CHAPTER 2

REVIEW OF LITERATURES
2. Review of Literature

2.1. Depression

A World Health Organization survey from primary healthcare system suggests a wide variation in prevalence of depressive disorder across 14 countries (from 1.6% to 26.3%) [111]. Depression is a mental health problem. It is a mood disorder in which feelings of sadness, anger or frustration interfere with the daily life for weeks or longer. Depression may be brought by alcohol or drug abuse, certain medical conditions (including underactive thyroid, cancer or long-term pain), steroidal drugs, abnormal sleeping, stressful life events, such as death of someone close to the family, divorce, loneliness (common in the elderly), relationship breakup etc. The symptoms of depression includes agitation, restlessness and anger, irritability, becoming isolated, fatigue and lack of energy, hopeless and helpless feeling, worthless, self-hate, loss of interest or pleasure in activities that were once enjoyed, change in appetite, often with weight loss or weight gain, thoughts of death or suicide, trouble concentrating, trouble sleeping or sleeping too much etc [1]. Cognitive impairments and diabetes are often observed during depression, in addition to mood disturbances and other motor, autonomic, endocrine and sleep-wake abnormalities [11, 112].

2.2. Co-morbidity of depression and diabetes with cognitive dysfunction

Many physiological alterations occur in the body during major depression. Many tissues, hormones, neurotransmitters and cytokines work together in order to rescue a stress response to maintain normal homeostasis [113-114]. The most important anatomical structures of brain involved are the hypothalamus, pituitary and the adrenal gland, constitute the HPA axis. Amygdala and prefrontal cortex also modulate the HPA axis. Depression is associated with the hyperactivity of the HPA axis [2-3]. During depressive episodes or stressful situations the hippocampus, amygdala and the prefrontal cortex are decreased in size, similarly to the effects shown during stressful conditions. The hormone cortisol is the end product of activation of the HPA axis. A meta-analysis investigated cortisol responses to stress, found that uncontrollable stressors likely to be associated with elevations in cortisol [3]. Stressors evoked drastic elevations in cortisol are important for energy mobilization to deal with impending threats; however, elevated levels of corticosteroids may also lead to deleterious health outcomes via associated increases in inflammation, oxidative stress, brain atrophy etc. Numerous studies have been conducted on structural effects of the glucocorticoids in
hippocampus and beyond. It has been reported that, glucocorticoids beyond a certain threshold level rendered corticosteroid receptor-bearing hippocampal neurons more vulnerable to excitotoxic neurotransmitter such as glutamate. A direct neurodegenerative effects of corticosteroids in hippocampal neurons, leading to reduced cell numbers. Hyperactivation of HPA axis is known to induce neuritic neurodegenerative process, reduce neurogenesis and associated with cognitive dysfunction [4-5].

The hormone insulin is secreted by the pancreatic β-cells, plays a major role in the stage that controls differentiation, especially by stem cells, into almost all of the cells that compose the body or the organ systems including hippocampus. Prolonged elevation of glucocorticoids inhibits insulin secretion from pancreatic β-cells, decrease glucose uptake and utilization, stimulates glucagon secretion, hepatic glucose production, decreased body weight and induces type 2 diabetes like state [6-7].

**Figure 1:** Effect of depression on all cause mortality in patients with diabetes, reprinted from [45], Copyright © 2015, with permission from Elsevier.

Literature showed that depression and diabetes are co-morbid, but it is not yet known whether depression increases the risk of diabetes or diabetes increases the risk of depression. Recent studies suggest that both cases are possible. In addition to possibly increasing the risk for depression, diabetes might make the symptoms of depression worse. The stress of managing diabetes daily and the effects of diabetes on the brain exacerbates the symptoms of depression [12]. At the same time, some symptoms of depression may reduce overall physical and mental health, not only increasing the risk for diabetes but making diabetes symptoms worse.
Studies have shown that people with depression and diabetes have more severe diabetes symptoms than people who do not have diabetes [6-7, 13].

