1 Chapter 1: General Introduction

1.1 Introduction

Diabetes mellitus and depression are greater burdens for the modern healthcare system (Stuart and Baune, 2012). Diabetes mellitus is a metabolic disorder characterized by hyperglycemic condition and is one of the most chronic and progressive syndrome, related with both physical as well as psychological comorbidities (Alberti and Zimmet, 1998). One of the main psychological comorbidities is depression, arises due to alteration in activity of the central nervous system. It is characterized by depressed mood, sadness or distress, less energy, feelings of guilt, sleep, appetite alteration, and problem with concentration which negatively affects the quality of life in person experiencing these symptoms (Drevets, 2001). Globally, depression is the fifth major cause of disability in 2016, contributing 34.1 million disability-adjusted life years (DALYs) (Vos et al., 2017). On the other hand, diabetes mellitus contributed 28.6 million DALYs with a rise of 23.6% between 2006 and 2016 (Vos et al., 2017). A recent review revealed that as compared to healthy individuals, the diabetic patients have twice the chance of developing depression (Semenkovich et al., 2015). It is found that there is a bidirectional relationship between diabetes and depression, it means that depression may cause diabetes and conversely diabetes to result in development of depression (Renn et al., 2011, Mezuk et al., 2008a, Fisher et al., 2008, Demakakos et al., 2014, Sevick et al., 2007, Golden et al., 2008). Recent reports revealed that depression is a common comorbid condition frequently observed in type 2 diabetic patients, one out of four patients experience depressive disorders (Semenkovich et al., 2015, Egede et al., 2016). It has been observed that the diabetic patients are more vulnerable to depressive disorder due to ignorance of self-care of patients or by healthcare

practitioners (Bădescu et al., 2016, Lin et al., 2010). In other words, psychological problems or diseases like affective disorders are frequently underrecognised by both patients, as well as the healthcare professionals (Lloyd, 2008), resulting in a lack of its treatment (Chen et al., 2013).

On the other hand, studies showed that major depressive disorder can occur eight years before the development of diabetes, hence, major depressive disorder is a risk factor for the development of diabetes in later life (Lustman et al., 1988, Knol et al., 2006, Williams et al., 2006). However, in diabetes comorbid depression, the exact neuropsychological mechanism is not been fully described. With the advancement of the current treatment strategies researchers becoming more concern about the prevalence and relevance of the diabetes comorbid depressive disorders but the psychological disorders associated with the diabetes are not fully studied. This is mainly due to underestimated, underdiagnosed, and undertreated by healthcare professionals, despite its high prevalence with adverse health outcomes and quality of life impairment (Bădescu et al., 2016). Within the past few decades, it has been observed that the treatment strategies for the depression in diabetic patients have been improved by the intervention of the use of current treatment strategies (Cezaretto et al., 2016). Moreover, evidence showing that in spite of the presence of pharmacological therapies, the antidepressant drugs are useful in limited number of patients, and also require a long time (weeks to months) to get a therapeutic response (Fava and Davidson, 1996, Little, 2009). Therefore, there is an unmet need to develop therapeutic strategies to treat both diabetes mellitus and comorbid depression.

An update on diabetes comorbid depression includes essential highlights on the diagnostic features, prevalence, pathophysiology, clinical outcomes, management, and pharmacological treatments. As a part of the literature review, PubMed, Google Scholar, and ScienceDirect databases were searched for English language peer-reviewed published studies with keywords relating to diabetes mellitus and depression. Additional references were identified using cross-references of clinical research association with diabetes and depressive complications, clinical trials and preclinical studies for compilation of literature.

1.2 Diabetes comorbid depression

1.2.1 Diagnostic features

Diabetes mellitus is known as hyperglycemic condition associated with deregulation of carbohydrate, protein, and fat metabolism, which is due to impaired insulin release, insulin sensitivity or both (AmericanDiabetesAssociation, 2017). Diabetes mellitus is generally classified as type 1, type 2, and gestational diabetes. Briefly, type 1 diabetes mellitus is due to insulin deficiency, type 2 is due to insulin resistance, and gestational diabetes is due to pregnancy related metabolic changes (AmericanDiabetesAssociation, 2018). The common symptoms may include thrust, tiredness, weight loss, genital itching, blurred vision, increase micturition, and decrease wound healing process (Awasthi et al., 2016). The diagnostic criteria may include; first, fasting plasma glucose levels more than ≥126 mg/dL; second, impaired glucose tolerance test which is two hours plasma glucose levels ≥200 mg/dL after ingestion of 75 g of anhydrous glucose; third, HbA1c level ≥6.5% of glucose in glycohemoglobin (Moran et al., 2018).

Depression is commonly known as psychological disorder associated with the serious mental illness, which directly affects the quality of life. The common symptoms include feeling of sadness, loss of interest, appetite, sleepiness, concentration, and suicidal thoughts (Fried and Nesse, 2015). Diagnosis of diabetes comorbid depressive symptoms by healthcare practitioners done by any one of the popular screening scales that includes first, the Center for Epidemiological Studies-Depression Scale (CES-D); second, the Beck Depression Inventory (BDI), and third, the Patient Health Questionnaire (PHQ-9) (Heeramun-Aubeeluck et al., 2012). It has been found that depression in type 2 diabetes mellitus was best predicted by CES-D scale (Mezuk et al., 2008b). Moreover, BDI scale has good sensitivity of predicting depression in more than 70% of the depressed patients (Wang and Gorenstein, 2013, Hiroe et al., 2005). On the other hand, PHQ-9 measurement is based on Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) for severe cases of depressive criteria (Fawzi et al., 2019). Other screening instruments comprise of the following scales: the World Health Organization- Five Well Being questionnaire (WHO-5) (Awata et al., 2007), the Psychomatic Evaluation of Problem areas in Diabetes (PAID) (Miller and Elasy, 2008), and the Hamilton Rating Scale for Depression (HAM-D) (Das et al., 2013).

1.2.2 Prevalence

The prevalence of type 1 diabetes was found to be 5-10%, type 2 diabetes has 90-95%, and gestational diabetes has 1-14% occurrence (AmericanDiabetesAssociation, 2014). Diabetes mellitus and depression have been considered a major public health problems, affecting more than 422 millions of people with diabetes, and 350 million people with depression globally (Hu et al.,

2015, WorldHealthOrganization, 2016). The occurrence of depression in type 1 diabetic patients was found to be 32.1% (Gendelman et al., 2009), with type 2 diabetic patients, occurrence was found to be 41% (Datta, 2011) with gestational diabetes patients, occurrence was found to be 14.7% (Ross et al., 2016). In a population-based cohort study, it has been observed that high prevalence of depressive disorder in diabetics patients (Holt et al., 2009, Zhang et al., 2005). According to World Health Organization (WHO) survey, it has been reported that depression is the 4th leading complication in women while it is 7th leading complication in men (Üstün et al., 2004). A meta-analysis study showed that 11% of patient with diabetes mellitus experience some form of depression and 31% of them experience clinically relevant depression (Heeramun-Aubeeluck et al., 2012). The same study showed that rate of undiagnosed depression in diabetic patients is approximately 45% (Heeramun-Aubeeluck et al., 2012). A WHO survey on adult humans aged above 18 years living in 60 different countries revealed that the prevalence of depression in diabetes mellitus patients is 2% for one year (Moussavi et al., 2007). A separate meta-analysis study showed that rate of depression is more in type 2 diabetes mellitus patients (Rasbach and Schnellmann, 2006). A study by International Diabetes Federation (IDF) showed that 280 million people suffered from diabetes mellitus in 2010 and the number of sufferer will increase to 439 million by 2030 (Heeramun-Aubeeluck et al., 2012).

1.2.2.1 **Diabetes comorbid depression in India**

A rising trend in prevalence of depression in diabetic patients has been reported in various parts of the world as well as in India. A recent study comprising Indian patients showed that depression is two times more prevalent in patients with T2DM

(26.3% vs. 11.2%) than in healthy controls (Rajput et al., 2016). In a population-based study in Chennai, it was found that the prevalence of depression was 23.4% (Poongothai et al., 2011). Raval et al. found a very high prevalence (41%) of depression in type 2 diabetes patients in a tertiary care hospital in Northern India (Raval et al., 2010). Another study conducted at a tertiary care center found that the prevalence of depression in T2DM patients to be 16.9% (Balhara and Sagar, 2011). The prevalence rate of depression was higher in diabetic patients with age between 41 and 60 years (Rajput et al., 2016). This may be because diabetic patients require adherence to a complex set of treatment regimens including daily multiple insulin injections, monitoring blood glucose level, adherence to specific dietary guidelines, and attending regular medical check-up (Bajaj, 2018). Furthermore, a number of risk factors including female gender, family dysfunction, and stressful experience in this age group may increase the likelihood of depression (Rehman and Kazmi, 2015). These results correlate with a previous report that showed that higher prevalence rate of depression in age group of 31–59 years (Larijani et al., 2004). In a much recent study, Sunny et al. found a higher rate of depression in diabetic patients with age ≤ 60 years (Sunny et al., 2019).

Clinical studies have reported that suicidal ideation (Goldston et al., 1997) or suicidal attempt (Roy et al., 2010) are one of the most characteristic symptoms of depressive disorders. It has been reported that diabetes comorbid depressed patients are more vulnerable to suicidal ideation (26.4%) and suicidal attempt (13.3%) (Sarkar and Balhara, 2014). A cohort study showed that ratio of suicidal rates in diabetic patients ranging from 0.55% to 40% (Sarkar and Balhara, 2014). In type 1 diabetes mellitus patients the suicidal rate is 5-15 % (Patterson et al., 2007, Feltbower et al.,

2008). On the other hand, in type 2 diabetes mellitus the mortality rate is due to complications of diabetes or other medical illness (Sarkar and Balhara, 2014). In a non-interventional study, total 506 type 2 diabetic patients were evaluated for 18 months and found that 60% of them developed major depressive disorders (Naranjo et al., 2011). The high prevalence of comorbid depression over time highlights the need for diabetes distress screening and mental health checkup regularly, especially for women and younger adults (Fisher et al., 2008).

