Chapter-2
Literature Review

2.1 Diabetes mellitus

Diabetes mellitus is a metabolic disease resulting from modification in the accessibility and use of the pancreatic hormone insulin. In Greek the word **diabetes** meaning is siphon and refers to the marked loss of water by urination (Polyuria). The **mellitus** comes from the Latin word for sweet or honey. Virchow (1821-1902) and others described the lesions of pancreas which leaded to Minkowsky (1858-1931) to hypothesize that the pancreas is somehow involved in diabetes mellitus, finally Frederick Banting and Charles Best (1921) confirmed that insulin is produced from the beta cells of pancreas and abnormal production of insulin is the main cause of diabetes mellitus (Guthrie and Guthrie 2008).

Diabetes mellitus or hyperglycemia is a condition in which excessive amounts of glucose merge in the blood plasma. It is a metabolic disorder resulting from defects in insulin secretion, insulin action or both which is characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism (WHO 1999). The autoimmune destruction of the pancreatic β-cells consequently causes the deficiency of secretion and action of insulin. Indeed abnormal action of insulin to the metabolism of carbohydrate, fats and protein on the target tissue consequently results the development of diabetes mellitus. (ADA 2014). Apoptosis is probably the main cause of progressive failure or death of β-cell. The execution of β-cell death arises through activation of mitogen-activated protein kinases, via triggering of endoplasmic reticulum (ER) stress and by the release of mitochondrial death signals. Elevated levels of chronic exposure of glucose and free fatty (FFAs) cause β-cell dysfunction and may induce β-cell apoptosis in type 2 diabetes (Cnop et al. 2005). The impact of a 20-50 % loss of β-cells function has been associated with the deposition of amyloids in islet cells and associated with disproportionate hyperproinsulinemia and late results to

progressive hyperglycemia (Porte and Kahn 2001).The progressive loss of pancreatic β-cell mass function and its resistance results in chronic hyperglycemia with the risk of micro and macro vascular complications (Fonseca 2009).

An amalgamate hypothesis whereby hyperglycemia and FFA induced activation of the nuclear factor –κB, p38 MAPK, and NH_2-terminal Jun kinases or stress-activated protein kinases, stress pathways along with the activation of the advanced glycosylation end products protein kinase C and sorbitol stress pathways play a main role in causing late complications in type1 and type 2 diabetes, along with impaired insulin secretion and insulin resistance in type 2 diabetes (Evans et al.2002).

2.1.1 Signs and Symptoms: The temporary diabetes mellitus is often asymptomatic. The chronic hyperglycemia can produce a complicated symptoms associated with acute or chronic hyperglycemia, polyphagia (hunger), polydipsia (thirst), polyuria (urination), blurred vision, fatigue, weight loss, poor wound healing, dry mouth, dry and itchy skin, tingling in feet or heels, erectile dysfunctions, recurrent infections, external ears infections (swimmer's ear), cardiac arrhythmia, stupor, coma, seizure and may be death (ADA 2006). The long term effects of diabetes mellitus leads to the renal failure with risks of ulcers, amputation, Charcot joints and cardiovascular, peripheral vascular and cerbrovascular diseases, autonomic dysfunctions, including sex dysfunctions, increase incidence of atherosclerotic cardiovascular, peripheral arterial and cerebrovascular disease (ADA 2004). The effects of diabetes mellitus comprised of long term dysfunction, damage and failure of various organs, particularly; eyes, heart, kidney, nerves and blood vessels (WHO 1999). The progressive development of the retinopathy with probable blindness, nephropathy with renal failure, neuropathy with risk of foot ulcers, amputations are the compilations of diabetes mellitus (DCCTRG 1993).

When the body does not have adequate insulin, it is not able to utilize the glucose; instead metabolic process of body starts to break down the fats for energy and increase total concentration of body ketones which is known as Ketoacidosis. The signs

and symptoms of the diabetic ketoacidosis (DKA) may include – deep and rapid breading, confusions or decreases the consciousness, dehydration due to glycosuria and osmotic diuresis, acute hunger and thirst, fruity smell of breathing, increased sadness and anxiety, heart attacks with coronary heart failure and death (Kitabchi et al. 2009). The blood glucose level more than 600mg/ dL without ketosis and with an effective plasma osmolarity more than 320 mOsm/L is known as Hyperosmolar Hyperglycemic State (HHS) and is results in the deficiency of insulin (Bhansali 2018). The proxy symptoms as hypoglycemia may also occur due to excessive medication for the insulin or dieting (ADA 2004).

2.1.2 Diagnostic criteria for Diabetes Mellitus: The classification and tests for diagnosis of diabetes mellitus were brought into order (NDDG 1979 and WHO 1980). The diagnostic fasting plasma (blood) glucose value is ≥7.0 mmol/L. The Impair Fasting Glycemia (IFG) is proposed to encompass value above the normal but below the diagnostic cut-off for diabetes, plasma <7.0 mmol/L, while whole blood plasma is 6.1mmol/L. The Gestational Diabetes Mellitus (GDM) includes gestational impaired glucose tolerance (Alberti & Zimmet 1998).

A fasting venous plasma glucose concentration of less than 6.1mmol/L (110mg/dL) has been known as normoglycaemia (WHO 1999), hyperglycemia generally refers the blood sugar above 140 mg/dL, but the hypoglycemia is the blood sugar below 70mg/dL. Severe hypoglycemia <40 mg/dL and admission A1C value ≥6.5% suggests pre-existing diabetes mellitus (ADA 2016).

On diagnosis and classification of diabetes mellitus the expert committee recognised an intermediate group of individuals whose glucose levels do not meet criteria for diabetes. The Impaired Fasting Glucose (IFG) who has Fasting Plasma Glucose (FPG) levels 100mg/dL (5.6mmol/L) to 125mg/dL (6.9mmol/L), or Impaired Glucose Tolerance (IGT) who has 2h values in the Oral Glucose Tolerance Test (OGTT) levels of 140 mg/dL (7.8mmol/L) to 199mg/dL (11.0mmol/ L) (ADA 2014).

The Fasting Plasma Glucose (FPG) is the blood sugar when one has been fasting for at least 8 hours. It is especially checking up of blood sugar

early in the morning which ranges 80-130mg/dL. Postprandial Glucose (PPG) is the check up of blood sugar that about 2 hours after eating meal which is less than 180mg/dL and the Random Blood Sugar (RBS) is the check up of blood sugar more than 2 hour after eating the meal which ranges 79-140mg/dL and between 140 -200mg/dL is considered as pre-diabetes and >200mg/dl is considered as diabetes mellitus (ADA 2016).

