al.,* 2003). There is considerable evidence supporting the ability of high fructose diet to upregulate the lipogenesis pathway, leading to increased TG production. Insulin and glucose are known to directly regulate lipid synthesis and secretion. Insulin controls hepatic sterol regulatory element binding protein (SREBP) expression, which is a key transcription factor responsible for regulating fatty acid and cholesterol biosynthesis. SREBP binds to sterol responsive element (SRE) found on multiple genes and can activate a cascade of enzymes involved in cholesterol biosynthesis pathway such as HMG CoA reductase (Kok *et al.,* 1996) and fatty acid synthase (FAS) (Bennett *et al.,* 1995). Recently is has been established that, hormones such as insulin and platelet derived growth factor play a role in regulating these transcription factors. Expression of SREBP is enhanced by insulin in all 3 major insulin target tissues, liver, adipose and skeletal muscle (Kim *et al.,*1998; Forezt *et al.,*1999; Guillet-Deniau *et al.,* 2002;

Sewter *et al.,* 2002). Similarly, levels of SREBP are enhanced in presence of hyperinsulinemia (De Fronzo *et al.,* 1991; Matsuzaka *et al.,* 2004). Despite the fact that SREBP is directly stimulated via insulin signalling, the depletion of insulin and insulin signalling through streptozotocin (STZ) treatment paradoxically induces SREBP expression upon glucose, fructose or sucrose feeding. It would have been expected that, SREBP would be down regulated concomitantly along with the reduced insulin availability but, glucose feeding causes a short term peak induction, whereas fructose causes a gradual extended increase in SREBP activity, providing evidence that lipogenesis can be independent of insulin signalling, given carbohydrate and particularly fructose availability (Reaven 1991).



It has been apparent for some time that increases in dietary carbohydrate intake can raise blood pressure in experimental animals (Hall and Hall, 1966; Ahren *et al.*, 1980). In particular, the ability of sucrose feeding to accentuate the magnitude of the blood pressure elevation already present in spontaneously hypertensive rats has been

documented (Young and Landsberg, 1977; Preuss and Preuss, 1980; Fournier *et al.,* 1986). Since sucrose feeding stimulates sympathetic nervous system activity (Young and Landsberg, 1977), it has been suggested that sucrose induced increases in sympathetic activity may elevate blood pressure in susceptible animals (Young and Landsberg, 1981). Indirect support for this hypothesis was provided by the observation that, the rats with sucrose induced hypertension respond to alpha-adrenergic blockade with phentolamine, than do their normotensive controls (Bunang *et al.,* 1983). This point of view has recently received added support from the demonstration that, hypertension produced by sucrose feeding is associated with evidence of increased catecholamine secretion (Fournier *et al.,* 1986). However, the physiological effect of sucrose is not limited to an increase in sympathetic activity, and rats fed a high sucrose diet also become insulin resistanct and hyperinsulinemic (Reaven *et al.,* 1979; Wright *et al.,* 1983). Since several recent observations have documented an association between hyperinsulinemia and hypertension in humans (Lucas *et al.,* 1985; Modan *et al.,* 1985; Singer *et al.,* 1985), it seemed important to see if a similar phenomenon could be found in carbohydrate induced hypertension in rats. Fructose feeding can also cause insulin resistance and hyperinsulinemia in normal rats (Zavaroni *et al.,* 1980; Tobey *et al.,* 1982). If fructose also produces hypertension, it would suggest that the insulin resistance and hyperinsulinemia associated with either fructose or sucrose feeding are the common factors in the mechanism of carbohydrate induced hypertension. The mechanism of fructose-induced hypertension is not fully understood. Evidence has shown that, the increase in blood pressure associated with a high fructose intake is not due to an increased activity of the renin- Angiotensin Aldosterone system (Hwang *et al.,* 1989). Based on the findings in rats that exercise training or somatostatin infusion could

simultaneously attenuate fructose induced hypertension, insulin resistance and hyperinsulinemia, it was hypothesised that, insulin resistance and hyperinsulinemia played an important role in the pathogenesis of fructose induced hypertension (Reaven *et al.,* 1988, 1989). Simultaneously, reduction in blood pressure, hyperinsulinemia and insulin resistance could also be observed in fructose hypertensive rats following the treatment with either benfluorex (Storlien *et al.,* 1993), or vanadyl sulphate (Bhanot *et al.,* 1994). However, a definite casual relationship between the changes in blood pressure and the plasma insulin level following fructose treatment has not yet been established. The fructose hypertensive rat model is a widely used model of acquired hypertension, wherein feeding normal Sprague- Dawley rats a fructose enriched diet results in hyperinsulinemia, insulin resistance and hypertension (Reaven *et al.,* 1989; Verma *et al.,* 1994). This model is ideally suited to examine the relationship between metabolic aberrations and blood pressure independent of any genetic contribution.

Obesity and Diabetes:-

Although the causal relationship between diabetes and obesity is not fully understood, upper body obesity is clearly a strong risk factor for the development of diabetes (Pascot et al., 2001). A probable common link between diabetes and obesity is the adipocyte, which stores excess energy in the form of triglyceride, and releases free fatty acids in response to energy requirements such as fasting. In healthy individuals, excess fat is stored in adipocytes and only low amounts of triglyceride are maintained in non adipocytes. In obese individuals, the capacity for adipose tissue to accommodate excess lipid can be exceeded, resulting in the abnormal accumulation of lipid in other tissues. Elevated cellular triglyceride content in muscle, liver and

pancreatic islets is associated with physiological dysfunction (lipotoxicity) in those tissues and might be a primary contributing factor for the development of obesity related type 2 diabetes mellitus (T2DM) (Unger, 2002). Adipose tissue also functions as an endocrine organ, secreting hormones and cytokines that regulate metabolism in other tissues. Given these key roles of the adipocyte in regulating overall energy balance, an understanding of the molecular and cellular biology of the fat cell will be required to understand fully the causes of diabetes and obesity, and to develop therapies for their treatment.

Obesity is characterized by increased adipose tissue mass that results from both increased fat-cell number (hyperplasia) and increased fat-cell size (hypertrophy) (Couillard *et al.,* 2000). The number of adipocytes present in an organism is determined to a large degree by the adipocyte differentiation process, which generates mature adipocytes from fibroblast-like pre-adipocytes. Many of the molecular details of this process are now known. One of the first steps in the process of adipogenesis is the re-entry of growth-arrested pre-adipocytes into the cell cycle and the completion of several rounds of clonal expansion. The tumor suppressor retinoblastomaprotein (Rb) has been extensively studied for its role in this initial step of adipogenesis. Hansen *et al.* (1999) demonstrated that mouse lung embryonic fibroblasts (MEFs) derived from mice with targeted disruption of the gene encoding Rb, completely failed to undergo adipocyte differentiation (Hansen *et al.,* 1999; Chen *et al.,* 1996) Rb phosphorylation status correlates well with cell-cycle progression; hypo-phosphorylated in growth-arrested pre-adipocytes and hyper-phosphorylated in proliferating cells. Hypo-phosphorylated Rb is complexed with transcription factor E2F, and upon addition of adipogenic hormones, Rb rapidly undergoes hyper-phosphorylation by cyclin-dependent kinases (CDKs). This results in the dissociation

of Rb andE2F, allowing E2F to promote cell-cycle progression to S phase. Just before entering the terminal differentiation state, Rb returns to a hypo-phosphorylated state, sequestering E2F and causing cells to permanently exit from the cell cycle (Heibert *et al.,* 1992). Many cell-cycle-associated proteins (CDKs and their inhibitors, p18, p21 and p27) play crucial roles during the cell-cycle progression that precedes entry into the terminally differentiated state (Morisson and Farmer, 1999; Reichert and Eick, 1999). Two transcription factor families have emerged as the key determinants of terminal adipocyte differentiation: the CCAAT/enhancer-binding proteins C/EBP α, -β and -δ, and peroxisome proliferator-activated receptor γ (PPARγ, encoded by *PPARG*) (Rosen and Speilgelman, 2000; Darlington *et al.,* 1998). As cells undergo the differentiation process in response to adipogenic signals, the initial event is the rapid induction of C/EBP β and -δ expression (Yeh *et al.,* 1995). A potential role for C/EBP β and -δ proteins is to stimulate CDK inhibitor p21 expression by directly binding to the promoter region of the gene encoding p21 (Cram *et al.,* 1998). Increased p21 expression then leads to an inhibition of CDK-mediated Rb phosphorylation. A role for C/EBP β and -δ in the induction of PPARγ2, a key regulator of adipogenesis, has also been reported (Scharwz *et al.,* 1997; Elberg *et al.,* 2000). The importance of C/EBPβ during adipogenesis was demonstrated by loss-of function and gain-of-function genetic studies in mice. Over expression of either C/EBP β or -δ in pre-adipocytes enhanced adipogenesis (Darlington *et al.,* 1998; Yeh *et al.,* 1995), whereas embryonic fibroblast cells (MEFs) derived from mice lacking either C/EBP β or -δ had reduced levels of adipogenesis compared with the wild type (Tanaka *et al.,* 1997). Mice lacking both C/EBP β and -δ showed reduced white adipose tissue mass and reduced lipid staining in inter-scapular brown adipose tissue. Additionally, MEFs derived from C/EBP β and δ-double-knockout mice completely

failed to differentiate into mature adipocytes (Tanaka *et al.,* 1997). These studies demonstrated that C/EBP β and -δ play synergistic roles in adipocyte differentiation and maturation. The induction of C/EBP β and -δ is immediately followed by an increase in PPARγ and C/EBP α expression. PPARγ is a ligand-dependent nuclear receptor transcription factor. In mice, there are two isoforms, PPARγ1 and PPARγ2, which are derived from the same gene by alternative promoter usage and RNA splicing (Zhu *et al.,* 1995). The expression of both isoforms is highest in adipose tissue, but PPARγ2 is expressed selectively in adipocytes, whereas detectable levels ofPPARγ1 are observed in many other tissues, including liver, muscle, colon and macrophage (Auboeuf *et al.*, 1997; Mansen *et al.,* 1996; Tontonoz *et al.,* 1998). PPARγ2 is identical to PPARγ1 except that it contains an additional 30 amino acids on its N-terminus. However, the functional differences between these two isoforms in adipocyte biology are not fully understood. Ren *et al.* (2002) recently addressed this issue by blocking PPARγ2 expression in 3T3-L1cells using artificial zinc finger repressor proteins that specifically bind to the PPARγ2 promoter, selectively reducing the expression of that isoform. This study demonstrated that the level of PPARγ2 expression, but not PPARγ1, correlates with the degree of lipid accumulation. Cells with a 95% reduction in PPARγ2expression completely failed to undergo adipogenesis, whereas cells with a 50% reduction in PPARγ2expression produced a corresponding 50% loss in adipogenic capacity. In addition, exogenous delivery of PPARγ2 into PPARγ-deficient cells was able to completely restore the adipogenesis, whereas over expression of PPARγ1 had virtually no effect. These results strongly suggest that PPARγ2, but not PPARγ1, plays a key role in adipogenesis. This result is rather surprising given that these two proteins are identical except for their N-termini, appear to bind to the same DNA regulatory elements [PPAR-response elements