1.2.3 Pathophysiology

Diabetes comorbid depression is a consequence of chronic hyperglycemia induced oxidative stress, hypothalamic-pituitary-adrenal (HPA) axis alteration, neuronal damage, monoamine dysregulation, mitochondrial dysfunction, inflammation, and epigenetic modification (Figure 1.1).

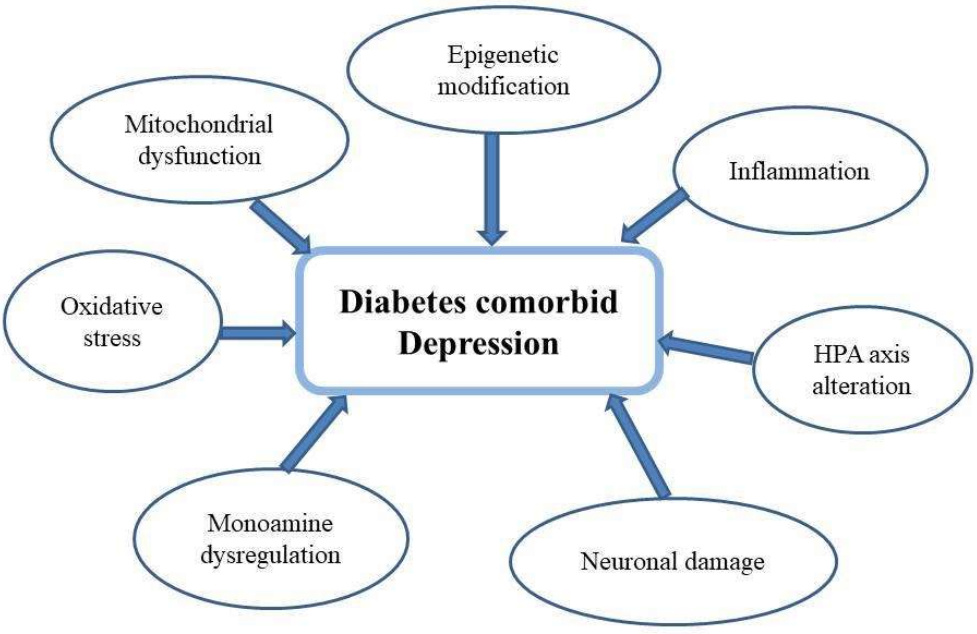

Figure 1.1: Pathophysiological factors involved in diabetes comorbid depression

1.2.3.1 Oxidative stress

During energy production, cells differ in their ability to use dioxygen (O_2) and generate reactive oxygen species (ROS) like hydrogen peroxide, hydroxyl radical, and/or reactive nitrosative species (RNS) such as nitric oxide and peroxynitrite (Valko et al., 2007). The ROS/RNS production is regulated by the activation of antioxidant systems of the body, which include non-enzymatic system (reduced glutathione and ascorbic acid) as well as enzymatic system (superoxide dismutase, catalase, and lipid peroxidase) (Valko et al., 2007, Halliwell, 2001, Joshi and Praticò, 2014). During hyperglycemic condition, oxidative stress occurs due to inequality between the production of reactive species (ROS and RNS) and the protective ability of antioxidant system. Glucose delivery and utilization in the mammalian brain is mediated primarily by a high molecular weight form of GLUT1 in the blood–brain barrier and GLUT3 in neuronal populations (Jurcovicova, 2014). Higher plasma glucose levels during hyperglycemia leads to enhanced entry of glucose into the brain through the non-insulin dependent GLUT receptors (Hwang et al., 2017). This blunted rise in brain glucose levels under hyperglycemia results in higher production of ROS which in turn leads to loss of balance between ROS and antioxidant mechanisms (Muriach et al., 2014b). Because of the reactive species, changes or disruption occurs in function of different macromolecules like proteins, lipids, and DNA including, those comprising the electron transport system and result in disruption of mitochondrial function (Sies, 1997, Madrigal et al., 2006, Naudi et al., 2012). Oxidative mechanism involved in the induction of diabetes comorbid depression is showed in Figure 1.2.

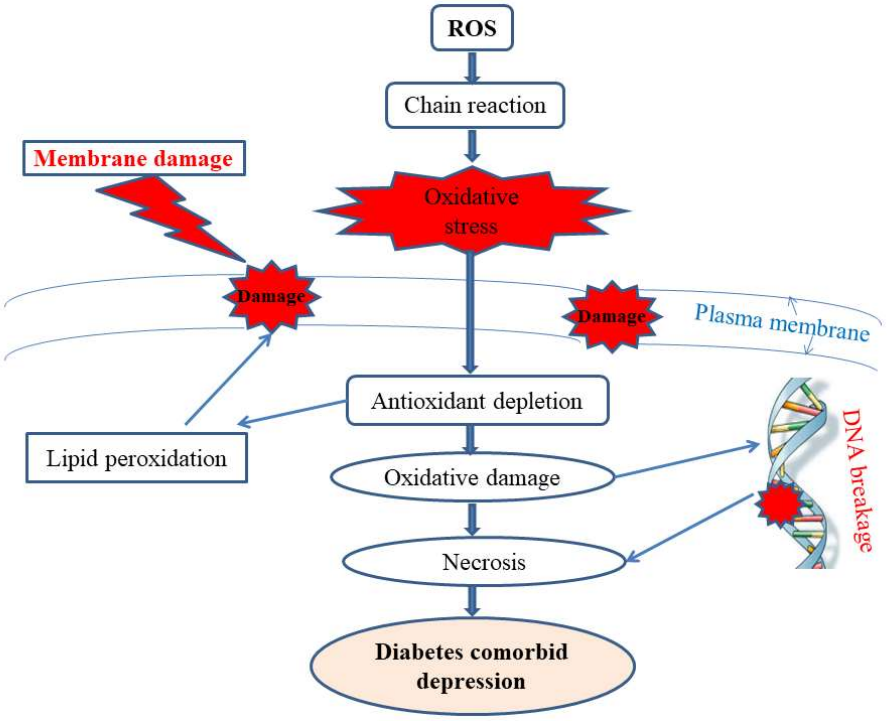

Figure 1.2: Oxidative mechanism for diabetes comorbid depression

The clinical examination of association between oxidative stress and diabetes comorbid depression yet to be made. However, in preclinical experiment, this correlation seems to play an important role in the pathophysiology of diabetes or depression or both (Shivavedi et al., 2017). Other causes for induction of oxidative stress is through polyol pathway, increased advanced glycation end products, increased activity of protein kinase C isoforms, and hexosamine pathway hyperactivity (Brownlee, 2005, Monnier, 2003, Giacco and Brownlee, 2010, Menezes Zanoveli et al., 2016). As we know that ROS production occurs by many ways such as non-enzymatic glycosylation reaction, the electron transport system in mitochondria, and membrane-bound NADPH oxidase. Neuronal and glial mitochondrial membranes possess monoamine oxidase A and B, and these oxidase

levels were elevated in depressive states (To et al., 2005). The by-products (hydrogen peroxide, ammonia, and aldehydes) of monoamine oxidase activity are toxic to the cells (Bortolato et al., 2008).

It has been reported that oxidative stress damages the cell structure through lipid peroxidation, deformation of carbonyl groups of proteins, and DNA damage caused by oxidation of the purine and pyrimidine bases (Valko et al., 2007, Sies, 1997). It has also been observed that lipid peroxidation occurs during hyperglycemic condition (Memon et al., 2000). Activation of leucocytes also activates generation of ROS by secreting a haeme protein called myeloperoxidase that results in lipid peroxidation (Zhang et al., 2002). The continuous generation of ROS results in development of various disorders like depression (Young and Woodside, 2001, Carvalho et al., 2015, Behr et al., 2012, Joshi and Praticò, 2014). It is found that brain is predisposed to different reactive species like ROS/RNS, because brain cells are rich in polyunsaturated fatty acids, which are highly liable to oxidative stress due to poor antioxidant defense system (Wang and Michaelis, 2010, Shichiri, 2014, Mangialasche et al., 2009, Valko et al., 2007). This disproportion affects brain areas such as hippocampus and prefrontal cortex which are involved in depression (Bremner et al., 2002, Frodl et al., 2002, Wayhs et al., 2013, de Morais et al., 2014). It was also reported that metabolism of monoamine neurotransmitters is responsible for protecting the brain against oxidative damage by scavenging reactive oxygen species (Liu and Mori, 1993). Different studies on humans suffering from depression revealed low levels of glutathione (GSH) in the prefrontal cortex of post-mortem brains (Gawryluk et al., 2011b), high activity of super oxide dismutase (SOD) in the serum (Khanzode et al., 2003), and low levels of antioxidant activity in plasma (Khanzode et

al., 2003, Sarandol et al., 2007, Cumurcu et al., 2009). In preclinical studies, it has been found that oxidative or nitrosative stress result in the pathogenesis of depression associated with diabetes (Wayhs et al., 2013, de Morais et al., 2014). Therefore, it has been suggested that oxidative stress in depressive patients results in high level of malondialdehyde (end product of lipid peroxidation), ROS generation, low levels of glutathione, low levels of ascorbic acid, and low levels of uric acid (Suzuki et al., 2001, Khanzode et al., 2003, Pedreanez et al., 2006, Gawryluk et al., 2011b, Gałecki et al., 2009).