2.1.3 Standard glucose abnormalities of Glycemia:

Hyperglycemia: >140mg/dL (7.8mmol/L)

Hypoglycemia:<70mg/dL(3.9mmol/L)

Severe hypoglycemia:<40mg/dL (2.2mmol/mol)

Admission A1C Value: ≥ 6.5% (48mmol/mol) suggests free existing diabetes mellitus (ADA 2016).

2.1.4 Types of diabetes mellitus:

The American Diabetes Association (ADA) summarized that the clinical diabetes is divided into four general subclasses. **Type-1** is primarily originates by autoimmune pancreatic β-cell destruction and characterized by absolute insulin deficiency. **Type–2** is characterized by insulin resistance and relative insulin deficiency. **Gestational Diabetes Mellitus** characterized by the high blood glucose level during the fragment. **Other Specific Types** of diabetes which is associated with identifiable clinical conditions or syndromes. In addition to these clinical categories, impaired glucose tolerance, impaired fasting glucose and a high glycohemoglobin (hemoglobin A1c [HbA1c] 5.7 to 6.4%) sometimes referred to as **prediabetes** which describes as intermediate metabolic states between normal glucose homeostasis and overt diabetes (Inzucchi & Sherwin 2011).

2.1.4.1 Type-1 diabetes mellitus: Type-1 diabetes mellitus is juvenile onset diabetes which results from the autoimmune destructions of the insulin producing β-cells in the pancreas. The absolute lack of insulin is due to breakdown of islet cells in pancreas and is genetically transmitted character to the offspring. Progression of type-1 diabetes or Insulin Dependent Diabetes Mellitus (IDDM) disease involves genetic as well as environmental factors. The environmental component of IDDM susceptibility is not well understood although viral infection has been suggested as a triggering event (Noble

et al. 1996). The development of type-1 diabetes mellitus is a series of stages beginning with genetic susceptibility and ending with complete or nearly complete β-cell destruction associated with the C-peptide secretion (Eisenbarth 2005). The human leukocyte antigen genotype, HLA-DQ on chromosome 6 is the major genetic factor for type-1 diabetes. The genotype HLA–DQB1*0302-A1*0301(DQ8) and HLA–DQB1*0201-A1*0501 (DQ2) confer the high risk for type-1 diabetes mellitus. The pathogenesis seems to be marked by auto antibodies against the glutamic acid decarboxylase (GAD65Ab), insulinoma associated protein-2 (IA2Ab) and insulin, alone or in combination are responsible for the type-1 hyperglycemic effects in the human being (Larsson et al. 2005). Islet cell autoantibodies are strongly associated with the development of type-1 diabetes. The appearance of autoantibodies to one or several of the autoantigens–GAD65, IA-2, or Insulin signals an autoimmune pathogenesis of β-cell killing. A β-cell attack may be best reflected by the emergence of autoantibodies dependent on the genotype risk factors, isotype and subtype of the autoantibodies as well as their epitope specificity (Pihoker et al. 2005). Some form of type-1 diabetes has no known etiologies. These patients have permanent insulinopenia and are prone to ketoacidosis and exhibit varying insulin deficiency between episodes, but have no evidence of autoimmunity and are not HLA associated. The rate of β-cell killing in infants is rapid and in adults it is slow (ADA 2010).

2.1.4.2 Type-2 diabetes mellitus: Type-2 diabetes accounts for approximately 90-95% globally. Previously it was referred as non-insulin-dependent diabetes, or adult-onset diabetes, encompass individuals who have insulin resistance or insulin deficiency. This form of diabetes is undiagnosed for years because it develops in slowly and at the earlier stages no enough symptoms of diabetes are noticed. It develops enough macrovascular and microvascular complications. The insulin level may appear normal or elevated. It may lead the to reduction of body weight. Ketoacidosis rarely occurs in this type of diabetes (ADA 2010). The risk of this type of diabetes increases with the age, obesity and lack of physical activity. It occurs more frequently in women with prior

GDM and in individuals with hypertension or dyslipidaemia. It is also associated with a strong genetic predisposition (Zimmet 1992). Type-2 diabetes mellitus is a long term metabolic disorder which leads to the high blood sugar due to the abnormal diet. The dysfunction of the pancreatic β-cell relatively produces insufficient insulin. Thus the excess sugars contents of food supplement in digestive tract become indigestible and transfer to the blood stream randomly (Humphreys 1997).

Oxidative stress is produced under diabetic conditions and is likely involved in progression of pancreatic β-cell dysfunction. The low level of antioxidant enzyme expressions and pancreatic β-cells are vulnerable to oxidative stress. Oxidative stress and consequent activation of the JNK (Jun Amino-Terminal Kinases) pathway are involved in progression of β-cell dysfunction found in diabetes. Antioxidants may serve as a novel mechanism-based therapy for type 2 diabetes (Kajimoto and Kaneto 2004).

2.1.4.3 Gestational Diabetes Mellitus (GDM): It is the carbohydrate intolerance in the onset of pregnancy. A woman with the GDM increases the risk of future type 2 diabetes. In this aspect the new born infants may suffer with the risk of diabetes and obesity. According to the International Association of Diabetes and Pregnancy Study Groups (IADPSG), value of fasting plasma glucose (FPG), 1-h and 2-h plasma glucose concentrations meet or exceed 92mg/ dL, 180 mg/dL and 153mg/dL respectively with 75g oral glucose tolerance test (OGTT) (Banerjeeg et al. 2012). The change of hormonal activity in women body increases the nutrient-stimulated insulin responds despite a minor deterioration in glucose tolerance and it shows a consistent progressive insulin resistance. In pregnancy metabolic processes do not completely compensate, so the glucose intolerance occurs and a predisposition to type 2 diabetes mellitus results and gluconeogenesis increases in late gestational period (Butte 2000).

The cellular membrane protein, glycoprotein-1(PC-1) has known as an inhibitor of the Insulin Receptor Tyrosine Kinase (IRTK) activity. The GDM woman shows the increased PC-1 content and suggests the excessive phosphorylation of serine / threonine residues in muscle insulin

receptors and it may contribute to decreased IRTK activity. These post receptor defects in insulin signaling may contribute to the pathogenesis of GDM and increases the risk of type-2 diabetes mellitus (Shao et al. 2000).