It has been hypothesised that, increased risk of type 2 diabetes in depressive patients is believed to be the result from increased counter regulatory hormone mechanism, alterations in glucose transportation and increased inflammation [115]. These physiological alterations thought to contribute to insulin resistance and pancreatic β-cell dysfunction, resulting in the development of type 2 diabetes. Clinically, the symptoms depression is known to increases the risk of pre-diabetes and type 2 diabetes [116-119]. In a cohort study, 11,615 nondiabetic patients with depression symptoms (including fatigue, sleep abnormalities, feelings of hopelessness, increased irritability and loss of libido) were selected and followed for 6 years. Results of this cohort study revealed that depressive symptoms induced the incidence of type 2 diabetes and this relation was explained by metabolic, demographich, and lifestyle factors [119]. In another study, 2127 Swedish middle aged men and 3100 women with psychological distress were included and followed-up for 8-10 years, 245 men and 177 women showed pre-diabetes, and 103 men and 57 women detected with type 2 diabetes [116]. Earlier data reported that depression with co-morbid diabetes have higher hazard ratio (Figure 1). In animal models, depressive phenotype also induced significant hyperglycaemia, glucose intolerance, hypercorticosteronemia, cognitive deficits, immunosuppression and hypoinsulinemia [10-11]. Hyperglycemia induces oxidative stress in hippocampus, resulting in neurological disorder such as memory impairment, depression and anxiety [14-15]. Besides, hypercorticosteronemia associated with over production of reactive oxygen species and elevation of cytosolic calcium, resulting in subsequent increase in the calcium dependent death in neuronal cells [16]. Evidence suggests that elevated corticosterone level as observed in stress, induces dysregulation of IR stimulated trafficking of glucose transporter-4 (GLUT4), thereby decreased metabolic activities and plasticity of hippocampal neurons resulting in cognitive dysfunction [17-19]. In animal models, CUMS exhibits insulin resistance, hypoinsulinemia and hyperglycemia [11, 120]. Insulin acts as a growth factor in the brain and is a neuroprotective, activates the dendritic sprouting, regeneration and proliferation of stem cells. The impairment of insulin signaling in hippocampus might facilitate the development of Alzheimer's disease [121]. The complex pathophysiology of cognitive dysfunction during co-morbidity of depression and diabetes has been illustrated in Figure 2.
Although, cortisol is typically considered as anti-inflammatory, however chronic activation of the HPA axis lead to condition called glucocorticoid resistance where immune cells are no longer to hear cortisol signal, thus leading to increases in both cortisol and inflammation [3]. Reactive nitrogen species such as NO has been implicated in stress mediated inflammation and cognitive deficit [20]. In addition, NO impairs ATG by inhibiting the activity of S-nitrosylation substrates in rat primary cortical neurons [21]. In ATG deficient mice and flies, neurodegeneration is accompanied by the accumulation of ubiquitylated protein aggregates, similar to those observed in human cognitive decline [122]. Chronic fatigue syndrome, the symptom of depression is associated with decreased hippocampal BDNF mRNA expression and exacerbated hippocampal apoptosis and brain atrophy [123]. Study revealed that cognitive decline is associated with biological markers such as brain atrophy, circulating levels of BDNF and insulin-like growth factor 1 [124]. Earlier data showed that an intervening stress free period can reverse hippocampal atrophy and the associated hippocampus-dependent behavioural deficits [125].

Figure 2: The complex pathophysiology of cognitive dysfunction during co-morbidity of depression and diabetes. Hypercortisolemia, depression, diabetes and neuronal dysfunction have been implicated as major causes of cognitive impairment.