1.2.3.2 HPA axis alteration

The maintenance of the body's homeostasis during physical, emotional, or metabolic stress is primarily controlled by the HPA axis (Spiers et al., 2014). The HPA axis is stimulated by neurosecretory neurons in the paraventricular nucleus of the hypothalamus, which is responsible for the secretion of both corticotropin-releasing hormone and vasopressin into the portal circulation of the pituitary gland. These two elements act relatively on pituitary corticotropic cells to stimulate the secretion of adrenocorticotrophic hormone into the circulation. These adrenocorticotropic hormones are responsible for the synthesis and secretion of glucocorticoids, primarily cortisol, by the stimulation of melanocortin two receptor (MC2R) in the zona fasciculata of adrenal cortex (Spiga et al., 2011). The released glucocorticoids acts as a negative feedback mechanism and control the activity of HPA axis (Figure 1.3) (Gądek-Michalska et al., 2013, Reul and Kloet, 1985). Moreover, hyperactivity of the HPA axis would be involved in the pathophysiology of many diseases including major depression and diabetes (Cameron et al., 1984, Roy et

al., 1990, Roy et al., 1993, Tsigos and Chrousos, 2002, Champaneri et al., 2010, Martinac et al., 2014, Jacobson, 2014, Gold, 2015).

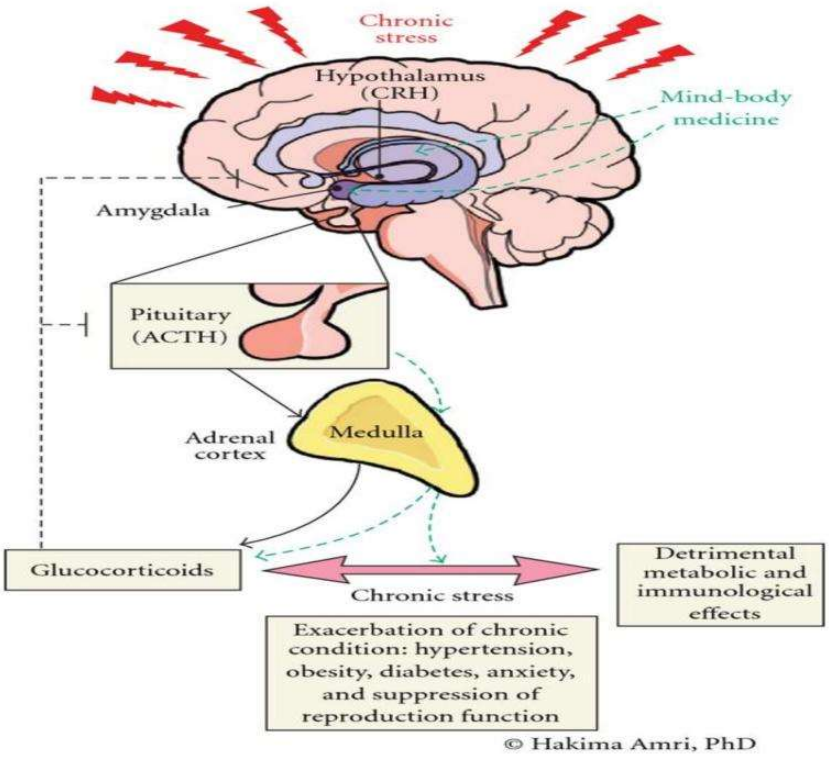

Figure 1.3: HPA axis alteration in diabetes comorbid depression (Benson and Stuart, 1993)

The stress responsive hypothalamic pituitary adrenal (HPA) axis has been implicated in the pathophysiology of anxiety and depression as well as cognitive functioning. The axis consists of stimulating forward and feedback inhibition loops involving the brain, pituitary, and adrenal glands, which regulates glucocorticoid production. Cortisol released from the adrenal glands, binds in brain with high affinity to mineralocorticoid receptors (MRs) and with lower affinity to glucocorticoid receptors (GRs). GR is distributed widely throughout the primate brain, whereas MR is heavily localized to the hippocampus (Patel et al., 2000). In addition, glucocorticoid

responsive elements are found in the regulatory regions of many genes in brain. Cortisol exerts its tonic influences predominantly via hippocampal MRs, whereas feedback actions at the level of the pituitary and activated brain areas such as the amygdala are mediated by GRs (de Kloet et al., 1999). The development of major depression has been postulated to reflect a dysregulation of MR and/or GR within the hypothalamic-pituitary-adrenocortical system (De Kloet and Reul, 1987). Intriguingly, in atypical depression, the HPA axis activity is more likely to be low (Jacobson, 2014). In diabetic animals, it was observed that diabetes was associated with increased basal plasma adrenocorticotropin hormone (ACTH) as well as corticosterone hormone (McMahon et al., 1988, Chan et al., 2002, Martinac et al., 2014, Chan et al., 2003). Interestingly, in diabetic patients, it has been reported that the level of cortisol secretion is particularly associated with diabetic complication development and severity (Chiodini et al., 2007, Gragnoli, 2014). Moreover, the capacity of glucocorticoids to modulate the HPA axis response through feedback loops appears to be altered in both diabetic and depressive patients (De Kloet et al., 1997, Heim and Nemeroff, 2001, Pariante and Lightman, 2008, Stranahan et al., 2010, Champaneri et al., 2010, Martinac et al., 2014). Evidence has shown that diabetic patients present a lack of stress-induced modulation in the HPA axis (Carvalho et al., 2015). In that way, greater attention to the HPA axis dysregulation and more consistent approaches to assessing the HPA function would be interesting to solidify the value of HPA axis alteration in diseases such as depression and diabetes or developing novel therapeutic strategies to treat them.

1.2.3.3 Neuronal damage

The process responsible for brain functions such as memory development, behaviour, and emotion is called the neuroplasticity (Askenasy and Lehmann, 2013). The brain functions are highly prone to environmental stress, HPA axis alteration, and inflammation (Leuner and Gould, 2010, Song and Wang, 2011). Different evidences from animal and human studies showed that the link between diabetes and depression occurs from the degeneration of hippocampal neuroplasticity (Barnard et al., 2013). It is also found from brain imaging of depressive patients that there is decrease in the size of neurons, loss of glial cells (Miguel-Hidalgo and Rajkowska, 2002), and decrease in size of hippocampus and prefrontal cortex (Drevets et al., 2008). Further, animal studies showed that continuous stress in animals is responsible for the loss of glia and degeneration of neurons in hippocampus and prefrontal cortex (Krishnan and Nestler, 2008). It was also found from different preclinical studies that survival rate of multipotent progenitor cells which are having the capability to differentiate and proliferate into new neurons are decreased by 80% in diabetic problems (Malberg et al., 2000), moreover, also reduction in the plasticity and volume of dendritic cells (Revsin et al., 2005, Saravia et al., 2002, Kim et al., 2003). From animal studies, it was reviewed that neurogenesis as well as synaptic plasticity, both are affected in type 1 and type 2 diabetes (Stranahan et al., 2008). It was estimated that there was 56% reduction in hippocampal cell growth in diabetic rats having depressive-like behaviour but, only 27% reduction in hippocampal cell growth was observed in animals having only diabetes (Wang et al., 2009). It was also concluded from previous study that rats with both diabetes and depressive-like behavior showed decreased cell survival and neuronal multiplication or growth (Wang et al., 2009). It

was further concluded that reduction in hippocampal cell differentiation is responsible for the depressive-like behavior and hyperglycemic condition is a risk factor to increase the chances of reduction in hippocampal cell differentiation (Adili et al., 2006, Musselman et al., 2003). The neuronal damage mechanism involved in the induction of diabetes comorbid depression is showed in Figure 1.4.

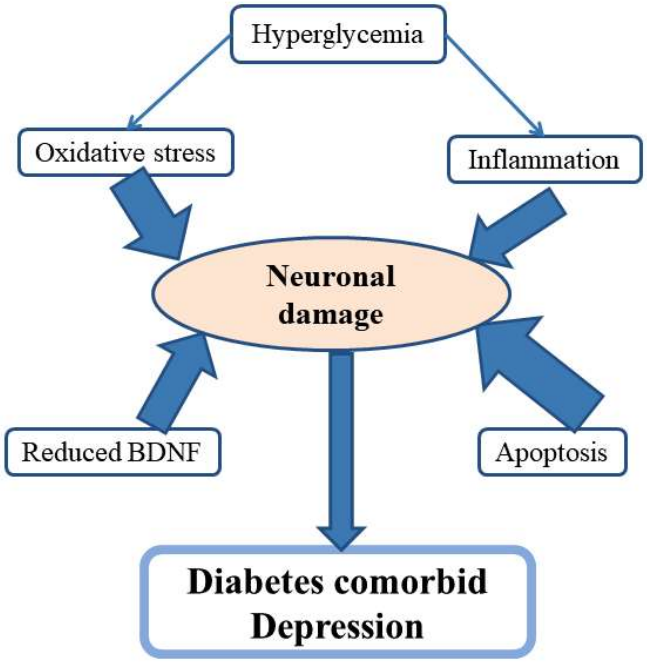

Figure 1.4: Neuronal damage mechanism for diabetes comorbid depression

As evidences showed that declination of neurogenesis is involved in the pathology of depression, some drugs like antidepressants caused increase in neurogenesis in the hippocampus (Airan et al., 2007, Dranovsky and Hen, 2006) through synthesis of BDNF and its TrkB receptor (Castrén and Rantamäki, 2010). BDNF is a neurotrophin found to be very essential for the growth, development, and maintenance of neural network (Autry and Monteggia, 2012). The mechanism involved in regulation of the neural network depends on alteration of synaptic