(PPREs)], and are expressed in similar amounts in adipocytes. Because the N-terminus of PPARγ2 contains a ligand-independent trans-activation function that is much stronger than PPARγ1 (Werman *et al.,* 1997), the two isoforms could differentially interact with the co-activators and co-repressors that mediate PPARγ transcriptional activity. Whether this function is crucial for the adipogenic role of PPARγ2 requires further study. What is the biological role of PPARγ1 in adipogenesis? One possibility is that PPARγ1, which is already expressed in pre-adipocytes, behaves as a priming factor (along with C/EBPβ and -δ) for the induction of PPARγ2, although no functional PPREs have been identified within the PPARγ2 promoter region. Another possibility is that PPARγ1 plays a role in the cell-cycle regulation, perhaps by modulating E2For its dimerization partners (the DP proteins) (Morisson and Farmer, 1999; Altoik *et al.,* 1997). Alternatively, PPARγ1 could be involved in the production of endogenous PPARγ ligands, which play a role in later stages of adipogenesis. Selectively blockingPPARγ1 expression without affecting the PPARγ2would be a useful way to determine the functional role of PPARγ1 in both adipose and non adipose tissue. During the later stage of differentiation, C/EBP α expression rises immediately after PPARγ2expression. The requirement for PPARγ and C/EBP α in adipose development has been demonstrated by a targeted gene-knockout strategy in mice. Homozygous knockout of either gene results in embryonic lethality and failure to develop normal adipose tissue (Kubota *et al.*, 1999; Barak *et al.,* 1999; Rosen *et al.,* 1999; Wang *et al.,* 1995). There has been an intense research effort to understand the relationship between these two transcription factors and the role they play in adipogenesis. Several studies have demonstrated that PPARγ2 and C/EBP α co-regulate each other's expression. Mice with reduced PPARγ expression owing to heterozygous gene-knockout displayed a drastically reduced level of C/EBP

α, even though no functional PPREs have been identified in the enhancer region of C/EBP α (Barak *et al.*, 1999). Likewise, mice with disrupted C/EBP α expression showed a reduced level of PPARγ (Wu *et al.*, 1999). This is probably because of direct binding of C/EBP α to the promoter region of PPARγ2 and activation of PPARγ gene transcription. The C/EBP α binding site in the PPARγ2 promoter was also shown to bind to C/EBP δ, but not C/EBP β (Elberg *et al.*, 2000).The crucial roles of PPARγ and C/EBP α were highlighted by the observation that over expression of either transcription factor in NIH3T3 cells is sufficient to convert these normally non adipogenic cells from fibroblasts into adipocytes (Freytag *et al.*, 1994; Tontonoz *et al.*, 1994). However, it was unclear whether either transcription factor, completely on its own, could induce adipogenesis. *CEBPA*-null MEF cells failed to undergo adipogenesis, but this defect could be successfully restored by over expression of PPARγ2 (Wu *et al.*, 1999). Rosen *et al.* (2002) performed the reverse strategy; forced C/EBP α expression in *PPARG*-null MEFs, and found that the cells remained defective for adipogenesis. These studies convincingly demonstrated that PPARγ2 is the ultimate key regulator of adipogenesis and C/EBP α might play more of an accessory role for PPARγ2 by inducing and maintaining PPARγ2 expression. The primary function of C/EBP α could be the regulation of genes involved in the metabolic actions of insulin, such as glucose transporter 4 (Glut4) (Wu *et al.*, 1999). Clearly, PPARγ and C/EBP α are key transcription factors in adipogenesis, acting synergistically to generate fully differentiated, insulin-responsive adipocytes (Wu et al., 1999; El-Jack *et al.*, 1999). In addition to the key role that PPARγ plays in adipogenesis, it also appears to carry out important metabolic functions in intact organisms. In patients with T2DM, activation of PPARγ by synthetic ligands, such as the thiazolidinediones (TZDs), results in a dramatic improvement in peripheral insulin

sensitivity and a reduction in plasma glucose concentrations (Willson *et al.,* 2001). Although the exact mechanism by which these drugs improve peripheral insulin sensitivity and reduce plasma glucose concentration is not fully understood, several general possibilities have emerged. First, TZDs might have a beneficial effect on metabolism by increasing fat-cell number and size, leading to greater lipid storage capacity and increased protection of non adipose tissues from the deleterious effects of excess lipid accumulation. Second, PPARγ agonists could act on the mature adipocyte to alter the production of adipose-derived hormones or metabolic signals that function to improve metabolic parameters in other tissues and organs, such as muscle, liver and pancreas. Third, itis also possible that TZDs exert their metabolic effects through direct action in non adipose tissues. The evidence that PPARγ ligands cause the generation of new adipocytes is supported by studies in obese Zucker rats treated with TZDs, where an increased number of small adipocytes, presumably generated by TZD-induced adipogenesis, were observed (Okuno *et al.,* 1998). An increase in fat-cell size after TZD treatment has also been seen in some rodent models (Yamauchi *et al.,* 2001) and in patients (Weyer *et al.,* 2000). Although it might seem paradoxical that an increase in adiposity could improve diabetes, the increased number and size of adipocytes, especially in the appropriate adipose beds, could improve the ability of adipose tissue to store excess lipid and reduce deleterious accumulation of triglyceride in muscle, liver and pancreatic islets (Yamauchi *et al.,* 2001). Consistent with this possibility is the observation that although T2DM patients undergoing long-term TZD treatment had an increased subcutaneous fat mass, they also showed a reduction in the amount of visceral fat (Mori *et al.,* 1999; Akazawa *et al.,* 2000). Visceral fat (mesenteric and omental adipose tissues) is known to be more lipolytic in response to catecholamine stimulation than is subcutaneous fat, and

delivers free fatty acids and other secreted factors efficiently to insulin-sensitive tissues such as liver and muscle, possibly causing an increase in insulin resistance (Bolinder *et al.,* 1982). Although intrinsic metabolic differences between subcutaneous and visceral fat are not completely understood, current evidence suggests that subjects with increased visceral fat are at considerably higher risk of diabetes and cardiovascular complications than are those within creased subcutaneous fat (Boyko *et al.,* 2000). These observations, plus the demonstration that PPARγ levels are higher in subcutaneous than in visceral fat, make it tempting to speculate that PPARγ activation by TZDs is fat-depot specific, and that drug treatment leads to a beneficial change in the proportions of key metabolically active adipose beds. A clear understanding of the physiology of different fat depots might make it possible to develop therapies that specifically reduce the size of fat beds that have deleterious effects on metabolism. This could theoretically be accomplished by inhibiting high-fat-induced hypertrophic obesity, perhaps using antagonists of PPARγ. Several PPARγ antagonists and partial agonists have been developed (Reed and Leff, 2002), but their activity in blocking high-fat-induced obesity has not been tested. Although it is possible that are modeling of fat distribution among the various adipose depots could be of therapeutic benefit, it is important to note that the relationship between body-fat content and metabolism is extremely complex. This is perhaps best illustrated by comparing metabolic parameters in different conditions where body fat content or distribution has been altered. For example, heterozygous *PPARG*-knockout mice (containing only one normal *PPARG* allele) have reduced body fat but are more insulin sensitive than are their normal littermates (Kubota *et al.,* 1999). By contrast, human syndromes of partial familial lipo-dystrophy in which mutations in one of several genes, including that encoding PPARγ, cause a loss of specific adipose tissue

beds (Cao and Hegele, 2000; Agarwal and Garg, 2002) are strongly associated with severe insulin resistance (Hegele, 2000). Finally, the outcome of TZD administration to T2DM patients appears to be as light increase in body-fat content and a significant increase in insulin sensitivity (Willson *et al.,* 2001). Although it is difficult to reconcile these disparate observations, they could all result from alterations in adipocyte metabolism that either change serum free fatty acid levels or change the production of adipose-derived hormones that affect peripheral insulin sensitivity. The role of adipose tissue as an endocrine organ, secreting hormones and cytokines (adipokines) that affect whole-body energy metabolism, has become a major focus of current metabolic-disease research. Multiple adipokine molecules have now been identified that have significant effects on whole-body energy homeostasis, feeding behavior and insulin sensitivity. The first clear adipokine described was leptin, the protein product of the *ob* gene, which, when mutated, causes severe obesity in mice (Zhang *et al.,* 1994). Leptin, secreted from adipose tissue, regulates body weight by acting directly in the CNS to inhibit feeding behavior. Leptin might also have direct effects on energy metabolism in peripheral tissues such as muscle, where it has been reported to cause an increase in fatty-acid oxidation rates (Minokoshi *et al.*, 2002). Although leptin behaves as an anti-obesity hormone in certain animal models, common human obesity does not appear to be a consequence of abnormally low leptin levels. The beneficial metabolic effects of PPARγ activation do not appear to result from an increase in expression of the gene encoding leptin. In fact, TZD-treated rodents showed increased food intake and adipose tissue mass, and reduced leptin expression in adipose tissue (De Vos *et al.,* 1996). It has been proposed that the inflammatory cytokine tumor necrosis factor α (TNF-α) also has adipokine-like activity under some circumstances. TNF-α production in adipocytes correlates within

creased obesity and insulin resistance (Hotamisligil *et al.,* 1993; 1995). Interestingly a mutual antagonism exists between TNF-α and PPARγ; TNF-α inhibits PPARγ expression in adipocytes, whereas PPARγ activation by TZDs can partially overcome the diabetogenic effects of TNF-α (Hotamisligil *et al.,* 1995; Szalkowski *et al.,* 1995), potentially explaining at least some of the insulin-sensitizing activity of PPARγ ligands. However, the proposed role for TNF-α in insulin resistance and diabetes is still unclear because infusion of TNF-α-neutralizing antibody into obeseT2DM patients did not alter insulin sensitivity (Ofei *et al.,* 1996). Another recently identified adipocyte-secreted hormone that might play a role in both obesity and diabetes is ACRP30 (adipocyte complement-related30-kDa protein, also called adiponectin or adipo Q).Originally identified as a secreted fat-specific protein whose expression was induced by adipogenesis (Scherer *et al.,* 1995; Hu *et al.,* 1996), ACRP30 levels were found to be reduced in obesity (Hu *et al.,* 1996) and increased by weight loss (Yang *et al.,* 2001). In addition, thegene encoding ACRP30 maps to a region on chromosome 3 that is associated with diabetes andthe metabolic syndrome (Vionett *et al.,* 2000; Kissebah *et al.,* 2000). Treatment of rodents with ACRP30 was found to increase muscle fatty acid oxidation (Freubis *et al.,* 2001), reverse insulin resistance (Yamauchi *et al.,* 2001) and improved hepatic insulin action (Berg *et al.,* 2001). ACRP30 is an excellent candidate for a fat-derived hormone that mediates the antidiabetic effects of PPARγ ligands because it has recently been demonstrated that levels of the ACRP30 are increased in patients treated with TZDs (Yang *et al.,* 2002) and that its expression in adipocytes is induced by PPARγ agonists (Combs *et al.,* 2002). Another potential candidate for an adipocyte hormone that could mediate some of the anti diabetic effects of PPARγ is the recently described molecule resistin [also known as adipocyte-secreted factor (ADSF or FIZZ3)] (Steppan and Lazar, 2002).