Large number of report underlines the connection between a high glucocorticoid level in the blood, a depressed like state and cognitive dysfunctions. Psychiatric and cognitive symptoms resembling major depression have been observed in patients receiving chronic glucocorticoid therapy as well as in patients suffering from Cushing's disease [126-128]. Modulation of neuronal plasticity by corticosteroids has been established. One example is the induction of long term potentiation (LTP) in the hippocampus, which shows a specific pattern: LTP is observed when corticosteroids are kept within normal physiological levels [129], but altered when corticosteroid levels are elevated (e.g. during stress) or presumably both glucocorticoid receptors and mineralocorticoid receptors are occupied [4]. LTP is a persistent increase in synaptic strength following high-frequency stimulation of a chemical synapse in the hippocampus, an important process for learning and memory formation. The hippocampus region of brain that regulates learning and memory processes is vulnerable to oxidative stress and hypercortisolemia [22-24], during the course of depression [25]. Hippocampal muscarinic receptors are known to modulate cognition. Thus, hippocampal cholinergic dysfunctions might underlie depression associated cognitive impairments [25]. In the central nervous system, mACHR1 is mainly coupled to phosphoinositide pathway through G_q protein, whereas mACHR4 preferentially coupled to the inhibition of stimulated adenylyl cyclase through G_{i/o} [130]. The mACHR1 has been considered one of the neurotransmitter receptors regulating hippocampal synaptic plasticity, which is known play a critical role in learning and memory. Cholinomimetic drug carbachol enhanced LTP of excitatory synaptic transmission in mouse hippocampal slices and this enhancing effect was abolished in mACHR1 knock-out mice [131]. Administration of selective mACHR1 antagonists induces spatial memory impairment in Morris water maze task [26]. Besides, mACHR4 function as presynaptic autoreceptor in hippocampal neurons to inhibit ACh release [27], which is known to be involved in learning and memory processes [28]. It has been reported that, upregulation of mACHR4 in striatal neurons inhibits locomotor activity in mice [29]. Apart from alteration in mACHRs, chronic stress activates AChE, resulting in reduced amount of ACh in synaptic cleft [30]. Direct acting mACHR1 agonists and indirect acting muscarinic cholinergic agonists such as AChE inhibitors, have shown cognition-enhancing properties [132]. Further, selective
blockade of mAChR4 induces presynaptic autoreceptor desensitization in hippocampal neurons and have a valuable therapeutic target in cognitive functions [27]. Besides muscarinic cholinergic system, it has been reported that depression modulates BDNF and MAPK levels, which is known for its modulation in synaptic plasticity and transmission [31-32]. Further, abnormalities in the hippocampal cholinergic system could represent depression phenotype [133].

Since the number of dentate gyrus hippocampal cells is also dependent on postnatal neurogenesis, observations that stress or hypercorticalism negatively regulate hippocampal neurogenesis [134]. Depression results in increased neurodegeneration and decreased hippocampal neurogenesis [33]. It has also been demonstrated that glucocorticoid treatment induces arrest of the neural cell cycle [34] and apoptosis in neuronal progenitors and mature neurons [35]. On the basis of numerous observations, it has been shown that high glucocorticoid levels initiate dendritic atrophy and synaptic loss in hippocampal neurons [125, 135-136]. Suppression of neurogenesis affects mood [5], fear conditioning, synaptic plasticity [36] and memory [37]. Shh, a mitogenic protein considered widely in cancer biology, has been implicated in neurological disorders and its vital role in neuronal regeneration has attracted several researchers globally in the field of neuroscience. An overview of Shh signaling has been given in Figure 3.

In the absence of Shh ligand, downstream hedgehog signaling is maintained in a repressed state by the activity of hedgehog receptor Ptc. Ptc is 12-transmembrane domain protein (display transporter like structure), whose intracellular loop is localized at the base of primary cilium [137-138]. Although the mechanism by which Ptc represses the downstream signal transduction has not been fully elucidated, report has shown that, free Ptc (unbound by Shh) acts sub-stoichiometrically to suppress 7-transmembrane domain protein-Smo activity in primary cilium and thus is critical in specifying the level of pathway activity [139]. Like Ptc, hedgehog-interacting protein (Hhip) is one of the inhibitory ligands that bind to Shh with high affinity and participate in an inhibitory mechanism of hedgehog signal by sequestering, modifying or degrading the Shh ligand at the cell surface [140-141]. Ptc induces rapid receptor-mediated endocytosis of Shh targeted by lysosomes for degradation [142], whereas Hhip appears to only physically sequester Shh at the cell surface [143]. Gli signals are also negatively regulated by binding to cytoplasmic protein suppressor of fused