structure and its deficiency is responsible for the occurrence of depressive-like behavior (Autry and Monteggia, 2012). BDNF is also involved in the survival and maintenance of neuron, formation of different synapses and development of neural circuitry (Lee et al., 2012). BDNF is involved in activity-dependent neuronal plasticity, such as learning and memory (Malcangio and Lessmann, 2003). Although animal studies clearly demonstrate that a decline of BDNF does not produce depressed mood or behavior, evidence from clinical studies tells us that decreased activity of BDNF or a neuronal dysfunction occurs in the brain of patients with major depression (Lee and Kim, 2010b). Major depression is associated with impaired neuronal plasticity. Suicidal behavior can be a consequence of severely impaired neuronal plasticity in the brain (Lee and Kim, 2010b). Antidepressant treatments promote several forms of neuronal plasticity, including neurogenesis, synaptogenesis and neuronal maturation and also increase BDNF activity, which can develop the antidepressant response (Lee and Kim, 2010b). BDNF could play an important role in the modulation of neuronal networks. Such neuronal plastic change can positively influence mood or recover depressed mood (Lee and Kim, 2010b). Studies showed that neurogenesis is responsible for maintenance of the brain function during stressful conditions as well as maintenance of corticosterone level (Snyder et al., 2011). It was found that there is high level of corticosterone in hyperglycemic condition which is responsible for the development of depression (Duman et al., 1997). Alteration in BDNF in hippocampus and prefrontal cortex is also responsible for high levels of glucocorticoids, which is responsible for the development of depression (Duman and Li, 2012, Duclot and Kabbaj, 2015). It was found that BDNF concentration is decreased in the hippocampus of patients with diabetes comorbid depression (Krabbe

et al., 2007). In different diabetic rodent model, it was found that there is positive response in body processes like metabolic functions e.g., less food intake, and more utilization of blood glucose in body after treatment with BDNF (Nakagawa et al., 2000, Nakagawa et al., 2002). Different studies also showed that the action of BDNF in maintenance of metabolic functions and glucose concentration was reproduced by administration of BDNF through intracerebroventicular, proposed that BDNF act directly on CNS, mainly hypothalamus. Taken together, BDNF plays a critical role in diabetes comorbid depression because of the beneficial effect on the brain.

1.2.3.4 Monoamine dysregulation

The catecholamine or monoamine hypothesis explains that the pathophysiology of depression involves the deficiency of neurotransmitter such as noradrenaline, serotonin, and dopamine (Schildkraut, 1965, Delgado, 2000). Some studies showed that an imbalance in levels of neurotransmitters like glutamate (excitatory) and gamma-aminobutyric acid (inhibitory) play an important role in depression (Altamura et al., 1995, Levine et al., 2000, Catena-Dell'Osso et al., 2013). The brain imaging studies of depressive patient showed that depression occurs because of decreased concentration of GABA in the prefrontal and occipital cortices (Maciag et al., 2010, Rajkowska et al., 2007). Moreover, the role of neuropeptides such as substance P, neurokinin, corticotropin releasing factor, vasopressin, neuropeptide Y, and galanin in depression has been reported (Holmes et al., 2003). Other receptors involved in depression are glucocorticoid receptor, opioid receptor, and cannabinoid receptors (Marazziti and Dell'Osso, 2008, Marazziti et al., 2009, Paschos et al., 2009).

In contrast, many researchers criticized the monoamine hypothesis because it does not explain many observations. For example, the monoamine levels were increased in synaptic cleft within two days of drug administration but, it was found that depressive symptoms were minimized only after 2 to 3 weeks (Schildkraut, 1965). Other drawbacks are, antidepressants are known for the treatment of depression while ineffective in panic disorder and compulsive disorder. It is also evident that drugs that enhance monoamine (serotonin, noradrenaline, dopamine) levels does not show complete response against depression (Krystal et al., 2002). Despite drawbacks, it was observed that alteration of central neurotransmitter system was seen in hyperglycemic conditions (Gupta et al., 2014, Abraham et al., 2010, Li and France, 2008, Umeda et al., 2007). As tryptophan is responsible for the synthesis of serotonin, it was observed that concentration of tryptophan was low in both diabetic and depressive conditions (Maes et al., 1990, Cowen et al., 1989, Herrera et al., 2003). Evidence obtained from brain areas of hyperglycemic animals showed that monoamine level was altered as compared to non-hyperglycemic rats (Ezzeldin et al., 2014). The changes in serotonin level depend on brain regions of hyperglycemic animals. For example, serotonin levels were low in thalamus/hypothalamus, cerebellum, and brainstem while there is high level in cerebral cortex and midbrain. As a result of changes in serotonin level in different areas of brain, changes in activity of serotoninergic receptors were observed in diabetes comorbid depression (Abraham et al., 2010).

1.2.3.5 Mitochondrial dysfunction

We know that mitochondria is the power house of cell and provides energy to cell (Dorn II, 2013). During normoglycemic condition, ATP is generated by the

electrochemical proton gradient through ATP synthase, and the leakage of 0.1% of total oxygen consumption from respiratory chain is responsible for the generation of reactive species (Kalogeris et al., 2014). In hyperglycemic condition, there is large intracellular concentration of glucose, which causes excessive flux of electron transfer donors (NADH and $FADH_2$) into mitochondrial respiratory chain (Malferrari et al., 2019). As a result, the mitochondrial inner membrane partially inhibits electron transport in complex 3 and accumulates electrons in ubisemiquinone (Turrens, 2003), leading to generation of superoxide ions (Nishikawa et al., 2000, Trumpower, 1990). Mitochondrial dysfunction mechanism involved in the induction of diabetes comorbid depression is showed in Figure 1.5.

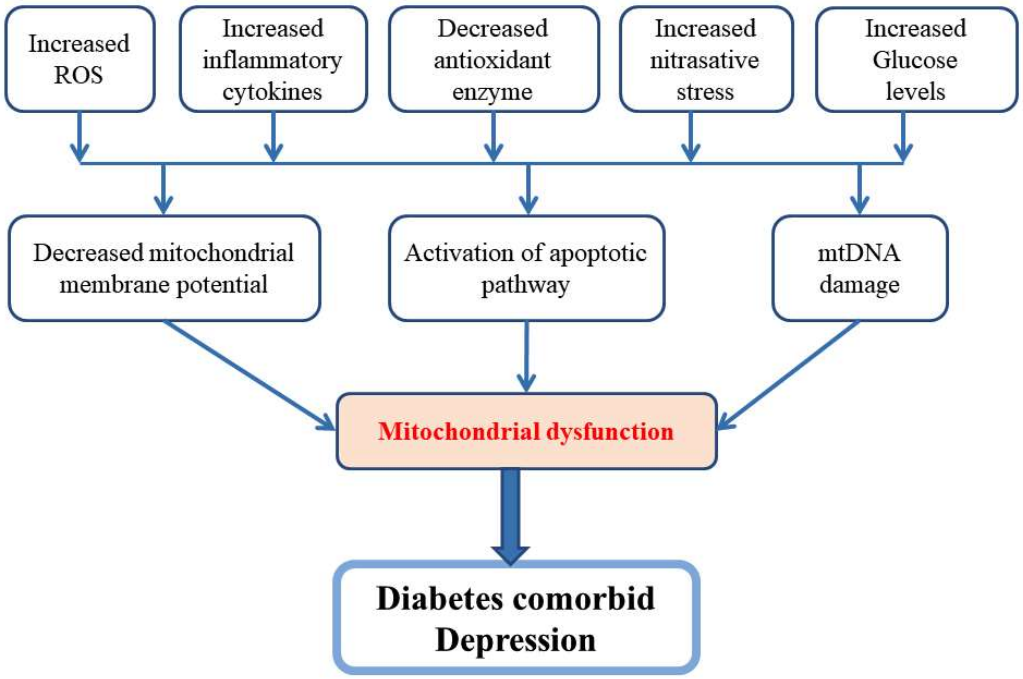

Figure 1.5: Mitochondrial dysfunction mechanism involved in the induction of diabetes comorbid depression

Mitochondria could play a role in the dampened plasticity associated with depression. Depression is associated with abnormalities in intracellular second messenger signal transduction cascades resulting from 5HT and NE receptor activation (Perez et al., 2000, Popoli et al., 2000) and dysregulated and desensitized monoamine receptors (Hamon and Blier, 2013). These observations can be related to mitochondrial dysfunction because ATP is needed for the activation of downstream signaling following the binding of neurotransmitters to receptors (Moretti et al., 2003). ATP is also necessary to attend to the energy demands of vesicle transport and neurotransmitter release (Vos et al., 2010). Furthermore, patients with mitochondrial diseases or mitochondrial DNA (mtDNA) mutations and polymorphisms often present symptoms characteristic of mood disorders (Suomalainen et al., 1992, Onishi et al., 1997, Fattal et al., 2006). Higher rates of mitochondrial biogenesis are needed for neuronal differentiation (Calingasan et al., 2008) and therefore, dysfunctional mitochondria could result in impaired neuroplasticity in depressed patients.

Taken together, the hyperglycemic condition increases the metabolic flux through the mitochondrial electron transport chain, leading to ineffective electron transfer to the oxygen molecules, which results in generation of free radicals and superoxide ions (Brownlee, 2005).