2.2 Pancreas

Pancreas is an important organ of the digestive system which is located in the left upper abdomen directly behind the stomach and next into the small intestine. Horizontal extending pancreas is divided into the three regions, the wide end head attached with the duodenum, body the middle part of the pancreas and narrower tail attributed to the hilus of spleen (Habal et al. 2002). The pancreas is comprised of the network of tubes or the pancreatic ducts. Pancreas has two main ducts; the main pancreatic duct and accessory pancreatic ducts (Villasenor et al. 2010). Pancreas secretes the digestive hormones and enzymes. This enzyme reaches in the gastrointestinal tract through the pancreatic ducts. These enzymes release into the Major papilla (Ampulla of Vater) (Slack 1995). The pancreas has two functions these are endocrine and the exocrine (Li 2016).

2.2.1 Endocrine (Internal Hormonal Role): It produces the chemicals or hormones into the blood stream (Slack 1995) that regulates the blood sugar. The endocrine components constitute approximately 4.5% of the pancreas volume for maintaining the homeostasis (Li 2016). The human pancreas islets of Langerhans reports a proportion of ~60% β-cells which secretes insulin, ~30% α-cells secretes glucagon, <10% δ-cells secretes somatostatin, <5% γ-cells which secretes pancreatic polypeptide and a few ε-cells which secretes ghrelin (Ionescu-Tirgoviste et al. 2015).

2.2.2 Exocrine (External Digestive Role**):** About 85% mass of the pancreas functions as an exocrine that secretes the pancreatic juices containing multiple enzymes (Li 2016). It produces enzymes into the digestive tract that help to digest the food such as amylase which breakdown the carbohydrate or starches into glucose, protease which breaks down the protein into amino acids and lipases breaks down the fats. These digestive enzymes are very powerful and are covered with a protective layer inside the pancreas. Once the enzymes reaches to the vater the wrapped protective layers removes and the enzymes become active.

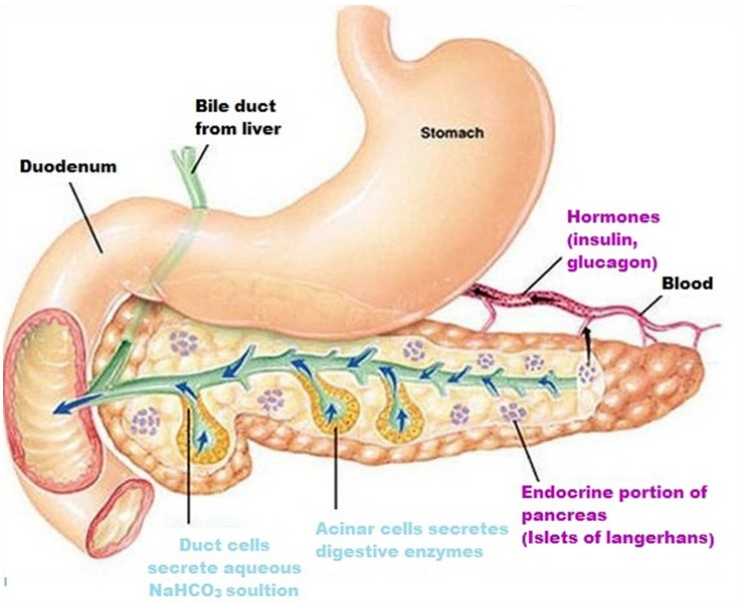


Fig-2.1: Structural feature of Pancreas (DOTE Anatomy topics)

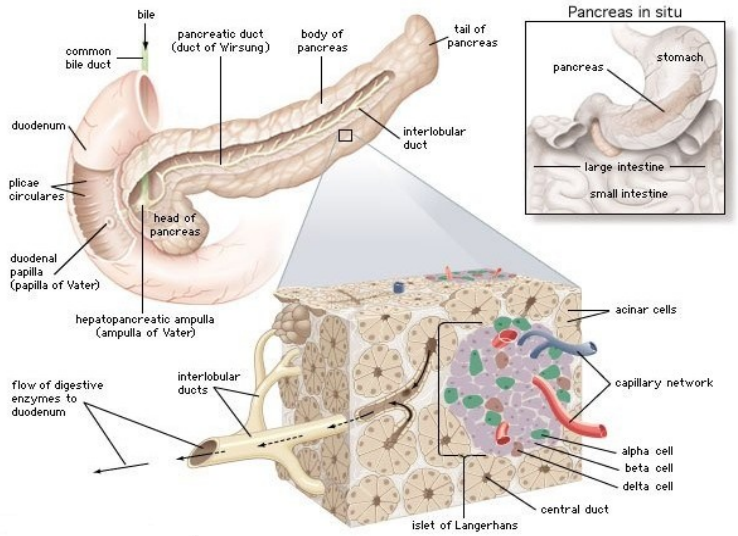


Fig-2.2: Cellular Structure of Pancreas (2003 Encyclopedia Britannica inc.)

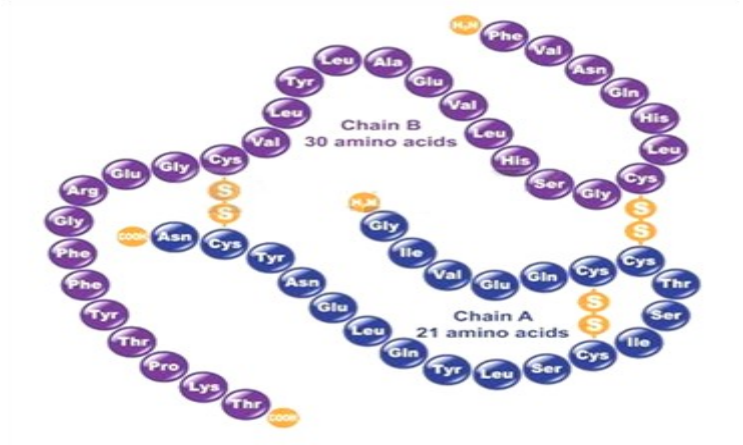


Fig-2.3: Structure of Human Insulin (123RF#13207592)

In the mean time the bile from the gall bladder also releases to the Major papilla and breaks down the fats into the smaller fat droplets which are further digested by the lipase (Hasan et al. 2006).