Resistin was over expressed in rodent models of diet-induced obesity and reduced by TZD treatment (Steppan *et al.,* 2001). In addition, treatment of normal mice with resistin induced insulin resistance and glucose intolerance (Steppan *et al.,* 2001). These data suggest that resistin acts in a converse manner to ACRP30, increasing insulin resistance and promoting the development of diabetes. However, by contrast, Way *et al.* (2001) reported that resistin expression was suppressed in several rodent models of obesity and was increased by PPARγ ligands. Furthermore, there was no correlation between adiposity and adipocyte resistin mRNA expression in isolated human adipocytes (Savage *et al.,* 2001). Given these contradictory findings, additional work is required to clarify the role of resistin as a potential link between obesity and insulin resistance.

Leptin Mechanism:-

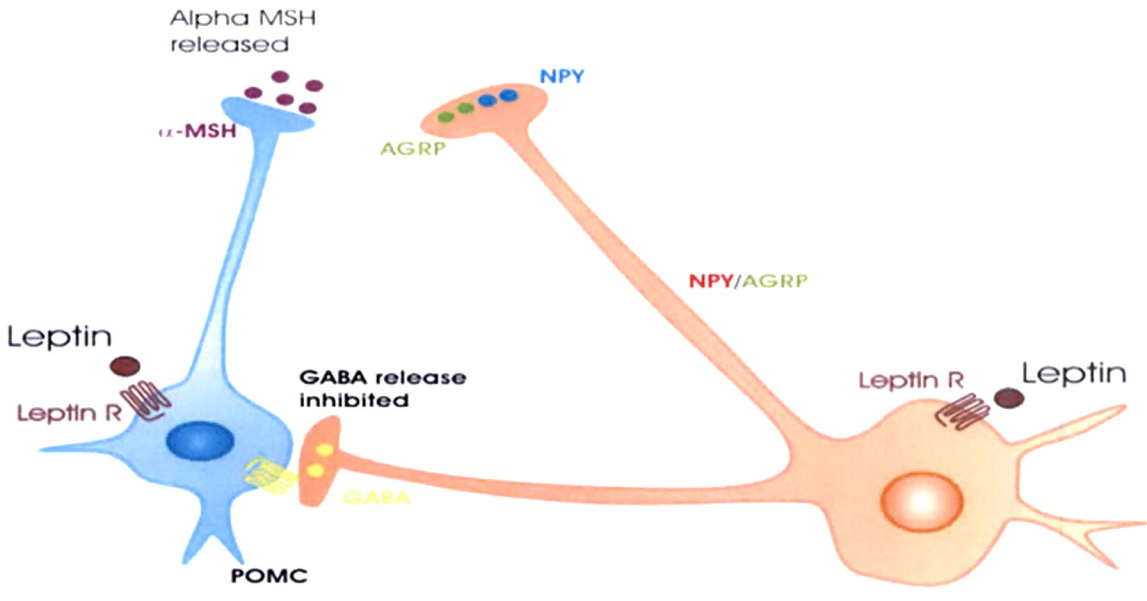
Leptin (Greek *leptos* meaning thin) is a 16 kDa protein hormone that plays a key role in regulating energy intake and energy expenditure, including appetite and metabolism. It is one of the most important adipose derived hormones (Brennan and Mantzoros, 2006). The *Ob (Lep)* gene (Ob for obese, Lep for leptin) is located on chromosome 7 in humans. The effects of leptin were observed by studying mutant obese mice that arose at random within a mouse colony at the Jackson Laboratory in 1950 (Ingalls *et al.,* 1950). These mice were massively obese and excessively voracious. Ultimately, several strains of laboratory mice have been found to be homozygous for single-gene mutations that causes them to become grossly obese, and they fall into two classes: "ob/ob", those having mutations in the gene for the protein hormone leptin, and "db/db", those having mutations in the gene that encodes the

receptor for leptin. When ob/ob mice are treated with injections of leptin they lose their excess fat and return to normal body weight. Leptin itself was discovered in 1994 by Jeffrey M. Friedman and colleagues at the Rockefeller University through the study of such mice (Zhang *et al.,* 1994). Human leptin is a protein of 167 amino acids. It is manufactured primarily in the adipocytes of white adipose tissue, and the level of circulating leptin is directly proportional to the total amount of fat in the body. In addition to white adipose tissue—the major source of leptin—it can also be produced by brown adipose tissue, placenta (syncytiotrophoblasts), ovaries, skeletal muscle, stomach (lower part of fundic glands), mammary epithelial cells, bone marrow, pituitary and liver (Margetic *et al.*, 2002). Leptin has also been discovered to be synthesised from gastric chief cells and P/D1 cells in the stomach (Bado *et al.*, 1998). Leptin acts on receptors in the hypothalamus of the brain where it inhibits appetite by counteracting the effects of neuropeptide Y (a potent feeding stimulant secreted by cells in the gut and in the hypothalamus); counteracting the effects of anandamide (another potent feeding stimulant that binds to the same receptors as THC, the primary active ingredient of marijuana); and promoting the synthesis of α-MSH, an appetite suppressant. This inhibition is long-term, in contrast to the rapid inhibition of eating by cholecystokinin (CCK) and the slower suppression of hunger between meals mediated by PYY3-36. The absence of a leptin (or its receptor) leads to uncontrolled food intake and resulting obesity. Several studies have shown that fasting or following a very-low-calorie diet (VLCD) lowers leptin levels (Dubuc *et al.,* 1998; Pratley *et al.,* 1997; Weigle *et al.,* 1997). It might be that on short-term leptin is an indicator of energy balance. This system is more sensitive to starvation than to overfeeding (Chin-Chance *et al.,* 2000). That is, leptin levels do not rise extensively after overfeeding. It might be that the dynamics of leptin due to an acute change in energy balance are

related to appetite and eventually to food intake. Although this is a new hypothesis, there are already some data that support it (Keim *et al.,* 1998; Mars *et al.,* 2006). In March 2010, researchers reported that mice with type 1 diabetes treated with leptin alone or in conjunction with insulin did better (blood sugar didn't fluctuate as much, cholesterol levels went down and they didn't form as much body fat) than mice with type 1 diabetes treated with insulin alone, raising the prospect of a new treatment for diabetes (Wang *et al.*, 2010). Leptin binds to neuropeptide Y (NPY) neurons in the arcuate nucleus, in such a way that decreases the activity of these neurons. Leptin signals to the brain that the body has had enough to eat, or satiety. A very small group of humans possess homozygous mutations for the leptin gene that leads to a constant desire for food, resulting in severe obesity. This condition can be treated somewhat successfully by the administration of recombinant human leptin. However, extensive clinical trials using recombinant human leptin as a therapeutic agent for treating obesity in humans have been inconclusive because only the most obese subjects who were given the highest doses of exogenous leptin produced statistically significant weight loss. It was concluded that large and frequent doses were needed to only provide modest benefit because of leptin's low circulating half-life, low potency, and poor solubility. Furthermore, these injections caused some participants to drop out of the study due to inflammatory responses of the skin at the injection site. Some of these problems can be alleviated by a form of leptin called Fc-leptin, which takes the Fc fragment from the immunoglobulin gamma chain as the N-terminal fusion partner and follows it with leptin. This Fc-leptin fusion has been experimentally proven to be highly soluble, more biologically potent, and contain a much longer serum half-life. As a result, this Fc-leptin was successfully shown to treat obesity in both leptin-deficient and normal mice, although studies have not been undertaken on human

subjects. This makes Fc-leptin a potential treatment for obesity in humans after more extensive testing (Friedman and Halaas, 1998; Heymesfeild *et al.,* 1998; Lo *et al.,* 2005). Thus, circulating leptin levels give the brain input regarding energy storage so it can regulate appetite and metabolism. Leptin works by inhibiting the activity of neurons that contain neuropeptide Y (NPY) and agouti-related peptide (AgRP), and by increasing the activity of neurons expressing α-melanocyte-stimulating hormone (α-MSH). The NPY neurons are a key element in the regulation of appetite; small doses of NPY injected into the brains of experimental animals stimulates feeding, while selective destruction of the NPY neurons in mice causes them to become anorexic. Conversely, α-MSH is an important mediator of satiety, and differences in the gene for the receptor at which α-MSH acts in the brain are linked to obesity in humans. Although leptin is a circulating signal that reduces appetite, in general, obese people have an unusually high circulating concentration of leptin (Considine *et al.,* 1996). These people are said to be resistant to the effects of leptin, in much the same way that people with type 2 diabetes are resistant to the effects of insulin. The high sustained concentrations of leptin from the enlarged adipose stores result in leptin desensitization. The pathway of leptin control in obese people might be flawed at some point so the body doesn't adequately receive the satiety feeling subsequent to eating. A study published recently suggests that the consumption of high amounts of fructose causes leptin resistance and elevated triglycerides in rats. The high-fructose diet rats subsequently ate more and gained more weight than controls when fed a high fat, high calorie diet (Science News, 2008; Vaselli, 2008; Shapiro *et al.,* 2008). Leptin interacts with six types of receptors (Ob-Ra–Ob-Rf, or LepRa-LepRf) which in turn are encoded by a single gene, LEPR (Wang *et al.,* 1996). Ob-Rb is the only receptor isoform that can signal intracellularly via the Jak-Stat and MAPK signal transduction

pathways (Malendowicz *et al.,* 2006), and is present in hypothalamic nuclei. It is unknown whether leptin can cross the blood-brain barrier to access receptor neurons, because the blood-brain barrier is somewhat absent in the area of the median eminence, close to where the NPY neurons of the arcuate nucleus are. It is generally thought that leptin might enter the brain at the choroid plexus, where there is intense expression of a form of leptin receptor molecule that could act as a transport mechanism. Once leptin has bound to the Ob-Rb receptor, it activates the stat3, which is phosphorylated and travels to the nucleus to, presumably, effect changes in gene expression. One of the main effects on gene expression is the down-regulation of the expression of endocannabinoids, responsible for increasing appetite. There are other intracellular pathways activated by leptin, but less is known about how they function in this system. In response to leptin, receptor neurons have been shown to remodel themselves, changing the number and types of synapses that fire onto them. There is some recognition that leptin action is more decentralized than previously assumed. In addition to its endocrine action at a distance (from adipose tissue to brain), leptin also acts as a paracrine mediator.