Sufu protects full-length glioma-associated oncogenes (Gli) proteins from speckle-type POZ protein-cullin3 (SPOP-Cul3) mediated ubiquitination and degradation by the proteasome. In this way, Sufu functions as an adaptor to protect a pool of Gli2 and Gli3 that can be converted into activators and repressors. This aspect of hedgehog pathway is evolutionarily conserved and independent of the cilium [147-149]. Furthermore, without Shh ligand, the full length Gli2 and Gli3 translocate at low levels into and out of cilia, where protein kinase A (PKA) phosphorylate them at four to six sites in cilia [147, 150-151]. The phosphorylated residues provide a binding site for centrosomal β-transducin repeat-containing protein, which in turn recruits Skp1/Cullin1/F-box-ubiquitin ligase complex to target full-length, ∼190-kDa Gli3 and ∼185-kDa Gli2 for cleavage via the ubiquitin-proteasome pathway to generate the ∼83-kDa Gli3 (Gli3R) and up to some extent ∼78-kDa Gli2 (Gli2R) N-terminal repressor form, respectively, while the C terminals are assumed to be completely degraded [147, 152-153]. Gli3R then translocate to the nucleus, bind with hedgehog gene promoters and repress target gene expression prior to being degraded by speckle-type POZ protein-cullin3 (SPOP-Cul3) ligase complex [147].

The repression exerted by Ptc on Smo is relieved, when Shh binds Ptc, simultaneous localization of Smo to cilia occur [137, 144, 154]. Activation of Smo and its translocation to the primary cilium involve association of Smo with G-protein coupled receptor kinase 2 (GRK2) and β-arrestins and anterior-grade trafficking motor kinesin-II protein (Kif3A) [155]. Reports suggest that, phosphorylation of Smo by the GRK2 and recruitment of β-arrestin results in endocytosis of Smo in clathrin-coated pits [156].

(Sufu) [144-146].
Figure 3: An overview of Shh signaling. In the absence of Shh ligand the downstream signaling is off (A) and in the presence of Shh the downstream signaling is activated (B).

A recent study demonstrated that, serine/threonine kinases (CK1α) also plays a key role in phosphorylation of mammalian Smo, in which Shh signaling recruits CK1α to initiate Smo phosphorylation, and phosphorylation further increases the binding of CK1α and GRK2 to Smo, forming a positive feedback loop that further increase the level of Smo phosphorylation [157]. Further, β-arrestin promotes translocation of active (phosphorylated) Smo into the cilia by mediating its interaction with the Kif3A in mammalian cells [155, 158]. It has been recently proposed that Evc/Evc2, the products of two human disease genes responsible for the Ellis-van Creveld syndrome interact with phosphorylated Smo to form Smo/Evc/Evc2 complex, further transduces the hedgehog signal to activate Gli by antagonizing Sufu in the primary cilium [159-160]. However, the mechanism by which Shh dissociates Gli-Sufu complex is not well understood, but there is some evidence suggests that the Sufu-full length Gli2 and Sufu-full length Gli3 complexes accumulate in the primary cilium following exposure to Shh, and they independently synthesis new proteins. This leads to immediate trafficking of full length Gli2 and Gli3 into the primary cilium followed by their phosphorylation and dissociation from Sufu. These modifications allow full length Gli2 and Gli3 to be converted into Gli activator (GliA) forms [161-163]. At the same time, PKA phosphorylation is inhibited, preventing βTrCP/Cul binding and processing [147]. In contrast, pharmacological activation of PKA mediates Gli phosphorylation resulting into decline in GliA levels [164]. As result of these complex events, the full length activator form of Gli, also reffered as GliA, migrate towards the nucleus, where it binds with target gene promoter, leading to the activation of transcription factors of the Gli family (Gli1-3) and to the transcription of target genes including Ptch and Gli1 themselves [144, 165-166]. Following this, GliA is ubiquitinated by the SPOP/Cul3 interaction and degraded by proteasome [147]. Gli1 constitutes a convenient readout for the pathway activation, amplifies the hedgehog response and is itself a hedgehog-target gene [167]. Gli2 mainly functions as a transcription activator; however, it was shown to have repressor functions in some specific contexts such as skeletal muscle and CNS development [167-168]. Gli3 retains a bipotential activity, functions as a transcriptional repressor in dorsal interneuron, but also exerts activator functions during embryonic development [144, 167-169].