2.2.3 Insulin

In 1923 Frederick Grant Banting and John James Richard Macleod discovered the insulin for which they were awarded the noble prize in physiology and medicine (Holt & Hanley 2011). Insulin is a 51 amino acid anabolic peptide-hormone that is produced by β-cells of the pancreatic islets of Langerhans. Insulin consists of two chains (A & B) connected by disulfide bonds . Chain-A has the NH-2 terminal with polypeptide (GlyA1-IleA2-ValA3-GluA4 or AspA4) with COOH-terminal on another side. B-chain conserves the NH-2 terminal with polypeptide (GlyB23-PheB24-PheB25-TyrB26) with the COOH-terminal on another side (Yu et al. 2005).

Insulin is synthesised as preproinsulin (a 12 kdalton single chain polypeptide) and processed to proinsulin and then converted to insulin and C-peptide and stored in the secretory granules. Insulin synthesis is regulated at both transcriptional and translational process (Pittman et al. 2004). Insulin secretion involves the sequence of events in β–cells that lead to fusion of secretory granules with the plasma membrane. Insulin is produced primarily in response of glucose, while other nutrients such as free fatty acids and amino acids can augment glucose induced insulin secretion. In addition various hormones, like melatonin, estrogen, leptin, growth hormone and glucagon like peptide-1 also regulate insulin secretion. Although an increase in intercellular [Ca^{2+}] is the primary insulin secretary signal, cAMP signalling-dependent mechanisms are involved critically in the regulation of insulin secretion (Fu et al. 2013). The impairment of β-cells function to resistance of insulin secretion is influenced by the risk of genetic loci of various genetic subtypes of MODY (Maturity Onset Diabetes of the Young). It has extended to other monogenic diabetes like neonatal diabetes. It has also led to the identification of common risk variants via candidate gene like E23 polymorphism in KCNJ11 (Potassium Voltage-Gated Channel, Subfamily J, Member 11) or common variants in

the MODY genes. It was conferred by the polymorphisms in the TCF7L2 (Transcription Factor7-Like2) gene (Florez 2008).

2.2.4 Anatomy of pancreas

In pancreas artery supply is from the major branches of celiac artery including the splenic, gastroduodenal arteries and superior mesenteric artery. The splenic artery supplies the blood to the neck, body and tail of the pancreas through its branches. The superior and inferior pancreatico-duodenal arteries supplies to the head of the pancreas. The venous supply comes from the superior mesenteric and splenic veins which jointly becomes a portal vein (Habal et al. 2002).

The consequences of abnormal pancreas show diarrhea, bloating, flatulence, oily and foul smelling stool, weight loss, malnutrition, poor blood sugar control and diabetes. The risk of poor pancreas may arise through heavy alcohol consumptions, eating highly fatty diet, being overweight, consumptions of tobacco product which causes stress to the pancreas. There are also some genetic conditions which is the reason of poor pancreatic functions. To keep healthy pancreas it is necessary to eat pyramid of various diet like fruits, vegetables, fishes and white meats, less fatty foods, consumptions of less alcohol, maintain healthy weight by physical exercises, maintain the normal cholesterol and triglyceride levels (Willett 2011).

2.3 Oxidative Stress and Diabetes

Oxidative stress is defined as a disturbance in the prooxidants – antioxidant balance which leads to potential tissue damages. Oxidative stress results for an imbalance between free radical productions and antioxidant defenses (Kelly 2003). It is considered as a metabolic syndrome which may be a unifying link between diabetes mellitus and its complication including nephropathy, liver dysfunction, retinopathy, neuropathy and loss of immune potency. Free radical induced damage is responsible for the organ dysfunction (Hutchenson and Rocic 2012).

Oxidative stress is defined by Halliwell as a serious disparity of the production of reactive species and antioxidant defenses leading to the potential tissue damage. Oxidative stress produced by ROS or their inadequate removal causes the

pathogenesis of late diabetic complications. In unrestrained diabetes it varies the level of superoxide dismutase, the enzyme responsible for inactivating the superoxide radical, along with the levels of the antioxidants vitamin E and Lipoic acid (LA) are decreased, deficiency in erythrocyte catalase and an enzyme responsible for the removal of H_2O_2 increases frequency of diabetes (Evans et al. 2002).

The consequences of oxidative stress play a major role in the pathogenesis of both types of diabetes mellitus. Free radicals are formed disproportionately by glucose oxidation, no enzyme glycation of proteins (haemoglobin) and the subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanism lead to the damage of cellular organelles and enzymes, consequently increased lipid peroxidation and development of insulin resistance promote the developments of complications of diabetes mellitus (Maritim et al. 2003). Reactive oxygen species are produced via oxidative phosphorylation during anaerobic glycolysis, via the Schiff reaction during glycation, via glucose autoxidation and via hexosamine metabolism under supraphysiological glucose concentrations which is responsible for chronic oxidative stress and important mechanism for glucose toxicity. (Robertson et al. 2003).

There are multiple sources of reactive oxygen species (ROS) production in mitochondrial and non mitochondrial origins. ROS accelerates the four important molecular mechanisms which causes the hyperglycemia and induces oxidative tissue damage. These four pathways are activation of protein kinase C (PKC), increased hexosamine pathway flux, increased advanced glycation end product (AGE) and increased polyol pathway flux (Rolo and Palmeria 2006). The unifying mechanism for the pathobiology of diabetic complications has been proposed to be hyperglycemia-induced production of superoxide in the mitochondria (Marklund 1982). Diabetes has been shown to increase the production of reactive oxygen species and disrupt the mitochondrial antioxidant defense systems in rats with diabetes (Raza and John 2006). The oxidative stress caused by glucose-induced free radical

production is implicated in the development of insulin resistance in both type 1 (Tabatabaie et al. 2003) and type 2 diabetes (Robertson et al. 2003). The presence of antioxidants such as glutathione (GSH) has been shown to protect against the development of diabetes and diabetic complications (Van-Dam et al. 2001). Several studies have reported that diabetes mellitus (type 1 and 2) is accompanied by increased formation of free radicals and decreased antioxidant capacity, leading to oxidative damage of cell components (Bashan et al. 2009).

2.3.1 Free Radicals

Occurrence of high doses of radicals either due to their increased production or their inadequate removal results in oxidative stress leading to several metabolic disorders (Ben and Catalano1993). Most free radicals in biological system are oxygen derivatives i.e. reactive oxygen species (ROS) but there are also exist nitrogen derivatives i.e. reactive nitrogen species (RNS). Among ROS hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\cdot -}$) and hydroxide radical (OH^-) are the predominant (Seifried et al. 2007). Hydroxyl ions (OH^-), the most powerful free radical generated *in vivo* during the degradation reduction of superoxide and hydrogen peroxide (H_2O_2) catalyzed by transition metal ion (Halliwell 1978) acts as a final mediator of most of the free radical induced tissue damage because of their ability to react with every type of molecules (carbohydrates, proteins, nucleotides and lipids) found in the living system.