Hypertension:-

Specific to cardiovascular health, a significant effect of obesity is the increase in the development of peripheral vascular disease, a condition identified by decreased perfusion to peripheral limbs and tissues, causing edema, and leading to a decrease in function and progressive loss of tissue viability (Mensah *et al.,* 2004).In humans, this can lead to loss of mobility, chronic pain, and development of psychological depression (Wexler *et al*., 2006). Chronically, these complications can manifest with venous stasis leading to lower limb ulcerations, increases in venous thromboembolism, and a higher rate of pulmonary embolism (Hansson et al., 1999; Golghaber et al., 1997). Studies of the direct physiological effects of peripheral vascular disease have been limited in humans in the past due to the invasive nature of some procedures. However, ultrasound techniques with advanced Doppler technologies have translated to non-invasive means to investigate and track peripheral and coronary disease states (Abularrge *et al.,* 2005; Wyman *et al.,* 2005). Carotid intima-medial thickness (IMT) is a good predictor of adult obesity and cardiovascular

events (Ciccone et al., 2001; Heiss et al., 1991). As subjects age, the cholesterol deposits in the macrophage foam cells of the vascular intima become more extensive, and the resulting plaques become linked to acute coronary syndromes; this thickening of the intima-medial layer is highly correlated with adiposity, plaque lesions, and future cardiovascular events (Coleman *et al.,* 2006; Magnien *et al.,* 1998). In a recognized experimental model of metabolic syndrome, Obese Zucker rat (OZR), the perfusion of multiple tissues has been shown to be compromised (Stepp *et al.,* 2004; Gray, 1997; Frisbee, 2005). The direct mechanism of this decrease in perfusion, found in both humans and the OZR, seems to be multi-faceted: a combination of altered responsiveness to vasodilator and vasoconstrictor mechanisms, changes to the mechanical properties of the perfusing arteries, or a limit in the density/number of available micro vessels to supply the tissue (Frisbee, 2007). Conditions that result from obesity, such as metabolic syndrome, manifest compromised vasodilation in response to physiological or pharmacological challenges, for example, elevated metabolic demand or infusion of endothelium dependent agonists. One of the most heavily studied endothelium-dependent mechanisms studied has been the changes associated with nitric oxide (NO) production and release from the endothelium (Feletou et al., 2006; Ignarro et al., 1987). In a 2006 study of humans by Van Guilder et al., obese subjects were observed to have a reduced reaction to the vasodilator acetylcholine after intra-arterial infusion, relative to age matched lean subjects, while there were no differences to an intra-arterial infusion of sodium nitroprusside, a direct NO donor (Van Guilder *et al.,* 2006). Patients with metabolic syndrome also show blunted endothelium dependent dilation responses to infused vasodilators, relative to control patients, while endothelium-independent mechanisms remained intact (Steinberg *et al.,* 1996). These results have been shown in animal studies as well, as

both OZR and lean rats fed a high fat diet have been shown to have an impaired vasodilatory response of isolated micro vessels to endothelium-dependent NO agonists (Erdei et al., 2006; Frisbee et al.,2001). This lowered response is mediated by a NO signalling mechanism which can lead to a condition that can also impact muscle cell proliferation, platelet aggregation, macrophage action, and inflammatory markers. The NO signaling mechanism is based upon a balance of NO production, via NO synthase and NO removal systems, which can include the presence of scavenging via reactive oxygen species. While NO synthase activity has been maintained, or possibly increased under obese conditions; however Eringa et al. (2007) shows a decreases in e NOS protein levels in resistance arteries, which may link obesity with several confounding disease states (Katakam *et al.,* 2005; Fulton *et al.,* 2004; Karagiannis *et al.*, 2003; Eringa *et al.,* 2007). In addition, the obese animal has been shown to exhibit higher levels of oxidative stress markers than their lean counterpart (Pichhi *et al.*, 2006; Phillips *et al.,* 2005; Frisbee *et al.,* 2002). These animal models of obesity have shown improvements in the patterns of vasodilator reactivity when treated with antioxidants such as vitamin E, or an array of superoxide dismutase mimetics (Hodnett *et al.,* 2007; Frisbee, 2001). Thus, the decreased levels of NO noted in obesity may primarily reflect an increased scavenging via reactive oxygen species, resulting in the production of substances such as peroxynitrite (Frisbee, 2001; Chinen *et al.,* 2007). When arachidonic acid is introduced to the system, vasodilatory responses are attenuated in both gracilis and spinotrapezious arterioles of the OZR in comparison to the Lean Zucker rat (LZR) (Frisbee, 2001; Xiang *et al.,* 2006). The impaired responses appear to operate partially via an elevation in oxidant stress, but additional signaling pathways independent of acute alterations in oxidant tone appear to also be involved. Flow-mediated dilation initiates the release of endothelium-

derived relaxing factors, causing an increase in vessel diameter and therefore an increase in volume perfusion (Henrion, 2005). This dilation, an endothelium-dependent mechanism, has been shown to be impaired in obese patients, due to changes within the endothelium, which may be strongly linked to signaling mechanisms associated with chronic and evolving inflammation (Meyer *et al.,* 2006). Patients with metabolic syndrome have an impaired brachial artery flow-mediated dilation relative to normal control subjects while their response to an exogenous NO-donor was uncompromised (Hamdy *et al.*, 2003). Amelioration of the condition of metabolic syndrome through weight loss and exercise improves conduit reactivity, via brachial artery flow mediated dilation, although improvements to indices of resistance vessel reactivity are less sensitive to these improvements (Hamdy *et al.,* 2003; Raitakari *et al.,* 2004). Vasoconstriction is occasionally described in the literature as a non-vasodilation, or a decrease in a vasodilatory mechanism, specifically relating to a diminished NO production or signaling and leads to decreased muscle tissue perfusion. This is also the case within the realm of obesity-related vasoconstriction. Vasoactivity and vascular tone are based on a balance of circulating vasodilatory and vasoconstrictive factors; diabetic patients have shown increases in vascular constrictive factors and decreases in vasodilatory agents (Dandona *et al.,* 2003). Vasoconstriction to angiotensin-II is amplified in the obese animal (OZR), while reactivity to norepinephrine remains similar to the reactivity of the LZR; a similar pattern of reactivity has also been described in humans (Stepp *et al.,* 2007). Metabolic syndrome patients have shown increased levels of endothelin-1(ET-1), a potent vasoconstrictor, and an overall increase in response to vasoconstrictive agonists (Cardillo et al., 2002; Ferri et al., 1997). The profound impact mediated by prostanoid species and other metabolites of arachidonic acid on basal perfusion/resting vascular

tone as well as on hyperemic responses has been well established (Wilson and Kapoor, 1993; Beaty and Donald, 1979; Young and Sparks, 1979; Kilbom and Wennmalm, 1976). More recently, increased levels of vasoconstrictor prostanoids have also been suggested as a possible mechanism for the decreased vascular perfusion and dilator reactivity within obesity and the metabolic syndrome (Prasad *et al.,* 1999). In the OZR, an increase in vasodilation was noted when the prostaglandin H2/ thromboxane A2 receptor antagonistSQ-29548 was administered, suggesting that a chronic basal vasoconstrictor influence, mediated via the PGH2/TxA2receptor may contribute to impairments in organ perfusion and dilator reactivity (Xiang *et al.,* 2006). These changes to endothelial vasoconstrictor response with obesity may show an initial effect, while neurological mechanisms may show a longer lasting systemic role (Knudson *et al.,* 2007). Neural mediated vasoconstrictors have also been shown to play a part in basal vasoconstriction in hypertensive patients, due to a hyperactivity of the sympathetic nervous system (Esler *et al.,* 2001). Baseline and maximal diameters and arteriolar blood flow of obese animals are reduced compared with the lean counterparts, suggesting an increase in adrenergic tone and structural mechanisms to increase vascular resistance (Frisbee, 2006). Systemic adrenergic activity has not been found to be elevated when normalized to blood volume, instead, these changes may indicate a redistribution and possible remodeling of adrenergic activity within the obese animal, allowing increases in perfusion of the mesenteric system and decreases in perfusion of the skeletal muscles (Schreihofer *et al.,* 2005).

Phytotherapy:-

Diabetes mellitus is a metabolic disease as old as mankind and its incidence is considered to be high (4–5%) all over the world (Pickup and Williams, 1997). In spite

of the introduction of hypoglycemic agents, diabetes and related complications continue to be a major medical problem. Since time immemorial, patients with non-insulin requiring diabetes have been treated orally in folk medicine with a variety of plant extracts. Herbal medicines are popular remedies for diseases used by a vast majority of the world' population which have attained wide spread acceptability as therapeutic agents. The World Health Organisation has estimated that 80% of the world's population use botanical medicine for their primary health care needs. Since the availability of insulin and oral hypoglycemic drugs, traditional drugs for diabetes have almost declined. Sulfonylurea and metformin are valuable treatments for hyperglycemia in NIDDM but they are often unable to lower glucose concentrations to within the normal range, or to reinstate a normal pattern of glucose homeostasis (Bailey *et al.,* 1998). The use of these drugs is restricted by their pharmacokinetic properties, secondary failure rates, and accompanying side effects (Melander et al. 1988; De Smet 1997) and the World health organization expert committee on diabetes has listed as one of its recommendations that traditional methods of treatment for diabetes should be further investigated (WHO Expert Committee). Plants constitute an important source of active natural products, which differ widely in terms of structure and biological properties. They have a remarkable role in the traditional medicine in different countries. The protective effects of plant products are due to the presence of several components, which have distinct mechanisms of action; some of them are enzymes and proteins and others are low molecular weight compounds such as vitamins, carotenoids, flavonoids (Zhang and Wang 2002), anthocyanins and other phenolic compounds (Sanchez-Moreno et al. 1998). It is of interest in the context of flavonoid chemistry that attempts to "improve upon nature" have generally failed. Worldwide, over 1200 species of plants have been recorded as traditional medicine

for diabetes (Marles and Farnsworth, 1995). More than 400 different plant and plant extracts have almost been described for the treatment of diabetes throughout the world, but only a small number of these have received scientific and medical evaluation to assess their efficacy (De Smet 1997).