1.2.3.6 Inflammation

Different theories have been proposed to explain the occurrence of inflammatory response in diabetes comorbid depression condition. In diabetes comorbid depression, inflammatory response can occur in both peripheral as well as central tissue (O'connor et al., 2009, Fu et al., 2010, Haroon et al., 2012). One

hypothesis called as 'macrophage theory' of depression (Smith, 1991) was proposed, which involves activation of macrophages during immune response cause release of proinflammatory cytokines and the proinflammatory agents act on CNS cause sickness behavior responsible for depression (Dantzer et al., 2008, Dobos et al., 2012). Other studies showed that the administration of inflammatory agents like lipopolysaccharides and exogenous endotoxin in animals induces depressive-like behavior (Dobos et al., 2012, Anisman et al., 2008). Studies showed that stimulation of innate immunity is also linked to depression (Raison et al., 2006) and leads to generation of proinflammatory mediators like interleukin-6, tumor necrosis factor-α (Miller et al., 2002, Penninx et al., 2003, Lespérance et al., 2004). Patients (having inflammatory response) treated with interferon alpha showed depressive symptoms in 50% cases, which are having history of depression or decreased level of tryptophan (Raison et al., 2006). It was observed that lipopolysaccharide or interleukin-1 result in amplification of depressive-like behavior through induction of inflammation in central as well as peripheral tissues (O'Connor et al., 2005). It was observed that cytokines are able to penetrate through blood brain barrier (Gutierrez et al., 1993). Studies showed that concentrations of interleukin-6 and tumor necrosis factor-alpha (TNF-α) are increased in depression associated with diabetes (Dowlati et al., 2010). Further, IL-6 causes glucose intolerance while acting on peripheral tissues when animals received high fat diet (Hidalgo et al., 2010). These observations suggest that inflammatory response on peripheral tissue results in prediabetic state. Moreover, it was also observed that TNF-α and IL-6 are associated with both depression as well as production of adipokine (Dowlati et al., 2010). Inflammatory mechanism involved in the induction of diabetes comorbid depression is showed in Figure 1.6.

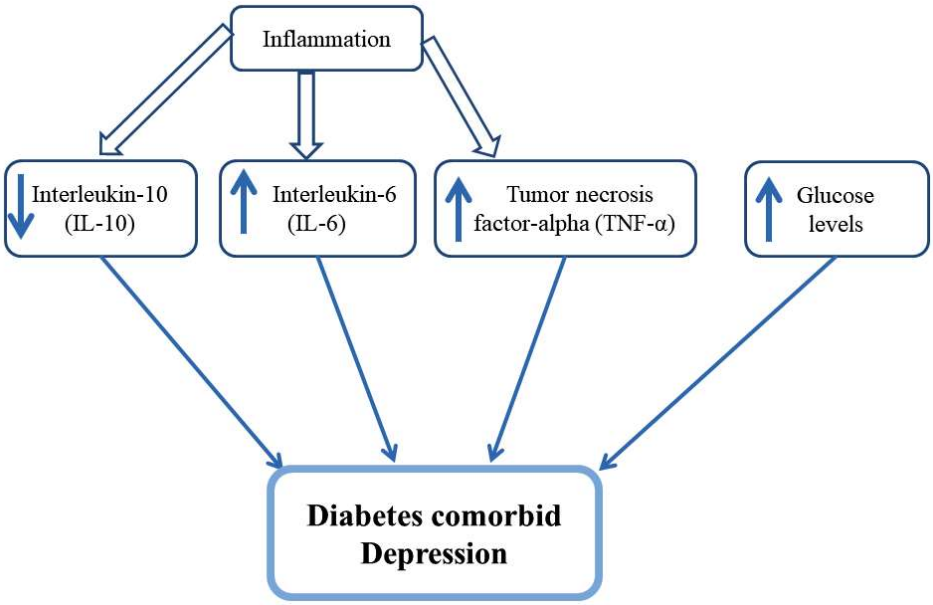

Figure 1.6: Inflammatory mechanism for diabetes comorbid depression

Different observations showed that type-2 diabetes is linked with obesity, depression (Luppino et al., 2010), and inflammation (Donath and Shoelson, 2011). Previously, it was believed that role of adipose tissues is only for storage of lipids but later, it was found that adipose tissues were also linked with production and release of adipokynesm, which activate immune system by several ways. The mechanism by which adipose tissues lead to production of cytokines is stimulation of NF-Kappa B and JNK pathways, hypoxia in the adipose tissue, activation of toll-like receptor, and by free fatty acids. Moreover, it was observed that in obese conditions, there is secretion of monocyte chemoattractant protein-1 (MCP-1) (Skurk et al., 2007). The MCP-1 causes production of M1/M2 macrophages in a balance way. However, in obese condition, more production of M1 macrophages ultimately lead to production of proinflammatory agents (Lumeng et al., 2008).

It was reviewed that HPA axis dysregulation and oxidative stress are also responsible for the production of inflammatory agents (Stuart and Baune, 2012). HPA axis dysregulation results in the production of glucocorticoids (Golden, 2007) and these hormones play a critical role in glucose metabolism and insulin expression. With immune suppression and reinforcement of inflammatory response, it was also reported that these hormones result in insulin resistance and diabetes (Golden, 2007). Additionally, it was observed that HPA axis dysregulation not only results in production of inflammatory agents but also affect sympathetic nervous system which in turn results in production of IL-6 that causes further release of proinflammatory agents (Champaneri et al., 2010).

Depression is associated with more production or high level of endogenous neurotoxin and low level of monoamines which are caused by overexpression of proinflammatory agents (Maes and Rief, 2012). It has been found that the activation of cell mediated immune response depends on an enzyme called indoleamine 2,3-dioxygenase, which affect tryptophan metabolism (Mellor, 2005). The enzyme causes increased metabolism of tryptophan leading to low level of tryptophan in plasma, hence decreased synthesis of serotonin and increased generation of neurotoxic substances such as kynurenic acid and xanthurenic acid (Li et al., 2018).

1.2.3.7 Epigenetic modification

Modification in gene expression by environment is known as epigenetic changes, dealing with changes in gene expression without changes in DNA sequence e.g., methylation of cytosine, histone post-translational modifications in chromatin (Kato and Natarajan, 2014). Recent evidence showed that genetic changes also play

important role in occurrence of depression in diabetes. Moreover, it has been observed that there is more expression of genes which are responsible for cell growth and stimulation of proinflammatory, proapoptotic agents through cellular transcription affected by hyperglycemic and depressive conditions (Lazarus et al., 2011, Forbes and Cooper, 2013). It has been examined that environment affects gene expression and these interaction of genes with environment play a significant role in occurrence of disease (Kato and Natarajan, 2014). Epigenetic modifications involved in the induction of diabetes comorbid depression are showed in Figure 1.7.

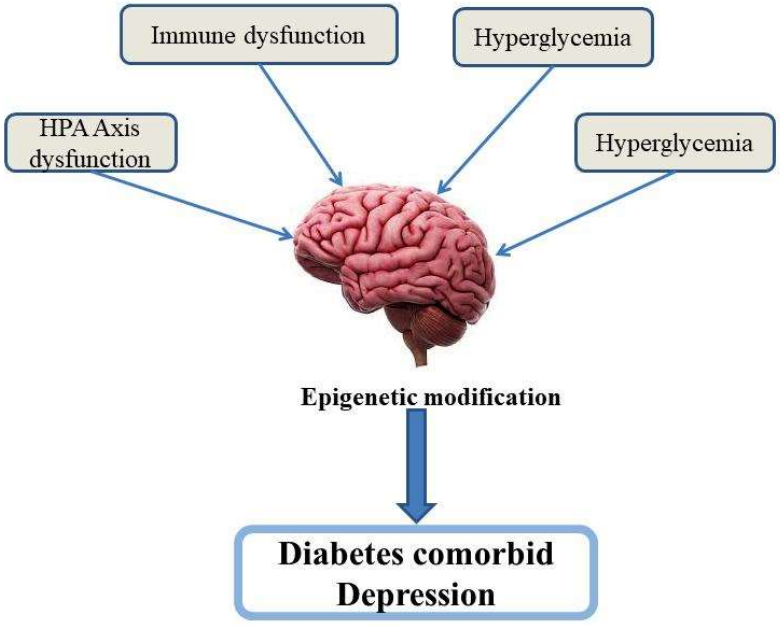

Figure 1.7: Epigenetic modification for induction of diabetes comorbid depression

Normally, the epigenetic changes are responsible for normal cell growth, differentiation, and genome stability. But, continuous presence of epigenetic alteration results in pathophysiology or occurrence of diseases like diabetes and depression (Bale et al., 2010, Reddy et al., 2015). Different evidence showed that epigenetic

changes are also responsible for the pathophysiology of disease (Kato and Natarajan, 2014, Picascia et al., 2015, Stankov et al., 2013, Peña et al., 2014, Duclot and Kabbaj, 2015), because there is more generation of advanced glycation end products and ROS in hyperglycemic conditions (Nowotny et al., 2015). These end products and reactive species act on many target cells by different signaling pathways resulting in expression of NF-kappa B (Mohamed et al., 1999). As a result, there is more production of cytokines leading to cell damage and inflammatory response (Rock and Kono, 2008). Studies reported that genes controlling phenotype and pathophysiology of disease may be different in different cells such as neuronal and/or smooth muscle (Reddy et al., 2015). Moreover, epigenetic changes are reversible and opposite to mutation process such as activation of genes epigenetically inactivated result in increased sensitivity to treatment. As a result, therapies or drugs which are responsible for epigenetic inactivation reversal are used as therapeutic treatment method e.g., miRNA inhibitors. (Reddy et al., 2015, Picascia et al., 2015, Baylin and Jones, 2011). In contrast, this process was criticized because of incomplete explanation of action of epigenetic drugs. Moreover, different results showed that epigenetic changes are cell-specific and results obtained from biopsies are difficult to interpret (Reddy et al., 2013). Additionally, other factors are also associated with diabetes like obesity, insulin resistance, and lifestyle are also responsible for epigenetic variation (Reddy et al., 2015). Hence, more information is required to know about the modification in genes due to environment for early detection of diseases and treatment of various diseases.