$Fe^{++} + H_2O_2 \qquad Fe^{+++} + OH^- + OH$

$Fe^{+++} + O_2^- \qquad Fe^{++} + O_2$

Hydroxyl ions($-OH^-$) can also be produced by Haber-Weiss reaction where superoxide and hydrogen peroxide react directly. Reactive intermediates like ferryl and perferryl might also be formed other than $-OH^-$ ions because *in vivo* the transitional metals (such as iron and copper) do not exist freely and remain bound with other molecules. In pathological conditions like inflammation ischemia reperfusion there is a breakdown of iron binding protein hampering the protective iron chelating effect by releasing iron that participate in the production of reactive $-OH^-$ radicals which in turn contribute to tissue damage (Halliwell et al. 2000).

Several exogenous environmental factors also promote for free

radical formation in the biological system. Xenobiotics often results in the formation of superoxide anions, which induce hepatic injury (Karapanayiotides et al. 2004). Atmospheric pollutants like ozone, nitrogen dioxide cause damage to alveolar lining through free radical generation subsequent manifestation of respiratory diseases. (Mustafa and Tierney 1978). Proteins, nucleic acid, carbohydrates and lipids are the main targets of the free radicals. Lipid peroxidation (LPx) i.e. reaction of hydroxyl ions with lipid is a marker for free radical damage to cell membrane (Lobo et al. 2010). The lipids are the major constituent of cell membrane occurring as a fluid mosaic bilayer with intertwined proteins that functions as receptors and transporters. Hydroxyl ions rapidly react with polyunsaturated fatty acids (Scarlata 2004). Malondialdehyde (MDA) is a physiologic keto-aldehyde produced by peroxidative decomposition of unsaturated lipids as a byproduct of arachidonate metabolism. The excess of MDA is produced as a result of tissue injury (Topçuoglu et al. 2009).

Proteins constitute the major 'working force' for all forms of biological work. Under pathological conditions reactive oxygen and nitrogen species (ROS & RON) are formed in higher fluxes. They cause cellular damage, an important part of which is the oxidation of amino acid residues of protein forming protein carbonyl (Butterfield et al. 1997), covalent modification of cysteine, lysine, and histidine residues by the lipid peroxidation product 4-hydroxynonenal (Papaioannou et al. 2001), nitration on tyrosine residues and glycation (Munch et al. 1997).

2.4 Antioxidant

Biological system has antioxidative defense mechanism to counter the ill effects of free radicals. Antioxidants are the molecules that can neutralize free radicals. Several findings regarding diabetes mellitus reveal the suppressed activity of antioxidative enzymes (Furukawa et al. 2004). The protective role of antioxidant against free radicals possessed by different mechanisms as follows (a) the catalytic systems to neutralise or divert ROS, (b) binding or inactivation of metal ions prevents generation of ROS by Haber-Weiss reaction, (c) suicidal and chain breaking antioxidants scavenge and destroy

ROS, (d) absorb energy, electron & quenching of ROS. (Sen and Chakraborty 2011).

2.4.1 Types of antioxidants:

Antioxidative defense mechanism to living body against the free radical induced oxidative stress can be classified into enzymatic and non-enzymatic based on the nature. Enzymatic antioxidant defenses include superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). Non-enzymatic antioxidants are represented by ascorbic acid (Vit C), α-tocopherol (Vit E), Glutathione (GSH), carotenoids, flavonoids and others. It is essential to both enzymatic and non enzymatic activities for the intercellular level to the living health (Valko et al. 2006). According to the mechanisms of action, antioxidants can be classified into – (a) primary or chain breaking antioxidant which are the free radical scavengers or acceptors and delay or inhibit the initiation or interrupt the propagation of autoxidation. (b) Secondary or preventive antioxidants slow down the rate of oxidation reactions which are chelators for prooxidants or catalyst metal ions, provide H to primary antioxidants, decompose hydroperoxide to non-radical species, deactivate the singlet oxygen, absorb UV radiation or act as an oxygen scavengers (Wanasundara and Shahidi 2005). Based upon the source antioxidants may be, *Endogenous antioxidants* like- Bilirubin, glutathione, lipoic acid, N-acetyl cysteine, NADPH and NADH, ubiquinone (coenzyme Q_{10}), uric acid, enzymes (SOD, CAT, GPx, GR. *Dietary antioxidants*- Vitamin C, Vitamin E, β-carotene and other carotenoids and oxycarotenoids (lycopene and lutein), polyphenols (flavonoids, flavones, flavonols, and proanthocyanidins). *Metal binding proteins*- Albumin (copper), ceruloplasmin (copper), metallothionein (copper), ferritin (iron), myoglobin (iron), transferrin (iron) (Sen and Chakraborty 2011).

2.4.2 Antioxidants against the diabetes mellitus: Diabetes mellitus is a global problem associated with increased formation of free radicals and decrease in antioxidant potential which results disturbed balance between radicals formation and

antioxidant protection in normal cell. Both insulin dependent (type1) and non-insulin dependent (type 2) are associated with increased oxidative stress. Hyperglycemia can also stimulate ROS formation from a variety of sources like oxidative phosphorylation, glucose autooxidation, NAD(P)H oxidase, lipooxygenase, cytochrome P_{450} monooxygenases and nitric oxide synthase (NOS). Several studies are reported the depletion of antioxidant enzyme levels in patients with diabetes. Oxidative stress has also implicated in the pathogenesis of cardiovascular disease, retinopathy, neuropathy, nephropathy and erectile dysfunction in diabetes (Valko et al. 2006).

Antioxidants can confer significant beneficial effects in diabetic patients. Antioxidant vitamins and supplements can help decrease the markers indicative of oxidant stress and lipid peroxidation in diabetic subjects. The intake of dietary antioxidant (total vitamin E, α-tocopherol, β-tocotrienol and β-cryptoxanthin) was associated with a reduced risk of type 2 diabetes. Phytochemicals with antioxidant activity like cinnamic acids, coumarins, diterpenes, flavonoids, lignans, monoterpenes, Phenylpropanoids tannins and triterpenes also proved beneficial to protect diabetes or protect diabetic complications. Several antidiabetic and other synthetic drugs with antioxidant activity like angiotensin, convertase inhibitors, angiotensin block receptors, melatonin, α-lipoic acid, glibenclamide, allopurinol, metformin, repaglinide, caffeic acid, phenethyl ester carvedilol are found beneficial in diabetes and to prevent diabetic complications due to their antioxidant activity. (Sen and Chakraborty 2011).