It is also worth noting that a number of drugs currently used to treat diabetes are historically derived from plant or fungal material. These include metformin (derived from *Galega officinalis*) (Oubr´e et al., 1997), acarbose (derived from *Actinoplances* spp.) (Marles and Farnsworth, 1995), and 4- hydroxyisoleucine (currently undergoing clinical trials, derived from *Trigonella feonum-feacum*) (Broca *et al.,* 2004).

Different traditional systems of medicine:-

In most societies today, allopathic and traditional systems of medicine occur side by side in a complimentary way. The former treats serious acute conditions while the latter is used for chronic illnesses, to reduce symptoms and improve the quality of life in a cost-effective way.

African traditional medicine

African traditional medicine is the oldest and perhaps the most diverse of all medicine systems. Africa is considered to be the cradle or Mankind with a rich biological and cultural diversity marked regional difference in healing practices. Unfortunately, the systems of medicines are poorly recorded and remain so to date. Yet the documentation of medicinal uses of African plants is becoming increasingly urgent because of the rapid loss of the natural habitats of some of these plants because of anthropogenic activities. The African continent is reported to have one of the highest rates of deforestation in the world. The paradox is that it is also a continent with a high rate of endemism with the Republic of Madagascar topping the list at 82%.

African traditional medicines in its varied forms, is a holistic involving both the body and the mind. The healer typically diagnoses and treats the psychological basis of an illness before prescribing medicines to treat the symptoms. Famous African medicinal plants include Acacia senegal (Gum Arabic), Agathosma betulina (Buchu), Aloe ferox (Cape Aloes), Aloe vera (North African Origin), Artemisia afra (African wormwood), Aspalanthus linearis (Rooibos tea), Boswellia sacra (Frankincense), Catha edulis (Khat), Commiphora myrrha (Myrrh), Harpagophytum procumbens (Devil_s Claw), Hibiscus sabdariffa (Hibiscus, Roselle), Hypoxis hemerocallidea (African potato), Prunus africana (African Cherry). Madagascar by herself has contributed with the Catharanthus roseus (Rosy Periwinkle) and has the potential of contributing more in view of the diversity of her flora and fauna.

American traditional medicine (North, Central and South)

In the US, just like in any other cultures, the indigenous healer or Shaman approaches illnesses by addressing both the physical and spiritual dimension of diseases. These Shamanistic ceremonies involve chanting, dancing and other rituals aimed at expelling evil forces so that the patient or the community as a whole can be healed. Early settlers learnt from native practices and they eventually adopted many of the herbal remedies, which later formed the basis of the Pharmacopeia of the United States. Among the famous medicinal plants of the United States are the Echinacea (Echinacea purpurea) and Goldenseal (Hydrastis canadensis). During most of the 20th century, herbs or botanicals have been regarded with scepticism and the practice of herbal medicine went into decline. Plants were viewed mainly as a potential source of pure chemical compounds for the development of medicine.

Central and South America

Just like Africa, Central and South American countries also have a rich and diverse healing culture, which are poorly known and have not been properly recorded. They will no doubt be a source of new herbal remedies in the years to come. South and Central America have made enormous contributions to agriculture and a large number of food crops (maize, potatoes, tomatoes, pumpkins, cassave, peanuts, sweet potato) originate from there. Traditional Indian medicinal herbs are also used extensively but the influence of Spanish, European, Indian and African culture is obvious. Famous examples of medicinal plants are: Cinchona pubescens (Peruvian bark), Erythroxylum coca (Coca), Ilex paraguariensis (Mate'), Myroxylon balsamum (Tolu balsam), Paullinia cupana (Guarana), Peumus boldus (Boldo), Psidium guajava (Guava), Spilanthes acmella (Brazilian cress), Tabebuia impetiginosa (Lapacho) and Uncarina tomentosa (Cat_s claw).

Australian and Southeast Asian medicine

This region has witnessed a resurgence of interest in traditional medicine and many countries now promote research into medicinal plants as a potential source of new remedies. The Aborigines had a complex healing system but much of the traditional knowledge in Australia was lost before it could be systematically recorded. In contrast, many healing places like Malaysia, Thailand, Vietnam, New Zealand, Borneo, and the Polynesian Islands remain intact and are being recorded and developed. A strong Chinese influence is being observed in most countries. Among the well-known medicinal products originating from this region are Croton tiglium (Purging croton), Duboisia hopwoodii (Pituri), Eucalyptus globulus (Bluegum), Melaleuca alternifolia (Tea tree), Myristica fragrans (Nutmeg and Mace), Piper methysticum

(Kava kava), Strychnos nux-vomica (Strychnine), Styrax benzoin (Benzoin) and Syzygium aromaticum (Cloves).

Chinese traditional medicine

With thousand years of medical practice, a great deal of valuable experience has been accumulated in the traditional Chinese medical system for diabetes therapy. In the traditional Chinese medical system, according to its clinical manifestations, diabetes mellitus is categorized as *Xiaokezheng* or *xiaodanzheng*, both of which mean diabetes. It is attributed to *yin*-deficiency diathesis, improper diet, emotional disorders, overstrain and excessive sexual activities. The main pathogenesis lies in consumption of *yin* fluid leading to endogenous dryness-heat in the body, with *yin* deficiency as the principal aspect and dryness-heat as the secondary aspect, and often with the presence of blood stasis and phlegm retention. If prolonged *yin* deficiency impairs *yang*, this will result in deficiency of both *yin* and *yang*

as well as deficiency of both *qi* and *yin*. Chinese doctors prescribe that diabetes would rather be treated through integrated care than only by lowering blood glucose (Zhu, 1982; Liu and Tang, 2000). The syndrome differentiation of the disease should aim at the predominance of *yin* deficiency or dryness heat. Generally at the onset, *yin* deficiency predominates. As the disease progresses, there appears the coexistence of *yin* deficiency and dryness-heat, and *yin* deficiency predominates again at the late stage. Clinically, the disease is classified as the following syndromes: fluid consumption due to lung heat, excessive fire in the stomach, deficiency of kidney *yin*, deficiency of both *qi* and *yin*, and deficiency of both *yin* and *yang*. The treatment is based on the principle of eliminating heat by nourishing *yin*, moistening dryness and promoting fluid production. According to the condition of the principal and secondary

aspects, deficiency and excess, as well as the location of pathological changes of the disease, the following methods are respectively adopted: clearing heat and purging fire, resolving phlegm to activate meridians, promoting blood circulation to remove blood stasis, removing dampness, nourishing the kidney to replenish *yin*, invigorating the spleen to tonify *qi*, replenishing both *yin* and *yang*, etc. Based on such a medical opinion, a general therapeutical rule is to promote blood circulation to remove blood stasis, and secondly to activate vital energy circulation, to clear away heat in the body, to invigorate liver and kidney to activate *yang*, and to invigorate the spleen and stomach to strengthen the body (Dai, 2000;

Xu and Lu, 2000). Traditional Chinese medical theory plays emphasis on integrated care of body, and then remove symptoms. Compound recipes are often used by Chinese doctors for diabetes treatment based on the fact that every herb in compound recipes can provide its special function, and lastly, form an integrated function for treatment of diabetes and complications. Single herb contains multi-ingredients, but these ingredients cannot play such a role as herbs in compound recipes. Most of western medicines, which are often made of a single chemical compound, are very effective for directly relief of symptoms, such as lowering blood sugar. So, in the Chinese medical system, it is considered that the efficacy of almost all western medicines to lower blood glucose is better than Chinese traditional medicines but not good for diabetic complications. Chinese medicines are more effective not only to treat and prevent diabetic complications but also at the meantime to lower blood glucose level. Therefore, Chinese doctors often make a combination of traditional medicine with western medicine, western medicine for reducing blood sugar, traditional medicine for integrated care of body (Cheng *et al.*, 1998).

Indian traditional medicine: AYURVEDA

Ayurveda is an ancient system of medicine practiced in India since the Vedic period, about more than 5000 years ago. The first recorded Ayurvedic medicine book, Charaka Samhita, was written in 600 BC (Schuppan *et al.,* 1999). The Ayurveda system relies strongly on preventive medicine and promotion of positive health. Ayurvedic preparations called Rasayanas are used to promote health. The Rasayanas are preparations from several plant extracts, which contain strong antioxidants and are used as rejuvenators or nutritional supplements (Govindarajan *et al.,* 2005; Sharma *et al.,* 1992; Thyagarajan *et al.*, 2002). Medicinal plant parts (roots, leaves, branches/stems, barks, flowers, and fruits) are commonly rich in phenolic compounds, such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins (Cai *et al.,* 2004; Ka¨hko¨nen et al., 1999; Larson, 1988). They have multiple biological effects including antioxidant activity (Tapiero *et al.,* 2002). The antioxidant properties of phenolic acids and flavonoids are due to their redox properties, ability to chelate metals and quenching of singlet oxygen (Rice-Evans *et al.,* 1996). Flavonoids, which are partly responsible for the pigmentation of flowers, fruits and leaves, are subdivided into flavanols, flavones, flavanones and anthocyanins based on the saturation of the flavan ring and also their hydroxylation. They occur mostly as glycosylated derivatives, sometimes conjugated with sulphate or organic acids (Youdim *et al.,* 2002). Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. 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 (Marles and Farnsworth, 1995). 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 officinalis. Thus, plants are a potential source of anti-diabetic drugs.