1.3 Management and treatment

In general, management of diabetes comorbid depression is important for improving the quality of life as well as removing the macrovascular as well as microvascular complications associated with diabetes comorbid depression (Gomez et al., 2001, Gonzalez et al., 2008, Rubin et al., 2010). The diabetes comorbid depressed patients benefit from the activities such as regular physical activity, getting help or support from family and friends, knowing about depression and diabetes, eating healthy food, involving in social activities, and consulting with doctors and other health professionals (Huang et al., 2013).

There are different treatment options available for depression. Cognitive behavior therapy (CBT), to identify and remove negative thinking pattern (DeRubeis et al., 1999). Interpersonal therapy (IPT), involves improving the relationship between chronic illness and need for long term therapy (Hetrick et al., 2016). Any medication such as over-the-counter products must be reviewed before using for depression. Antidepressants take long time (days to weeks) to work hence, treatment should not be stopped without the advice of the doctor (Anderson and Roy, 2013). Electroconvulsive therapy (ECT) is also found to be effective in diabetic patients (Tew Jr et al., 1999). ECT is safe for diabetic patients and can be used in diabetic patients intolerant to medications and suffering from life-threatening depression (Williams et al., 2006).

Pharmacological treatment involves selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs), and antihyperglycemic agents. Selective serotonin reuptake inhibitors play an important role in the treatment of comorbid

depression in type 2 diabetes patients as it also control blood glucose level (Deuschle, 2013). SSRIs decrease HbA1C level (Ghaeli et al., 2004, Markowitz et al., 2011), and improve sensitivity of insulin receptors (Sawka et al., 2000). Citalopram produces antidepressant activity without affecting body weight (Nicolau et al., 2013). TCAs are generally not recommended in diabetic patients (Deuschle, 2013) due to their adverse effects such as orthostatic hypotension, urinary retention, and QT interval prolongation (Tesfaye et al., 2011). Antihyperglycemic agents such as insulin regimens show good response in diabetes (Wu et al., 2015). Glycemic control directly affects mood and improves depressive behavior (Lustman and Clouse, 2005). Pioglitazone reduces depressive-like behavior and decreases microglial stimulation as well as neuronal damage (Kemp et al., 2012). The glycemic control can be achieved by healthy diet, oral hypoglycemic agents, and insulin therapy (Menezes Zanoveli et al., 2016).

Over the past few years, researchers have successfully described high prevalence of diabetes and its psychological sequelae (De Groot et al., 2016). Diabetes increases the risk of depression (Celano et al., 2013). Patients suffering with diabetes comorbid depression were incompletely diagnosed as physician may not focus on the psychological problems associated with diabetes such as loss of interest in regular activities, memory impairment, and concentration difficulties (Biessels et al., 2014, Petrak et al., 2015). Hence, there is under-recognition of psychiatric disorder associated with diabetes. The diagnosis of psychopathological disorders requires the expertise of an experienced physician using validated diagnostic criteria and multiple observations of the patients (Starkstein et al., 2014). Preclinical research using animal models based on negative behavioral symptoms of depression, anxiety

such as fear, sadness, and hopelessness would help in investigating the underlying molecular mechanisms of psychological sequelae of diabetes. Most of the available studies proposed general hypothesis of association between diabetes and depression (Menezes Zanoveli et al., 2016, Gragnoli, 2014). However, there is an emerging need for both preclinical and clinical improved study designs and expansion of the research on the biological determinants and pathophysiology of diabetes comorbid depression. Management psychopathological symptoms of diabetes comorbid depression requires further investigation which should include pilot studies of innovative behavioral interventions and large-scale randomized clinical trials of drugs that are safe for patients with diabetes (Menezes Zanoveli et al., 2016). The pathophysiology of diabetes comorbid depression is complex and requires great efforts for understanding the pathological mechanism behind this complication and there should be early diagnosis of the disease for better and effective treatment of the disease (Petrak and Röhrig, 2018).

1.4 Metformin

Metformin (N,N-dimethylimidodicarbonimidic diamide) was first discovered in *Galega officinalis* in the early 90s (Bailey, 2017, Oubre et al., 1997) and later became popular in modern medicine as an oral hypoglycemic agent for the management of type 2 diabetes mellitus (Miller et al., 2013). In 1957, Stern discovered the clinical efficacy of metformin when working in Paris and observed that the metformin was related to glucose lowering capacity (Sterne, 1958). Metformin act through reducing liver glucose output and enhancing glucose uptake in the peripheral tissues (Rojas and Gomes, 2013). These effects are mediated by the activation of an upstream kinase

(liver kinase), which in turn regulates the downstream kinase, i.e. adenosine monophosphatase protein kinase (Viollet et al., 2006). Adenosine monophosphatase protein kinase phosphorylates a transcriptional co-activator resulting in its inactivation which accordingly downregulates transcriptional actions that promote synthesis of gluconeogenic enzymes (Kim et al., 2008). The antidiabetic efficacy, safety profile, and compatibility with other antidiabetic agents make metformin the first-line glucose-lowering agent against type 2 diabetes mellitus (Garber, 1997). It is clear that metformin does not increase body weight as compared to other oral antidiabetic agents, and may help to limit the weight gain linked with insulin- or sulphonylurea-based regimens (Viollet et al., 2012). This observation supports present recommendations relating to the suitability of metformin in patients with type 2 diabetes irrespective of body weight (DeFronzo et al., 2005).

1.4.1 Chemistry of metformin

Metformin (Figure 1.8) is a white hygroscopic crystal-like powder with a bitter taste (Saxena et al., 2010). Chemically, it is 1,1 dimethyl-biguanide hydrochloride with a antidiabetic mode of action comparable to other biguanides (Saxena et al., 2010). This small molecule is soluble in water and 95% alcohol while it is practically insoluble in ether or chloroform (Saxena et al., 2010). It undergoes negligible hepatic metabolism and is excreted by the kidney with a half-life of about two hours (Mubeen and Noor, 2009). Several studies reported on crystallographic structure of metformin and its derivatives-namely metformin hydrochloride, N,N-dimethylbiguanidium nitrate, and metal complexes with metformin (Trouillas et al., 2013). The IUPAC name of metformin is N,N-dimethylimidodicarbonimidic diamide (Trouillas et al., 2013).

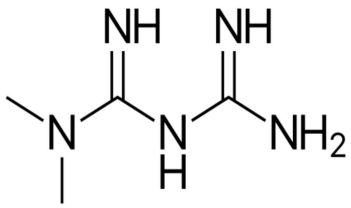

Figure 1.8: Structure of metformin

The reaction of dimethylamine (1) and 2-cyanoguanidine (2) with heating involves the synthesis of metformin (3) (Figure 1.9). According to the procedure described in 1975 in Aron patent and the pharmaceutical manufacturing encyclopedia, equimolar amounts of dimethylamine and 2-cyanoguanidine are liquefied in toluene with cooling to make a concentrated solution, and an equimolar quantity of hydrogen chloride is slowly added. The mixture begins to boil on its own, and after cooling, metformin hydrochloride precipitates with a 96% yield (Werner and Bell, 1922).

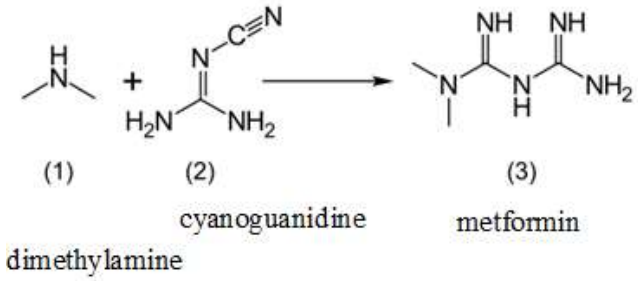

Figure 1.9: Synthesis of metformin

1.4.2 Pharmacokinetics of Metformin

1.4.2.1 Absorption and bioavailability

Metformin is absorbed incompletely and mostly from the small intestine (Marathe et al., 2000). Oral absorption of metformin occurs in 6 hours with 50 to 60% bioavailability (Pentikäinen et al., 1979). The difference between absorbed and available drug may reflect binding to the intestinal wall or minor presynaptic

clearance of the drug. The lack of dose proportionality with increasing doses is indicated by single oral doses of metformin HCl 500 mg to 1500 mg, and 850 mg to 2550 mg, which is due to decreased absorption rather than an alteration in elimination (Hasan et al., 2013). Food decreases the extent of absorption of metformin, as shown by nearly a 40% lower mean peak plasma concentration, 25% lower area under the plasma concentration versus time curve, and a 35 minutes' prolongation of time to peak plasma concentration (Brookes et al., 1991).

1.4.2.2 Distribution

Data obtained in mice indicate that the tissues of the small intestine may represent an important depot for accumulation of metformin (Wilcock and Bailey, 1994). Binding to plasma proteins does not occur but an increase in the blood: plasma metformin concentration ratio over 24 hours has been observed after single oral dose indicates a slow association of the drug with blood cells (Tucker et al., 1981).