2.5 Screening models for anti diabetic activity

2.5.1 Laboratory rats as an experimental model: The laboratory rats with white fur and red eyes are the organism used for the scientific research. Albino rats are breed in the scientific conditions in laboratory for different fields in research (Wolf, 2013). The uses of experimental Wister rat model started in 1906 in Wister institute. Now-a-day's about 51 known species of the Rattus of both albino and pigmented types are available. There are distinguished differences between wild and laboratory rodents. In example laboratory rats have smaller adrenals

and preputial glands, earlier sexual maturity, no reproductive cycle seasonability, better fecundity and shorter life span than their free-ranging wild counterparts (Sengupta 2013). Currently Wister rats and Sprague-Dawley rats are becoming most popularly used laboratory rats worldwide (Krinke 2000).

Almost all disease–linked human genes are currently known of having equivalent genes within the rat's genome making them suitable for the research tool. Rats share 90% of the genome with human beings (Gibbs et al. 2004). The blood and organs tissues of the animal are used for the diagnosis. The histopathology of macroscopic, microscopic, Biochemi-cal immunologic and molecular examination of the blood and organ tissues is served for the disease and disorder diagnosis (Kohn and Clifford 2002). Measuring of antioxidative endogenous enzymatic activities can be diagnosed from the tissue samples of the experimental animals (Chen et al. 2000). The most commonly used animal models to test drugs with potential antidiabetic activities are as follows-

2.5.1.1 *In vivo* animal models of diabetes mellitus:

(i) Pharmacological induction of diabetes; Streptozotocin (69%) and alloxan (31%) are the most frequently used drugs to induce diabetes of laboratory animals (Mythili et al. 2004).

(ii) Surgical models of diabetes; It is the technique of complete removal of pancreas to induce diabetes of animals and exploring the effects of natural products (Choi et al. 2004).

(iii) Genetic models of diabetes;

(a) The model of animal strains that spontaneously develop diabetes is applied to evaluate the natural product in animal without the intrusion of side effects induced by chemical drugs like alloxan and Streptozotocin (STZ) (Rees and Alcolado 2005).

(b) Genetically engineered diabetic mice are allowed to produce over (transgenic) or under (knockout)–express proteins notion to play a key part in glucose metabolism (Clee and Attie 2007).

iv. Other models to evaluate the reduction of pancreatic β-cell mass; Progressive loss of pancreatic β-cell functions in the course of type 2 diabetes has to be focused for therapeutic targets in the improvement

of novel and potential drugs for enhancing pancreatic β-cell growth and or survival (Hansotia and Drucker 2005).

2.5.1.2 *In vitro* models of diabetes mellitus:

i. In vitro studies on insulin secretion; This is the conventional antidiabetic agents which can affect several pathways of glucose metabolism such as insulin secretion, glucose uptake by target organs as well as nutrient absorption. Recently, incretins (Hansotia and Drucker 2005), and transcription factor such as peroxisome proliferator activated receptors (PPAR) are targets of modern therapy (Rosenson 2007).

a) Studies using isolated pancreatic islet cell lines- The pancreatic β-cells of type 2 diabetes exhibit a typical 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 animals which can be obtained by collagenase digestion technique, followed by adequate separation and transference to appropriated culture medium (Zhao et al. 2005).

b) Studies using insulin secreting cell lines- To study the natural products, bioengineering technologies has developed the facilitating technique to appropriate cultured cell lines of RIN, HIT, beta-TC, MIN6 and INS-1 cells to know the mechanisms of both secretion and β-cells dysfunction (Poitout et al. 1996).

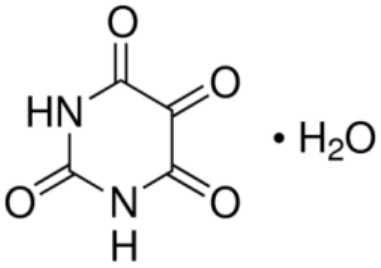
ii. In vitro studies on glucose uptake; To evaluate the effects of natural products upon glucose uptake and insulin resistance pathways the cell lines of adipocyte such as murine 3T3 -L1 cells (Jarvill-Taylor et al. 2001) and rat L6 muscle are engineered to over express GLUT4 (Maddux et al. 2001).

2.5.1.3 Diabetes accelerated atherosclerosis: It do not exhibit the arteriosclerosis unless hyperglycemia is associated with severe hyperlipidemia. It is the model of diabetic nephropathy, a microvascular complication by a fatty diet (Wu and Huan 2007).

2.5.2 Induction for Diabetes Mellitus: The most possible inductions of diabetes mellitus to experimental animals can be done by

alloxan and streptozotocin.

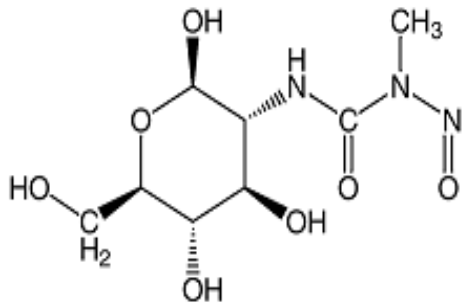
2.5.2.1 Alloxan induced diabetes: Alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetrone) is an oxygenated pyrimidine derivative. It was isolated by Brugnatelli in 1818 and got its name by Friedrich Wöhler and Justus von Liebig in 1838. This causes insulin dependent diabetes mellitus (alloxan diabetes) when administered to rodents and many other animals. The alloxan model of diabetes was first described in rabbits by Dunn, Sheehan and McLetchie in 1943 (Sharma et al. 2010). It is a diabetogenic cytotoxic triketone compound of glucose analogue that selectively destroys the pancreatic β-cell (Elsner et al. 2008). Alloxan is a strong oxidizing hydrophilic and unstable substance. Its molecular formula is $C_4H_2N_2O_4$ and molecular mass is 142.06g/M. Its IUPAC name is mentioned as 2,4,5,6-tetraoxypyrimidine or 2,4,5,6-pyrimidinetetrone (Veeranjaneyulu and Subrahmanyam 2016) or 5,6-dioxyuracil (Szkudelski 2001). The superoxide radical forms by the establishment of a redox cycle by the induction of alloxan and the products of its reduction and dialuric acid. With a massive increase in cytosolic calcium concentration, the radical undergoes dismutation to hydrogen peroxide which causes rapid destruction of the pancreatic β-cell (Fröde and Medeiros 2008). Chemical structure of Alloxan monohydrate is $C_4H_2N_2O_4 . H_2O$:



In laboratory animals diabetes can be induced with Alloxan (ALX). ALX is a potent generator of reactive oxygen species (ROS). Glucose transporter 2 (GLUT2) and Glucokinase (GK) are target molecules for ALX. Diabetes results from irreversible damage of insulin-producing β-cell. Free radicals involve in the production of DNA lesions by the alloxan thus produces the hyperglycemia in animals. (Szkudelski 2001)

2.5.2.2 Streptozotocin induced diabetes: STZ, a glucose analogue, was originally isolated by Herr et al. in 1960 from the culture of boil bacterium *Streptomyces achromogenes*. STZ is notably a deathly toxic to insulin and cause the insulin dependent diabetes mellitus by the necrosis of the β-cells of

pancrease. Its IUPAC nomenclature is [2-deoxy-2-(3-methyl-3-nitrosourea) 1-D-glucopyranose] and molecular formula represents $C_8H_{15}N_3O_7$ and the molecular weight is 265g/M (Veeranjaneyulu & Subrahmanyam 2016). Chemical structure of Streptozotocin is-



The glucose transporter2 (GLUT2) mediates glucose uptake into β-cells of pancreas which also mediates the cellular uptake of STZ. In pancreatic β-cells STZ causes fragmentation of DNA through formation of free alkylating radicals which leads to reduction of nucleotides and related compounds particularly NAD^+ in the cellular levels for which causes a rapid necrosis of β-cells. (Thulesen et al. 1997).

2.6 Remedial Approaches for Diabetes mellitus:

The major objective in treating the diabetes mellitus is to control blood sugar (glucose level) within the normal range with minimum expedition to low or high levels (Chamberlain et al. 2016). An important strategy to reduce the burden of diabetes mellitus and its complication includes the measure of preventions, change of life style and effective pharmacological managements (Alberti et al. 2007 and Crandall et al. 2008). There are several therapeutic approaches for diabetes mellitus which includes sulphonylureas which increases insulin release, (Proks et al. 2002) metformin which reduces hepatic glucose production (Foretz et al. 2010) peroxisome proliferator activated receptor-γ (PPARγ) activators (thiazolidinediones) which increase insulin sensitivity (Wang et al. 2006) α-glucosidase inhibitors which interfere with glucose absorption and insulin itself. The α-glucosidase inhibitors can retard the liberation of d-glucose from dietary complex carbohydrates and delay glucose absorption, resulting in reduced postprandial plasma glucose levels and suppression of postprandial hyperglycemia. Recently many α-glucosidase inhibitors of natural phytoconstituents such as flavonoids, alkaloids, terpenoids, anthocyanins,

glycosides, phenolic compounds, and so on, have been isolated from the plants lead compound use against diabetes (Kumar et al. 2011).

The plasma insulin concentration measurements are established for metabolic process. The insulin response after the oral glucose administration was significantly higher than the insulin response after intravenous glucose application mimicking oral glucose excursions. This phenomenon is termed as 'incretin effect' and the humoral factors contributing to the stimulation of post prandial insulin secretion is called as 'incretins'. Glucagon like peptide-1 (GLP-1) is a tissue specific post translational product of preproglucagon that is produced in the small intestinal L-cells, whereas in the pancrease glucagon is predominantly derived from preproglucagon. GLP-1 stimulates insulin secretion and inhibits glucagon secretion in a strictly glucose dependent manner and can therefore normalize glucose in type 2 diabetes (Gallwitz 2016).

2.6.1 Managements of diabetes mellitus: The measurements of plasma glucose remain the sole diagnostic criterion for diabetes mellitus. Examining of glycemic control is performed by measurements of their own plasma or blood glucose with glucometer or by the laboratory analysis of glycated haemoglobin. The noninvasive glucose monitoring, genetic testing, autoantibodies, micro albumin, proinsulin, C-peptide and other analyte are the important for analysis (Sacks et al. 2002). One should attain the goals of medical nutrition therapy (MNT) to promote and support the eating patterns, emphasize a variety of nutrient-dense foods in appropriate proportion sizes to achieve body weight goals, attain glycemic lipid, blood pressure goals and prevent or mend the complications of diabetes. To manage diabetes the PPG controlling is important to one to get closer to A1C goal (ADA 2016).

One should encourage replacing of the refined carbohydrates and added sugars with whole grains, legumes vegetables and fruits. Regular physical activities, leave out of cigarettes smoking, regular examination of blood pressure, A1C, urine, skin, intensive change of life style are essential for the management of diabetic patients (Nathan et al. 2009).

2.6.2 Drugs used for diabetes

treatments: Diabetes mellitus is often treated with insulin sensitizers, secretagogues and external insulin delivery insulin analogues. A number of compounds for treatment of diabetes are currently at different stages of development.

i. Drugs that promote the production of body insulin (Insulin Secretagogues):

- Sulfonylurea's; e.g. Acetohexamide, Carbutamide, Chlorpropamide, Metahexamide, Tolazamide, Tolbutamide, Glibenclamide, Glibornuride, Gliclazide, Glipizide, Gliquidone, Glisoxepide, Glimepiride.
- Benzoic acid derivatives; e.g. Repaglinide.

ii. Drugs that reduce the glucose production by liver-

- Biguanides; e.g. Metformin (Glucophage).

iii. Drugs that help the body respond to insulin (Insulin Sensitizers)-

- Thiazolidinediones; e.g. Pioglitazone, Rosiglitazone, Lobeglitazone.

iv. Drugs that reduce post prandial glucose concentrations-

- Glucosidase inhibitors; e.g. Acarbose, Miglitol, Voglibose.

v. DPP-IV inhibitors (Dipeptidyl peptidase-4)-

- Sitagliptin, Vildagliptin, Saxagliptin, Linagliptin, Anagliptin, Tenegliptin.

However, all these therapeutics receipts different drawback of several side effects. (He et al.2015).