Phyto-Compounds and their mode of action:-

Phytochemistry deals with the chemistry of plant metabolites and their derivatives. The metabolic system of a plant may be regarded as being constituted of regulated processes within which biochemical conversions and mass transfer take place. Understanding in this field has advanced to a stage in which definite metabolic processes, biosynthetic pathways and their interconnections are distinguished and studied in the context of their function and genetic control. The metabolic performance of living organisms can be distinguished into primary metabolism and secondary metabolism. Primary metabolism is associated with fundamental life processes common to all plants. It comprises processes such as photosynthesis, pentose cycle, glycolysis, the citric acid cycle, electron transport, phosphorylation and energy regulation and management. Primary metabolites are produced and converted molecular entities, needed in anabolic pathways to build, maintain and reproduce the living cell. In catabolic pathways, primary metabolites (and food products) provide the chemical energy and precursors for biosynthesis. Primary and secondary metabolisms are interconnected in the sense that the biosynthesis of accumulating secondary metabolites can be traced back to ubiquitous primary metabolites. However, in contrast to primary metabolites, secondary metabolites represent features that can be expressed in terms of ecological, taxonomic and biochemical differentiation and diversity. The biosynthesis and accumulation of secondary

metabolites provide a basis for biochemical systematics and chemosystematics. In addition, the wide molecular diversity of secondary metabolites throughout the plant kingdom represents an extremely rich biogenic resource for the discovery of novel drugs and for developing innovative drugs. Not only do plant species yield raw material for useful compounds; the molecular biology and biochemistry provide pointers

for rational drug development. Primary and secondary metabolites can be classified on the basis of their chemical structure into much the same categories of chemical compounds: Carbohydrates, Lipids, Amino acids, Peptides, Proteins, Enzymes, Purine and pyrimidine derivatives. Within such classes of compounds, secondary metabolites generally show greater individuality and diversity in their molecular structure than primary metabolites. Certain compound classes also appear to be extraordinarily rich in secondary metabolites, e.g. the structurally diverse groups of alkaloids, phenolics, acetogenins and terpenoids. Ubiquitary primary metabolites belonging to these compound classes seem to be restricted to only a limited number of key compounds functioning as biosynthetic precursors. Most of the plant compounds that have been found to be medicinally useful and interesting tend to be secondary metabolites. Also, despite enormous structural diversity, nature just uses a few building blocks, e.g. Shikimic acid and Shikimate, to create this chemo-diversity. Shikimic acid accounts for the synthesis of many aromatic amino acids including phenylalanine, tyrosine, tryptophan as well as organic acids like benzoic and gallic acids and aldehydes like vanillin, benzaldehyde. The basic building blocks are the acetate (C2), isoprenoid (C5) and phenylpropanoid (C9) units. The acetate unit is used in the polyketide biosynthesis and is particularly well developed in microorganisms. The isoprenoid

pathways lead to all terpenoids by coupling two or more C5 units. The terpenoids are found in all organisms.

Alkaloids: The term _alkaloid_ has been defined as a cyclic organic compound containing nitrogen in a negative oxidation state, which has limited distribution in living organisms. Based on their structures, alkaloids are divided into several subgroups: nonheterocyclic alkaloids and heterocyclic alkaloids, which are again divided into 12 major groups according to their basic ring structure. Mescaline is an example of a non-heterocyclic or pseudo-alkaloid, Tetrandrine is another example of a bisbenzylisoquinoline alkaloid while Solasodine is a triterpene alkaloid. Free alkaloids are soluble in organic solvents and react with acids to form water soluble salts. There exceptions like Berberine, which is a quartenary ammonium alkaloid. Most alkaloids are solids except for Nicotine, which is a liquid. Alkaloids, usually having a marked physiological action on humans or animals, are believed to be waste products and a nitrogen source. They are thought to play an important role in plant protection and germination and to be plant growth stimulants. Alkaloids are more common in dicotyledons than in monocotyledons. Families reported to be rich in alkaloids are: Liliaceae, Amaryllidaceae, Apocynaceae, Berberidaceae, Leguminosae, Papaveraceae, Ranunculaceae, Rubiaceae and Solanaceae. Alkaloids are pharmaceutically significant, e.g. morphine as a narcotic analgesic, Codeine in the treatment of coughs, Colchicine in the treatment of gout, quinine as an anti-malarial, quinidine as an anti-arrythmic and L-hyoscyamine (in the form of its racemic mixture known as atropine) as antispasmodic and for pupil dilation.

Phenols are among the largest group of secondary metabolites. They range from simple structures with one aromatic ring to complex polymers such as tannins and lignins. Example of phenolic classes of pharmaceutical interests are: (1) Simple

phenolic compounds: These compounds have a monocyclic aromatic ring with an alcoholic, aldehydic or carboxylic group. They may have a short hydrocarbon chain. Capsaicin, isolated from Capsicum sp., is a vanillyl amide of isodecenoic acid and is marketed as an analgesic. Eugenol is widely used in dentistry due to its anti-bacterial and anti-inflammatory and local anaesthetic activities.

Tannins: The chemistry of these compounds is very complex. The distinction made in the literature between hydrolysable and condensed tannins is based on whether acids or enzymes can hydrolyse the components or whether they condense the components to polymers. Although not altogether watertight, this distinction largely corresponds to group based on gallic acid and those based on flavane-related components. Several vegetable tannins have been discovered but only the tanning constituents of the most important groups will be reported here, i.e. the group of gallotannins and ellagitannins, the group of proanthocyanidins. Gallotannins and ellagitannins are esters of gallic acid or its dimers digallic acid and ellagic acid with glucose or polyols. Tannins are able to react with proteins. Upon being treated with a tannin, a hide absorbs the stain and is protected against putrefaction and thereby becomes converted into leather. Although tannins are widespread in plants, their role is still unclear. They may be an effective defence against herbivores. Tannins are used against diarrhoea and as an antidote in poisoning by heavy metals. Their use declined after the discovery of hepatotoxic effects of absorbed tannic acids. Recent studies have reported that tannins have anti-cancer and anti-HIV activities.

Coumarins and their glycosides: Coumarins are, shikimate-derived, benzo-apyrone derivatives that are present in plants both in a free state and as glycosides. They have limited distribution in the plant kingdom and have been used in chemotaxonomy in order to classify plants. They give a characteristic odour of new-mown. They are

found in the following plant families: Apiaceae, Rutaceae, Asteraceae and Leguminosae. Common derivatives are: Umbelliferone, Herniarin, Aesculetin, Scopoletin, Fraxin and Chicorin. Quinones are oxygen-containing compounds that are oxidized homologues of aromatic derivatives and are characterized by a 1,4-diketo-cyclohexa- 2,5-diene pattern (paraquinones) or by a 1,2-diketo-cyclohexa-3,5-diene pattern (ortho-quinones). In naturally-occurring quinones, the dione is conjugated to an aromatic nucleus (benzoquinones), or to a condensed polycyclic aromatic system: naphthalene (naphthoquinones), anthracene (antraquinones), 1,2-benzanthacene (anthracyclinones), naphthodianthrene (naphthodianthrone), pyrelene, phenanthrene and abietane-quinone. Naphthoquinones and anthroquinones have some importance medicinally. Flavonoids are compounds that are responsible for the colour of flowers, fruits and sometimes leaves. The name refers to the Latin word _flavus_ meaning yellow. Some may contribute to the colour by acting as co-pigment. Flavonoids protect the plant from UV-damaging effects and play a role in pollination by attracting animals by their colours. The basic structure of flavonoids is 2-phenyl chromane or an Ar–C3–Ar skeleton. Biosynthetically they are derived from a combination of the Shikimic acid and the acetate pathways. Small differences in basic substitution patterns give rise to several subgroups. In the plant, flavonoids can either occur as aglycones or as O- or C-glycosides. Recently, flavonoids have attracted interest due to the discovery of their pharmacological activities as anti-inflammatory, analgesic, anti-tumour, anti-HIV, anti infective (anti-diarrhoeal, anti-fungal), anti-hepatotoxic, anti-lipolytic, anti-oxidant, vasodilator, immunostimulant and anti-ulcerogenic. Biologically active flavonoids comprise of hesperidin and rutin for decreasing capillary fragility and quercetin for its anti-diarrhoeal activity. Several

plants have been tested for their anti-diabetic potential. For most of them, the findings have been based on the ethno-botanical claims.

Anti-diabetic Plants:-

The present non-exhaustive list gives an overview of some plants with well-known profiles of anti-diabetic claims.

Aegle marmelos (Rutaceae) (Bael fruit)

This plant originating from India is used against diabetes. In Mauritius, the bark decoction, is drunk by people suffering from diabetes. The tests effected on the aqueous extracts of the root bark, as used by people in India, (1 ml/100gm) showed hypoglycaemic effect which peaked (44%) at 3 h in normal fasted rats. In addition, the same extract completely prevented peak rise or blood sugar at 1 h in OGTT. The hypoglycaemic activity was reduced upon storage of the extract. Aqueous extracts of the leaves (1 mg/kg for 30 days) significantly controlled blood glucose, urea, body weight, liver glycogen and serum cholesterol or alloxanized (60 mg/kg IV) rats as compared to controls and this effect was similar to insulin treatment (Ponnachan *et al.,* 1993). When fed as aqueous leaf extract (1gm/kg/day) to STZ (45 mg/kg IV) diabetic rats for 2 weeks, it decreased malate dehydrogenase levels (an enzyme known to increase in diabetes) in comparison to diabetic controls. The extracts were equi-effective in comparison to insulin in restoring blood glucose and body weight to normal levels (Seema *et al.,* 1996). It must be reported that aqueous leaf extracts admistered orally for 28 days also normalized STZ (45 mg/kg body weight) induced histo-pathological alterations in the pancreatic and kidney tissues of rats (Das *et al.,* 1996).

Allium sativum (Liliaceae) (Garlic)

This perennial herb is cultivated almost throughout the world and is used as a food ingredient. Experiments have shown that an oral administration of 0.25gm/kg of ethanol, petroleum ether, ethyl ether extract of Allium sativum cause 18.9, 17.9, 26.2% reduction of blood sugar in alloxan-diabetic rabbits (150 mg/kg). Oral administration of 0.25gm/kg allicin (isolated from Garlic) produced hypoglycaemia comparable to tolbutamide in mildly diabetic rabbits (glucose level ranging from 180–300 mg%) while it showed no effect on severely diabetic animals (>350 mg%) (Grover et al., 2002). Aqueous homogenates of garlic (10 ml/kg/day) administered orally to sucrose fed rabbits (10gm/kg/day) in water for 2 months) significantly increased hepatic glycogen and free amino acid contents, decreased fasting blood sugar, triglyceride levels in serum, liver and aorta and protein levels in serum and liver in comparison to sucrose controls. It has been shown also that oral feeding or garlic extracts (100 mg/kg) increased cardiovascular functions in STZ rats, prevented abnormality in lipid profile and increased fibrinolytic activities with decreased platelet aggregation. Plasma insulin level increased with concomitant decrease in plasma glucose levels. In addition, daily oral feeding of the same dose for 16 weeks showed anti-atherosclerotic effects in STZ diabetic rats. Thus garlic may prevent diabetic cardiovascular complications (Patumraj et al., 2000).