1.4.2.3 Metabolism

Metformin was reported not to undergo metabolism in either healthy volunteers or patients with diabetes, although in one study 20% of an intravenously administered dose was unaccounted for metabolism (Sambol et al., 1996). It is therefore possible that some metabolic transformation may occur in humans, but no metabolites or conjugates have been identified (Sambol et al., 1993).

1.4.2.4 Elimination

Metformin does not bind to plasma proteins and rapidly excreted unchanged in the urine with a mean half-life of 4.0 to 8.7 hours in healthy volunteers (Scheen,

1996). However, the patients with kidney impairment have prolonged half-life and correlates with creatinine clearance (Garraffo et al., 1997). Ranges of values for renal and total clearance (CLR and CLT) are reported to be 20.1 to 36.9 L/h and 26.5 to 42.4 L/h, respectively, indicating active tubular secretion of metformin (Figure 1.10) (Scheen, 1996). Renal clearance is correlated with creatinine clearance and after intravenous administration, the majority of the dose is excreted within 8 hours (Tucker et al., 1981).

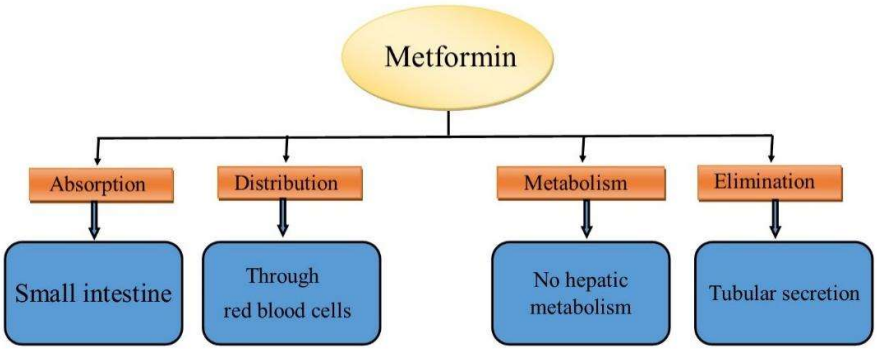

Figure 1.10: ADME of metformin

1.4.3 Role of metformin in diabetes

Metformin helps lowering blood glucose levels by improving the way the body handles insulin, by preventing the liver from making excess glucose, and by making muscle and fat cells more sensitive to available insulin (Klip and Leiter, 1990). Metformin not only lowers blood glucose levels, it also lowers blood cholesterol, and triglyceride levels, and does not cause weight gain (Qaseem et al., 2012). Overweight, high lipid, and high cholesterol levels increase the chance of developing micro and macro vascular complications, which is the principal cause of death in people with type 2 diabetes mellitus (Giugliano et al., 1996). Another advantage of metformin is

that it does not cause hypoglycemia (Nasri and Rafieian-Kopaei, 2014). The immediate-release formula of metformin is usually taken two to three times a day, with meals and the extended-release formula of metformin is taken once a day, with the evening meal (Gusler et al., 2001). The foremost common side effects of metformin are nausea and diarrhea that usually go away over time (Foss and Clement, 2001). A more serious side effect is a rare however probably fatal condition called lactic acidosis, during which high levels of lactic acid build up in the bloodstream (Bolen et al., 2007). Lactic acidosis is observed in people with kidney disease, liver disease, or congestive heart failure, or in those who drink alcohol regularly (Bolen et al., 2007).

1.4.4 Mechanism of metformin in diabetes

Metformin decreases hyperglycemia principally by suppressing glucose production by the liver i.e. hepatic gluconeogenesis (Figure 1.11) (Wang et al., 2012). Individuals with type 2 diabetes have three times the normal rate of gluconeogenesis and metformin treatment decreases this by over one-third (Hundal et al., 2000). The mechanism of metformin involves inhibition of the mitochondrial respiratory chain (complex I), activation of adenosine monophosphate-activated protein kinase (AMPK), inhibition of glucagon-induced elevation of cyclic adenosine monophosphate (cAMP) with reduced activation of protein kinase A (PKA), inhibition of mitochondrial glycerol phosphate dehydrogenase, and an effect on gut microbiota (Madiraju et al., 2014).

Activation of AMPK, an enzyme that plays an important role in insulin signaling, whole body energy balance, and the breakdown of glucose and fats, is

required for metformin's inhibitory effect on the production of glucose (Suwa et al., 2006). Activation of AMPK is required for an increase in the expression of small heterodimer partner, which in turn inhibit the expression of the hepatic gluconeogenic genes (Chanda et al., 2009). Metformin increases the concentration of cytosolic AMP (Zhang et al., 2007). Increased cellular AMP has also been proposed to explain the inhibition of glucagon-induced increase in cAMP and activation of protein kinase A (Miller et al., 2013).

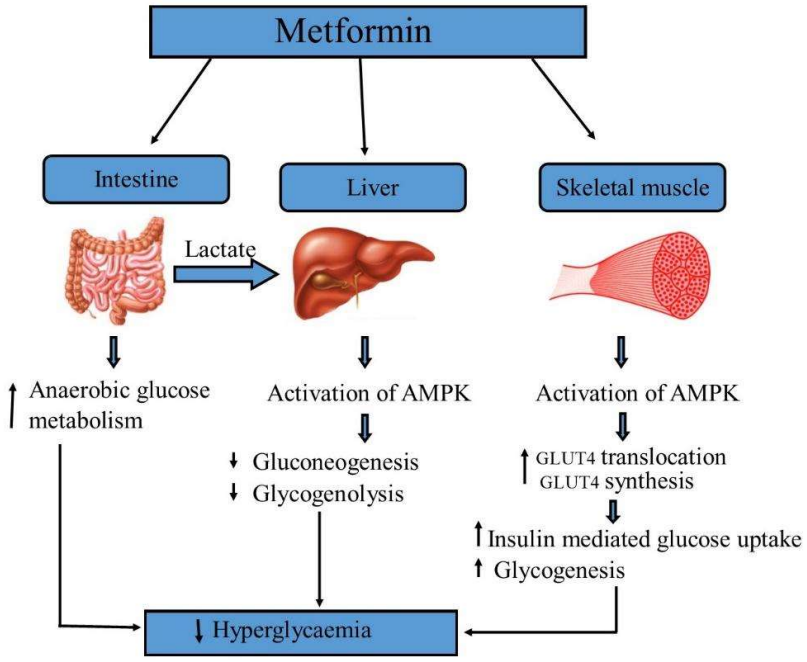

Figure 1.11: Mechanism of action of metformin in diabetes

Metformin and other biguanides may antagonize the action of glucagon to reduce fasting glucose levels (Rena et al., 2013). Metformin induces a profound shift in the fecal microbial community profile in diabetic patients and this may contribute to its mode of action possibly through an effect on glucagon-like peptide-1 secretion (Kim et al., 2008). Metformin increases AMPK activity in skeletal muscle and

increases GLUT4 translocation to the plasma membrane, resulting in insulin-independent glucose uptake (Rena et al., 2013). Some metabolic actions of metformin do appear to occur through AMPK-independent mechanisms; the metabolic actions of metformin in the heart muscle can occur independent of variations in AMPK activity and may be mediated by p38 MAPK- and PKC-dependent mechanisms (Saeedi et al., 2008).

Along with suppression of hepatic glucose production, metformin enhances insulin sensitivity, increases peripheral glucose uptake (by bringing the phosphorylation of GLUT4 enhancer factor), reduces insulin-induced suppression of fatty acid oxidation, and reduces absorption of glucose from the gastrointestinal tract (Rena et al., 2013). Metformin enhances peripheral use of glucose through improving insulin-insulin receptor interaction (Rena et al., 2013). The increase in insulin binding after metformin treatment has also been established in patients with noninsulin-dependent diabetes mellitus (Fantus and Brosseau, 1986).

1.4.5 Central nervous system regulation

The relationship between metformin and dementia has recently been reported in cellular, animal, and epidemiological studies (Huang et al., 2014). Metformin can pass through the blood-brain barrier and have specific pharmacological effects on the central nervous system (CNS) (Łabuzek et al., 2010). However, the exact mechanism and sites of its action in the CNS remain uncertain. Recently, metformin has drawn attention of researchers because of its possible beneficial effects on the CNS. Metformin has been shown to protect against apoptotic cell death in primary cortical neurons, promote neurogenesis, and reduce CNS-based inflammation (Nath et al.,

2009). In contrast, one study reported that metformin could deregulate β-secretase (BACE1) promoter activity and induce more than two times the normal production of β-amyloid peptide, the protein that forms toxic brain plaques in Alzheimer's disease (Chen et al., 2009). Epidemiological evidence (Biessels et al., 2006) suggests that diabetes increases the risk of dementia; diabetes and dementia are two of the most common and overwhelming health problems in the elderly. “Diabetes dementia” is probably a mix of vascular and neurodegenerative dementia (Huang et al., 2014).

Metformin may directly affect the hypothalamus neurons that control feeding behavior (Chau-Van et al., 2007). It has been reported that oral metformin administered to diabetic rats have elevated concentrations of the drug in the cerebrospinal fluid (Łabuzek et al., 2010). Indeed, metformin reduces food intake by reducing the orexigenic peptides, neuropeptide Y, and agouti-related protein in the hypothalamus (Lv et al., 2012). As metformin regulates the interaction of insulin resistance and adenosine AMPK in the liver, skeletal muscle, and adipose tissue, it is reasonable to note that metformin mediates anorectic effects by changing hypothalamic AMPK (Lv et al., 2012). In response to low blood glucose or caloric deficit, ghrelin secreted by the stomach stimulates food intake by increasing neuropeptide Y and agouti-related protein neural activity via AMPK (Lv et al., 2012). As metformin lowers blood glucose and body weight, it is not surprising to see reports that indicate higher ghrelin levels in humans with type 2 diabetes (Lv et al., 2012). This apparent discrepancy between weight loss and elevated ghrelin is likely explained by the fact that metformin blocks ghrelin-induced activation of AMPK in the brain without affecting ghrelin level (Kola, 2008). Signal transducer and activator of transcription 3 were recently reported to regulate feeding, and STAT3 activation is

also associated with the leptin receptor (Kola, 2008). Metformin has been shown to enhance STAT3 signaling in the hypothalamus and inhibit NPY and AgRP expression, highlighting that metformin controls food intake by affecting multiple appetite regulatory pathways (Duan et al., 2013).