2.6.3 Diabetes and herbal treatments

Nature still oblige as the man's primary source for the cure of ailments. The preventive medicine shows the importance of functional nutrition in reducing the risk factor of certain chronic diseases (Middha et al. 2013). Traditional medicine and medicinal plants contributes to the discovery of powerful source of new drugs (Rates 2001). Herbal treatments are popularly increasing world wide as the complementary or alternative medicine supposedly less frequent side effects when compared with modern western medicines. The efficacy of the herbal drug is well documented and is interesting to note that some European countries, United

States, Japan etc. have adopted Ayurveda into their mainstream medical system (Torwane et al. 2014). Several medicinal plants and their formulations are used for treating the diabetes in Ayurvedic medicine. In India, indigenous treatment of diabetes mellitus has been reported since the time of Charka and Shusrutha. The most potent hypoglycemic effective plants belong to the families of Leguminosae, Lamiaceae, Liliaceae, Cucurbitaceae, Asteraceae, Moraceae, Rosaceae, Euphorbiaceae and Arliaceae. Several plants have been used as dietary adjunct for treating the number of diseases even without any proper knowledge on their functions and constituents (Patel et al. 2012).

The Plants like *Coccinia indica, Tragia involucrata, Gymnema sylvestre, Pterocarpus marsupium, Trigonella foenum-greacum, Moringa oleifera, Eugenia jambolana, Tinospora cordifolia, Swertia chirayita, Momordica charantia, Ficus glomerata, Ficus benghalensis, Vinca rosea, Mucuna prurita, Terminalia bellirica, Azadirachta indica, Zingiber officinale, Aegle marmelos, Cinnamomum tamala, Ocimum sanctum, Salacia oblonga, Cassia auriculata, Curcuma longa, Andrograpis paniculata, Emblica officinalis* etc. are mainly used herbs for antidiabetic formulations (Jarald et al. 2008). Plants generally produce many secondary metabolites which are biosynthetically derived from primary metabolites and many pharmaceutical drugs which have been directly or indirectly playing an important role in the human society to combat diseases. Plant secondary metabolites can be structurally divided into five major groups; Polyketides, Isoprenoids Alkaloids, Phenylpropapoids and Flavonoids (Oksman-Caldentey and Inze 2005). The polysaccharides, peptides, alkaloids, glycopeptides, triterpenoids, amino acids, steroids, xanthone, flavonoids, lipids, phenolics, coumarins, iridoids, alkyl disulphides, inorganic, ions and guanidines etc. are the phytoconstituents which are reported as antidiabetic activity (Jarald et al. 2008).

The proven antidiabetic and related beneficial effects used in the treatments of diabetes mellitus are *Allium sativum, Eugenia jambolana*, *Momordica charantia, Ocimum sanctum, Phyllanthus amarus, Pterocarpus marsupium, Tinospora*

*cordifolia, Trigonella foenum-graecum, Withania somnifera etc. (*Modak et al. 2007).

Phytochemicals are the major source of antioxidants. These phytochemicals are generally redox active molecules and hence they are active to maintain redox balance, therefore defined as antioxidants. The plant chemicals are generally classified as primary or secondary constituents, depending on their role of metabolism. Primary constituents include, the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophyll's etc., whereas secondary constituents includes alkaloids (amino acids derivative), terpenes (group of lipids) and phenolics (carbohydrates derivative). The Secondary constituents are antioxidant phytoconstituents or metabolites found naturally in plants such as fruits and vegetable. Plants produce an extremely impressive array of antioxidant compounds such as carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols, and tocotrienols to prevent oxidation of the susceptible substrate (Sen and Chakraborty 2011).

The selected portions of specific plant tissues are used for the extraction and separations of the medicinally active compounds with the selective solvents known as *menstruum* through standard protocols. Preparations of relatively complex mixtures of metabolic compounds are obtained by decoctions; infusions fluid extracts, tinctures, pilular (semisolid) extract or powdered extracts etc. which are popularly known as *galenicals* (Tiwari et al. 2011).

2.6.3.1 Hodgsonia heteroclita

Hodgsonia was named for Brian Houghton Hodgson in 1853 by British botanists Joseph Dalton Hooker and Thomas Thomson. (Kocyan 2007). It is believed to have originated in Northeast India, China and Malaysia (Hu 1964; Zeven and Zhukkovsky 1975). It shows a wide distribution from southern temperate Asia to tropical Asia and found to occur in Bangladesh, China, India, Malaysia and Nepal (Semwal et al. 2015). In India, *H. heteroclita* is encountered in the hilly areas of Assam, Manipur, Meghalaya, Nagaland, Arunachal Pradesh, Tripura and Sikkim (Arora and Hardas 1977). The tribal communities of North-east India were

very much acquainted with the different uses of *H. heteroclita*. The seed cotyledons are consumed in different forms as either wholly or with other accessories like meat fish or along with other edible vegetables. The seed oil is used for cooking food and preparation of other food items and beverages. In Manipur and Nagaland the crushed seeds are used for the treatment of intestinal worms and the fruit pulp is used to prevent bacterial infections (Changkija 1999). The tribal people of Tripura uses leaf juices on fresh cuts and wounds to heal (Semwal et al. 2015). The seed decoction is used for the uterine disorders (Sharma et al. 2001). The fruit pulp is used by the Bodos as a remedy against the diabetes (Swargiary et al. 2013). In Malaya and Java the *Hodgsonia* dried leaves are burnt and inhaled the smoke or the juice of young stems and leaves are squeezed into the nostrils to allay nose irritation (Hu shing-ying 1964). The boiled leaves and the resulting liquid taken internally reduce the nose complaints and fevers (Arora and Hardas 1977). In Sarawak, *Hodgsonia* oil is used to anoint bodies of mothers after child birth (Hu 1964). The ashes from the burnt leaves are used to heal wounds (Schreitera et al. 2007). The methanol extract of leaf and fruit showed effective against both gram negative and gram positive bacteria. *H. heteroclita* have been proposed a potential source of natural antioxidants. The methanolic extract of *H. heteroclita* fruit extract exhibited a strong scavenging effect on DPPH (Basumatary et al. 2015). *Hodgsonia* was a traditional oil plant used by the mountain tribes of Southwest China which was identified by Chinese scientists as promising commercial oil plant because they show very high seed-oil content between 72% and 77% high degrees of unsaturated fatty acids, as well as proteins. They are also reported to have a very pleasant taste (Schreitera et al. 2007). Apart from this, the seed oil is much valued and uses as the vital formulations of many medicines (Semwal et al. 2015).