Aloe barbadensis (Asphodelaceae) (Aloe vera)

This plant is cultivated widely as an ornamental plant locally but in many countries, Aloe vera is cultivated on commercial scale for its gel and plant extracts. The latter are recommended in Ayurveda for managing painful conditions and it is also mentioned in other Pharmacopeias, namely the Arabic Pharmacopeia, as being useful in managing diabetes. Extracts of aloe gum effectively increased glucose tolerance in

both normal and diabetic rats. Chronic but not single administration of the leaf exudates at a certain dose (500 mg/kg PO) showed significant hypoglycaemic effect in alloxan-diabetic mice. Nonetheless, single as well as chronic administration of the bitter principle (5 mg/ kg IP) showed significant hypoglycaemic effect in the same model. The hypoglycaemic effect of single dose of the bitter principle was extended over a period of 24 h with maximum hypoglycaemia observed at 8h while chronic administration (exudates twice daily and the bitter principle once a day for 4 days) showed maximum reduction in plasma glucose level at the 5th day. Hypoglycaemic effect of aloe and its bitter principle is mediated through the stimulation of synthesis and or/release of insulin from the b-cells of Langerhans (Ajabnoor, 1990).

Momordica charantia (Cucurbitaceae) (Karela, Bitter gourd)

The juice, extracted from the various plant parts (fruit pulp, seeds, leaves and whole plant), is very common folkore remedy for diabetes. When tested on laboratory animals, M. charantia has shown hypoglycaemic as well as anti-hyperglycaemic activity. Polypeptide-p isolated from fruit, seeds and tissue of M. Charantia showed potent hypoglycaemic effects when administered subcutaneously to gerbils, langurs and humans. The aqueous extracts of M. charantia improved OGTT after 8 h in normal mice and reduced hyperglycaemia by 50% after 5 h in STZ diabetic mice. In addition, chronic oral administration of extract to normal mice for 13 days improved OGTT while no significant effect was seen on plasma insulin levels. Another study carried out recently on M. charantia fruit extracts has shown that the latter had a direct impact on transport of fluid in vitro. Everted intestinal sacs from rats mounted in an organ bath containing Kreb solution was used. It was observed that M. charantia extract had a direct impact on water transport with increasing inorganic phosphate concentration with or without D-glucose in the buffer. In the control experiment, fluid

intake was greatly enhanced at high inorganic phosphate concentration (8–10 mM) in the presence of 5.5 mM D-glucose. The addition of 3.0 mg/ml M. charantia extract to the serosal side inhibits the uptake of fluid significantly. It has been hypothesized that an increase in inorganic phosphate enhances oxidative phosphorylation thereby increasing the fluid uptake across everted intestinal sacs of rats. This would point to the fact that M. charantia extracts reduced fluid absorption capacity and this may be because of interference with the carrier-mediated coupled entrance of glucose and Na+ across the brush-border membrane (Mahomoodally et al., 2004).

Murraya koenigii (Rutaceae) (Curry leaf, Carripoule)

The Curry leaf is an inevitable ingredient in Indian recipes. It is extensively used as a flavouring agent both in curries and chutney. It has been shown than an oral feeding of Murraya koenigii leaves diet (10% w/w) for 60 days to normal rats showed hypoglycaemic effect associated with increased hepatic glycogen content due to increased glycogenesis and decreased glycogenolysis and gluconeogenesis (Khan et al., 1995). Dietary supplement with curry leaves has been shown to increase lecithin cholesterol acyl transferase activity (Khan et al., 1996). Curry leaf powder supplementation (12 g providing 2.5 g fibre) for a period of 1 month in 30 NIDDM patients showed reduction in fasting and post-prandial blood sugar levels at 15-day period with no significant changes in serum glycosylated cholesterol fraction, serum lipids, lipoprotein cholesterol levels, uronic acid and total amino acids (Iyer and Mani, 1990).

Ocimum sanctum (Lamiaceae) (Tulsi, Holy Basil)

This herb, considered to be sacred by Hindus, is commonly planted next to temples generally. It is also an ornamental plant and is grown in gardens. The traditional pharmacopeia reports on the use of this plant against diabetes. In 1968, Dhar et al. In

Alarcon-Aguilera et al., 1998 reported hypoglycaemic effect of the ethanolic extracts of the leaf. The ethanol (70%) leaves extract or Ocimum sanctum has been shown to cause significant reduction of blood glucose level in normal, glucose fed hyperglycaemic and STZ (50 mg/kg IP) induced diabetic rats. This effect was 91.55 and 70.43%of that of Tolbutamide in normal and diabetic rats respectively. Diet containing leaf powder (1%) fed to normal and diabetic rats for 1 month significantly reduced fasting blood sugar, uronic acid, total amino acids, total cholesterol, triglycerides and total lipids (Rai et al., 1997). This plant has also demonstrated anti-oxidant and hypolipidemic effect (Kelm et al., 2000).

Syzygium cuminii (Syn. Eugenia jambolana) (Myrtaceae) (Jamblon, Java plum)

This herb, widely distributed throughout India and Africa, is commonly used against diabetes. The decoction of the dried leaves and bark as well as the seeds, have shown hypoglycaemic effect. Oral feeding of S. cuminii (170, 240, 510 mg/rat for 15 days) caused 50% reduction of blood glucose or normal fasted rats while chlopropamide showed 52% reduction. In addition, there was a 2.4, 6.8-fold and9.2-fold increase in cathepsin B activity (proteolytic conversion or pro-insulin to insulin) by plant extract and chlorpropaide respectively (Bansal et al., 1981). Oral administration of the fruit pulp extract to normoglycemic and STZ induced diabetic rats showed hypoglycaemic activity in 30 min possibly mediated by insulin secretion. In addition, the extract inhibited insulinase activity from the liver and kidney. Oral administration or the aqueous extract or the seeds (2.5 and5.0 mg/kg for 6 weeks) showed hypoglycaemic (>glibenclamide) and anti-oxidant activity. Daily administration of lyophilized powder of E. jambolana (200 mg/kg)showed maximum reduction or 73.51, 55.62 and 48.81 as compared to their basal values in mild (plasma sugar > 180 mg/dl, duration 21 days), moderate (plasma sugar > 280 mg/dl, duration 120 days) and severe (plasma

sugar > 400 mg/dl, duration 60 days) diabetic rats. In addition, the treatment also partially restored altered hepatic and skeletal muscle glycogen content and hepatic glucokinase, hexokinase, glucose-6-phosphate and phosphofructokinase levels (Grover et al.,2000).

Trigonella foenum-graecum (Apiaceae) (Fenugreek seeds)

This plant is a very commonly used herb in Indian cooking. It is also popular in traditional medicine as a hypoglycaemic agent. This hypoglycaemic effect of fenugreek seeds has been demonstrated in experimentally induced diabetic rats, dogs, mice and healthy volunteers (both IDDM and NIDDM). The isolated fibres, saponins and other proteins from the seeds were given with meals for 21 days to alloxan-diabetic dogs. Significant anti-hyperglycaemic, antiglycosuric effect along with reduction in high plasma glucagons and somastatin (Ribes et al., 1986) were observed. Oral administration of 2 and 8 g/kg of plant extract produced fall ($p < 0.05$) in blood glucose both in the normal as well as diabetic rats. 4-Hydroxyisoleucine, a novel amino acid, extracted and purified from fenugreek seeds, has been found to increase glucose-induced insulin release (ranging from 100 lmol to 1 lmol) through a direct effect on the isolated islets of Langerhans in both rats and humans. The pattern of insulin secretion was biphasic, glucose-dependent, occurred in the absence of any change in pancreatic alpha and delta cell activity and without interaction with other agonists or insulin secretion (such as leucine, arginine, tolbutamide, glyceraldehyde).In clinical trials, administration of fenugreek seed powder (50gm each with lunch and dinner) in insulin-dependent (Type 1) diabetic patient for 10 days significantly reduces fasting blood sugar and improved OGTT along with 54% reduction in glycosuria. In addition, it also showed significant hypolipidemic effect (Sharma et al., 1990).

Allium sativum (Liliaceae) Garlic

This perennial bulbous herb has been used since time immemorial as a culinary herb. It is particularly notorious because of its characteristic and persistent pungent smell and acrid taste. This is due to the number of sulphur compounds and the main one being alliin. The latter undergoes enzymatic hydrolysis by alliinase to produce allicin when the garlic pod is crushed. Allicin forms a wide range or compounds such as allyl methyl trisulphide, diallyldisulphide, ajoene and many others, which are volatile. Garlic has also been used in traditional medicine to treat asthma, bronchitis, as an expectorant, aphrodisiac, antihelmintic, anti-fungal and also to thin the blood. Experimental pharmacology has shown that the essential oil, water and ethanol extracts of the garlic bulb extract exhibits a wide range of anti-bacterial and anti-fungal activity against a wide range or pathogens. The antimicrobial and antihelminitic activities have been attributed to the presence of allicin. Ajoene and diallyl trisulphide also have anti-bacterial and anti-fungal activities. The properties for which garlic (both essential oil and extracts) is also well known for, are its ability to lower cholesterol and plasma lipids, lipid metabolism and atherogenesis, both in vitro and in vivo. Anti-hypercholesterolaemic and anti-hyperlipidaemic effects have been observed in various animal models after administration of garlic extracts. Clinical studies on serum lipids and lipoproteins reviewed 25 randomized controlled trials, with daily doses or garlic over a period or 12 weeks showed a12% average reduction or the total cholesterol and 13% in serum triglycerides. Meta analysis of the clinical studies confirmed the lipid-lowering and cholesterol actions of garlic.

Standardization and Quality control of Herbal drugs:-

Standardisation is a method of assuring a minimum level of active ingredients in the extract and is becoming increasingly important as a means of ensuring a consistent supply of high-quality phyto-pharmaceutical products. It can be defined as the establishment of reproducible pharmaceutical quality by comparing a product with established reference substances and by defining minimum amounts of one or several compounds or groups of compounds. In the field of phyto-medicines, standardization only applies to extracts. Standards for active ingredients to be used in medicinal products may be found in monographs and/or pharmacopeias. Although it is a well-known fact that these compounds are among the most important ones, other compounds are also important and their role and action must also be understood as they may well add to the pharmacological activity of the extract.