Another potential factor through which metformin affects food intake is related to the nonspecific toxic mechanisms that induce gastrointestinal stress (e.g. nausea, diarrhea, etc.) and taste disturbances (Palomba et al., 2008). The nucleus tractus solitarius (NTS) is embedded in the medulla oblongata and important for feeding behavior because this is the first synaptic contact for vagal afferent projections from the gastrointestinal tract (Rui, 2013). The hindbrain also contains the area postrema, where the blood brain barrier is circumvented, and accessible to feeding signals through both vagal afferents and circulating hormones [e.g. leptin, insulin, and glucagon-like peptide-1 (GLP-1)] (Akieda-Asai et al., 2014a). In addition, the NTS and area postrema are important neurocircuits which innervate multiple forebrain regions, including the hypothalamus, to modulate energy status (Van Daele and Cassell, 2009). The role of metformin on meal intake via changes in NTS and area postrema activity has received less attention compared to that of the hypothalamus, but recent work demonstrates that metformin increases c-FOS expression in the NTS, a marker of neural activation, and parallels reduced meal intake (Kim et al., 2013).

1.4.6 Regulation of gut-mediated satiety signals

Metformin induces weight loss by enhancing satiation signals secreted by the gut (Malin and Kashyap, 2014). Glucagon-like peptide-1 (GLP-1) is a hormone produced in the L-cells of the gastrointestinal tract in response to nutrient intake (Lim

and Brubaker, 2006), with secondary production in the nucleus of the solitary tract cell bodies of the brainstem (Alhadeff et al., 2012, Trapp and Richards, 2013). GLP-1 reduces appetite by acting on vagal afferents that reach the NTS and by directly decreasing hypothalamic AMPK activity, which is associated with elevated proopiomelanocortin (Poleni et al., 2012). Elevated GLP-1 in turn slows gastric motility and emptying in individuals and contributes to reduced carbohydrate absorption and circulating glucose (Salehi et al., 2010). Importantly, when midbrain transacted rats are studied to investigate the neural pathway from the hindbrain to the hypothalamus, it was demonstrated that the NTS alone has less prominent effect on food intake reduced by GLP-1 and leptin (Akieda-Asai et al., 2014b). These findings imply that both circulating and neural factors are likely to be involved in the regulation of food intake. Metformin potentially reduces hunger and affects carbohydrate absorption by raising GLP-1 through inhibition of dipeptidyl peptidase-IV (Green et al., 2006) and alteration in muscarinic and gastrin-releasing peptide related pathways (Malin and Kashyap, 2014, Mulherin et al., 2011). This elevated GLP-1 may also be influenced by metformin-induced changes in the gut microbiota (Malin and Kashyap, 2014), which have been strongly implicated in energy extraction, obesity, and diabetes. Moreover, it has been observed that metformin directly affects the enterocyte not only by increasing glucose utilization in the intestinal mucosa but also by altering immune signals in the gut that regulate energy homeostasis and insulin action (Shin et al., 2014). As peptide YY and cholecystokinin are not directly affected by metformin, higher GLP-1 in humans and likely changes in the gut flora appears to be an enteroendocrine mechanism influencing body weight (Rohde et al., 2016). Indeed, when metformin is co-prescribed with DPP-IV inhibitors

greater weight loss and glycemic control is observed in rodents and adults with type 2 diabetes (Cervera et al., 2008).

1.5 Ascorbic Acid

Ascorbic acid, a natural antioxidant, is primarily consumed through a diet rich in fresh fruits and vegetables (Carr and Frei, 1999). Ascorbic acid is a major free radical scavenger, therefore, prevents cellular damage induced by free radicals (Lobo et al., 2010) and provides protection against diseases (arthritis, atherosclerosis, cancer, diabetes, ischemia) that involves oxidative stress (Chambial et al., 2013, Rajendran et al., 2014). Besides, ascorbic acid acts as a cofactor in the biosynthesis of catecholamines, amino acids, and certain peptide hormones (Harrison and May, 2009). The benefits of ascorbic acid include free radical reduction, nitric oxide synthesis or release control, repression of reactive oxygen species production, and the induction of antioxidant enzymes (Smirnoff, 2000). In this context, it has been observed that the dietary antioxidant ascorbic acid is negatively associated with the presence of several disease conditions such as hypertension, gallbladder disease, stroke, cancers, and atherosclerosis, and also with the occurrence of obesity (Garcia-Diaz et al., 2010b, Flora, 2007, Bsoul and Terezhalmy, 2004). Among the beneficial effects of ascorbic acid on obesity-related mechanism, the modulation of adipocyte lipolysis, glucocorticoid release from adrenal glands, hyperglycemia improvement and glycosylation decrease in obese diabetic, and an inhibition of the inflammatory response have been documented (Johnston, 2005). Lipoprotein lipase is the most important substance involved in absorption and breaking down of triglycerides in blood (Daniels et al., 2009). One of the most important effects of ascorbic acid is that

it activates lipoprotein lipase (Kotze et al., 1973). It is believed that the lipolytic effect of ascorbic acid is via the mechanism that inhibits triglyceride accumulation (Senen et al., 2002, Frayn et al., 1995, Krotkiewski et al., 1983). Excess fibrosis and skin contractures may be ascribed to increase in collagen synthesis by ascorbic acid (Pinnell et al., 1987, Darr et al., 1993, Maione-Silva et al., 2019).

The various reports documented the role of ascorbic acid in steroidogenesis (Gupta et al., 2004). The decrease in level of adrenal ascorbic acid concentration occurs in the guinea pig having scurvy (Hasselholt et al., 2015). Isolated adrenal cells display decrease in steroidogenesis (Hasselholt et al., 2015)and lipid peroxidation with the addition of ascorbic acid (Sönmez et al., 2005). It has been documented that differing concentrations of ascorbic acid within the adrenal may exert a modulating role in the production and/or release of corticosteroids (Doulas et al., 1987). Various hormones, circulating compounds, and neurogenic signals have been examined for their potential ability to regulate adipose leptin secretion, including insulin, cAMP and glucocorticoids (Mick et al., 2000). Diaz et al. 2010 reported *in vitro* effects of ascorbic acid incubated with epididymal rat adipocyte metabolism and secretory functions (Garcia-Diaz et al., 2012). The glucose uptake inhibition observed in adipocytes without insulin treatment, and especially in adipocytes under insulin treatment, could be partially explained by the fact that dehydroascorbic acid possibly competes with glucose for GLUT1 (SLC2A1), GLUT3 (SLC2A3), and GLUT4 (SLC2A4) transporters, respectively (Rivas et al., 2008). However, it was reported that in primary cultures of rat hippocampal neurons, ascorbic acid accumulation inhibited the glucose transport inside the cytoplasm independently of this competition (Ailhaud, 1997). The lactate production inhibition by ascorbic acid is in agreement

with a study that described a decrease in lactic acid plasma concentration in rats with streptozotocin-induced diabetes (Rupérez et al., 2008). The lower glycerol release induced by ascorbic acid may indicate inhibited fat utilization in both insulin-treated and non-treated adipocytes (Garcia-Diaz et al., 2010a). It has been described that rats fed with high-fat diet with ascorbic acid supplementation presented a decreased isoproterenol-induced lipolysis compared with the rats fed with high-fat diet alone (Perez-Matute et al., 2007, Dyck, 2009, Garcia-Diaz et al., 2010a).

Regarding the secretion and expression of some adipokines, the important inhibitory effect of ascorbic acid is on leptin secretion (Garcia-Diaz et al., 2010a). The reduction in leptin secretion was accompanied by a decrease in body weight and adiposity (García et al., 2012). The leptin secretion inhibition was mainly due to specific effects of the ascorbic acid treatment over the adipocytes and not due to mass-reducing effects on the leptin secretor tissue (García et al., 2012). Further, it was reported that the leptin expression and secretion in cultured rat adipocytes were decreased by glucose uptake inhibition (Mueller et al., 1998). The positive correlation between leptin secretion and glucose uptake in both insulin-treated and non-treated cells suggests that the glucose uptake inhibition by ascorbic acid (Garcia-Diaz et al., 2012). It indicates that glucose utilization stimulates leptin production by driving the glucose metabolism to oxidation or lipogenesis, rather than anaerobic lactate production (Mueller et al., 1998).

1.6 Aim

The overall aims of the research conducted in this thesis were to evaluate the therapeutic potential of metformin and ascorbic acid against diabetes comorbid depression in rats.

Specifically, the aims were to:

1) Evaluate the efficacy of ascorbic acid monotherapy against diabetes comorbid depression in rats. (Chapter 2)

2) Evaluate the efficacy of metformin monotherapy against diabetes comorbid depression in rats. (Chapter 3)

3) Evaluate the efficacy of ascorbic acid and metformin combination therapy against diabetes comorbid depression in rats. (Chapter 4)

4) Evaluate the role of BDNF, caspase, NF-kB, and mitochondrial functions in the efficacy of ascorbic acid and metformin combination therapy. (Chapter 5)