Quality control

Microscopy is less important here as opposed to phytochemical methods. In the case of the crude drug, Thin Layer Chromatography is only feasible for some components like the flavonoids. The presence of vital minor compounds may be masked and may not be very visible. It is in this particular instance that the complementarity of analytical methods like HPLC and GC are of paramout importance for analyzing both the lead and the minor compounds.

Side-effects (toxicity) of plant extracts

Botanical secondary compounds are not benign molecules; ecologically speaking, these evolved as chemical defences that can repel, stun, poison or kill other species. It would be naive on anybodys behalf to think that every plant extract is necessarilysafe for human consumption. It is precisely for these reasons that poison center shave been

established across several continents. Nonetheless, it would be difficult to distinguish between an effective medecinefrom a deadly poison. The dosage is critical in these circumstances especially as some plants with a long history of use have been implicated as being potentially toxic. Among such plants are, amongst others, the Comfrey (Symphytum officinale) which has been reported to cause acute liver damage; Yohimbe (Corynanthe yohimbe) isused as a dietary supplement and aphrodisiac but at high doses, it is suspected able to cause kidney failure and death.

Adulteration

Over and above the dosage factor, is adulteration either accidentally or intentionally. Foxglove (Digitalis purpurea) has shown up as an adulterant in herbal mixture where it is not among the listed ingredients. Sometimes herbal remedies have been found to be tainted with heavy metals. In other instances, when one medicinal plant has been replaced by another similarly looking one, this led to disastrous consequences. Although Aristolochia species are used in Chinese medicine, the replacement by Aristolochia fangchi has caused the death of several patients who were attending a weight loss clinic in Belgium (Schaneberg and Khan, 2004).

Microbial contaminations

With requirements of Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) being improved more and more on producers of medicinal plants, Microbial contamination can be controlled. It is a fact that natural materials harbour a large number of spores and other microorganisms but nonetheless, the maximum number or microorganisms allowed is regulated in most Pharmacopeia. The European Pharmacopeia, for example, excludes the presence of Escherichia coli and Salmonella sp. and limits aerobic microorganisms up to 105 per g or ml and this includes up to 103 yeast and fungi per g or ml and up to 103 enterobacter per g or ml.

Due to economic constraints, providing modern medical healthcare in developing countries such as India is still a far-reaching goal. The most commonly used drugs of modern medicine such as aspirin, anti-malarial, anti-cancer, digitalis, etc. have originated from plant sources. Out of an estimated 250000 higher plants, less than 1% have been screened pharmacologically and very few in regard to DM. Therefore, it is prudent to look for options in herbal medicine for diabetes as well. Such an ethnomedical approach for diabetes is a practical, cost-effective and a logical for its treatment. The goals of medicine no matter to which group it belongs, are the same i.e. the welfare of the patient. One can look towards a future of integrated medicine and hope that research in alternative medicine will help identify what is safe and effective rather than marginalising, unorthodox medical claims and findings.

Most of the studies that have been reviewed include dietary interventions carried out on animals and only a few were conducted on humans. Since, these food materials form a major portion of our diet it is imperative that systematic studies be designed and carried out over a long duration in suitable populations so as to evolve major dietary recommendations.

Taxonomic classification

Kingdom: Plantae

Phylum : Tracheophyta

Class : Magnoliospida

Subclass : Dilleniidae

Superorder : Urticanae

Order ; Urticales

Family : Urticaceae

Genus : *Oreocnide*

Specific epithet : *integrifolia* (Gaudich) Miquel

Synonyms : Villebrunea integrifolia

Common name: Wild Rhea

- Assamese - Ban rhea, Bon rhea, Chho-oi-paroli
- Garo- Khilkhra, Sejugbu, Gingsining
- Khasi- Dieng teingbah, Tillejwat

Morphological Features: They are small trees or shrubs 5-20 m tall; bark grayish brown or brown gray; branchlets reddish brown; and petioles grayish brown velutinous, glabrescent, or sparsely appressed pubescent. Stipules linear, 1-2 cm; petiole 1-9 cm; leaf blade adaxially green, then light green, becoming grayish green when dry, elliptic, oblong, oblong-lanceolate, or oblanceolate, 8-33 × 3.5-12 cm, papery, 3-veined, basal pair reaching middle margin, secondary veins 8-12 pairs, reticulate, abaxial surface densely villous or sparsely pubescent on veins, or sometimes tomentose, adaxial surface glabrous, base rounded or obtuse, margin denticulate to middle, entire apically, apex caudate to long caudate-acuminate. Inflorescences in axils of fallen leaves or on older branches, dichotomously branched 2 or 3 times, 1.5-2.5 cm; glomerules 4-5 mm in diam. Male flowers: perianth lobes 4, oblong, connate 1/2 of length, ca. 1.2 mm; rudimentary ovary subclavate. Female flowers ca. 1 mm. Achene conic, ca. 1.5 mm, 3- or 4-ribbed, surrounded by a fleshy discoid cupule at base.

Flowering: Mar-May; Fruiting: Jul-Sep.

Distribution: Rain forests, valleys, 200-1400m; Bhutan, North east India, Indonesia, Laos, Myanmar, Sikkim, Thailand, Vietnam.

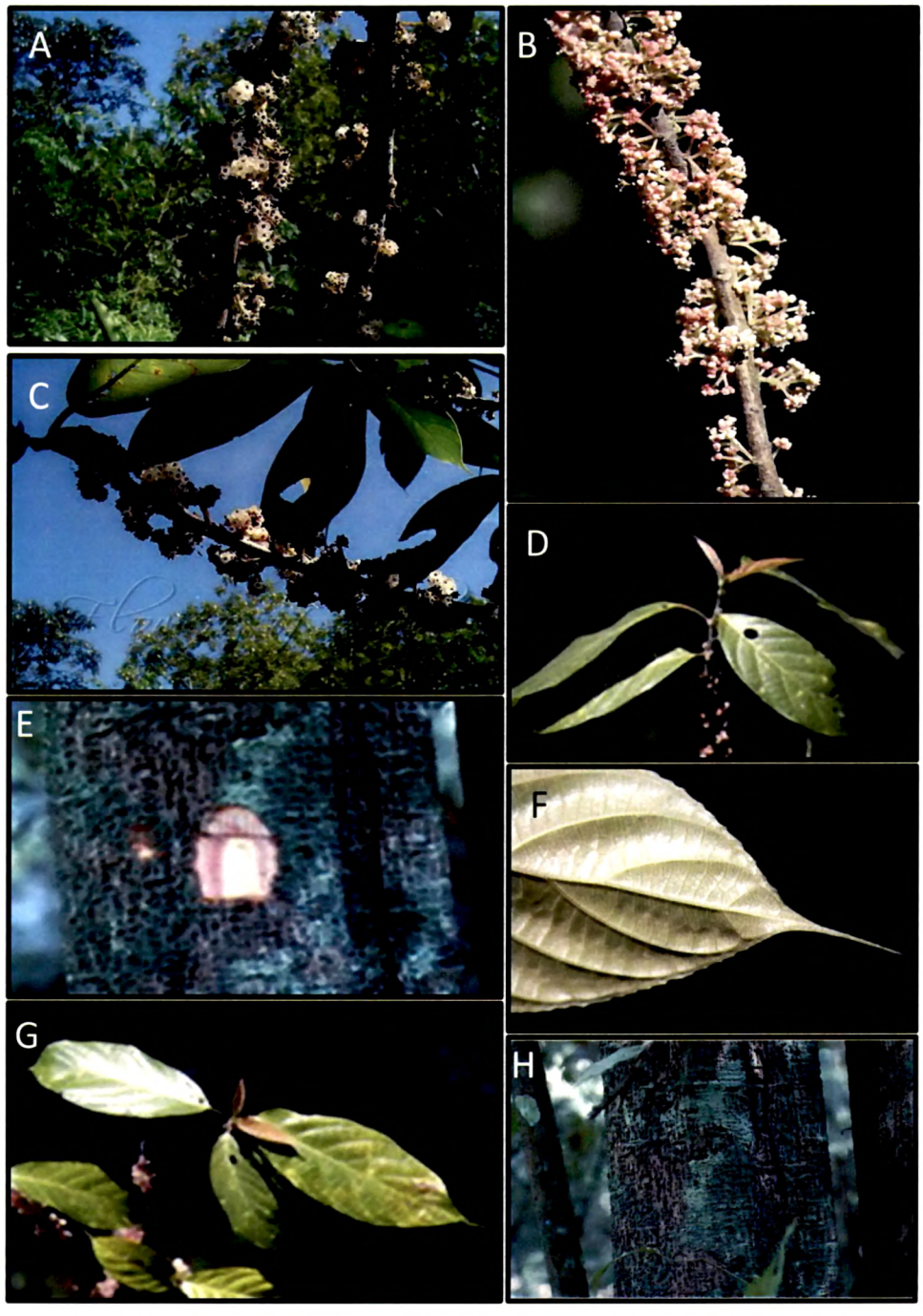
Medicinal uses: Leaf decoction used to alleviate diabetic symptoms, maintains blood pressure and ginger powder mixed with leaf powder for treatment of skin rashes

Commercial uses: Used for making ropes (*Boree*), fishing nets etc due to high fibre content.

Pharmacology: No previous scientific reports

Chemical constituents: No previous scientific reports

Oreocnide integrifolia (Gaud.)Miq



Images of *Oreocnide integrifolia* fruits (A,C) ; flowers (D,F,G) and bark (E,H)

Photo Courtsey : Prashant Awale, Juliana prosperi

Herbarium Sheet of *Oreocnide integrifolia* (Gaud.) Miq

OBJECTIVES:

1) To evaluate the dose and duration dependent hypoglycemic and hypolipidemic potential of *Oreocnide integrifolia* (Gaud.) Miq aqueous leaf extract (OI) on streptozotocin induced type-1diabetic model.
2) To assess the potential of OI extract on experimentally induced hypertension and insulin resistance using high fructose fed *Charles foster* rats.
3) To evaluate the mechanism of OI extract in terms of glucoregulation and its possible mediation through insulin signaling pathway and its role in leptin regulation in high fat diet induced type-2 diabetic *C57BL/6J* mice.
4) To screen the phytochemicals present using chromatography techniques and to evaluate the insulin secretogogue potential of OI extract & its fractions through bioactivity guided approach using *in-vitro* and *in-vivo* models.
5) To assess the potential of active fraction(s) for their beta-cell regeneration/islet neogenesis using pancreatectomized mouse model.