Shh interacts with its receptor Ptc1, which is observed to be present in the adult basal forebrain [170] and hippocampus [171]. The role of Shh on the proliferation of the cholinergic neurons [170] and induction of ATG [172] imply its critical role in neuronal/memory dysfunction associated with neurological disorders. Autophagy is a highly conserved pathway for degradation, by which intracellular macromolecules are delivered to lysosomes, where they are degraded into biologically active monomers such as amino acids that are subsequently recycled to maintain cellular homeostasis [173].

One of the etiological hypothesis attempting to explain the pathogenesis of depression is the monoamine theory, which hypothesised that depression is the result of decreased availability of monoamine neurotransmitters such as serotonin (5HT) and norepinephrine (NE) in the central nervous system [174]. Monoamine oxidases are the family of enzymes that metabolize the monoamines. This hypothesis derived from the observation that drugs acting on the synaptic concentrations of monoamines can attenuate the symptoms of depression, suggests that depletion of monoamine neurotransmitters is the important cause of depressive symptoms. Currently the etiology of depression seems to involve the monoaminergic receptors and the downstream signaling events that this receptors trigger, including the gene expression. At molecular level the abnormality would be in the signal transduction cascade or in appropriate gene expression [175].

A decline in hippocampal neurogenesis is observed following 5HT and/or NE depletion, while increased hippocampal neurogenesis is seen following elevation of monoamine levels [38, 176-178]. It has been reported that combined depletion of both 5HT and NE with parachlorophenylalanine resulted in a significant decrease in Smo and Ptc mRNA levels within the dentate gyrus subfield of the hippocampus. However, selective depletion of 5HT, using the serotonergic neurotoxin 5,7-dihydroxytryptamine or NE using the noradrenergic neurotoxin DSP-4, did not alter expression of Shh and its co-receptors Smo and Ptc [38].

2.3. Drug therapy

Antidepressants are the class of drugs used primarily in the management of depressive and anxiety disorders. This class of drugs is also used for the control of sexual dysfunction, eating disorders, enuresis, aggression and some personality disorders [179]. The most important classes of current antidepressant therapy are the selective serotonin reuptake inhibitors
(SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), NE-dopamine reuptake inhibitors (NDRI) and monoamine oxidase inhibitors (MAOIs). Other drugs used for the treatment of depression include buprenorphine [180], low dose of antipsychotics [181] and St. John's wort [71]. On the basis of literature the list of drugs comes under antidepressants are reported on Table 1 [179, 182].

Table 1: Classification of major antidepressant drugs.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Class</th>
<th>Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>TCAs</td>
<td>Amitriptyline, Butriptyline, Clomipramine, Desipramine, Dosulepin, Doxepin, Imipramine, Iprindole, Lofepramine, Nortriptyline, Protriptyline, Trimipramine</td>
</tr>
<tr>
<td>2.</td>
<td>SNRIs</td>
<td>Venlafaxine, Milnacipran, Duloxetine, Levomilnacipran, Desvenlafaxine, Sibutramine</td>
</tr>
<tr>
<td>3.</td>
<td>SSRIs</td>
<td>Citalopram, Escitalopram, Fluoxetine, Fluvoxamine, Paroxetine, Sertraline</td>
</tr>
<tr>
<td>4.</td>
<td>MAOIs</td>
<td>Isocarboxazid, Nialamide, Phenelzine, Tranylcypromine, Moclobemide, Pirindole, Toloxatone, Rasagiline, Selegiline</td>
</tr>
<tr>
<td>5.</td>
<td>NDRI</td>
<td>Aminopropion, Desoxypipradrol, Dexamphetamine, Difemetorex, Ethylphenidate, Fencamfamine, Prolintane, Tametraline</td>
</tr>
</tbody>
</table>

Antidepressants are also known to improve neurocognitive functions depending on clinical, social and emotional factors [95]. Antidepressant medications involve in serotonin modulation has the potential to cause serotonin toxicity which induce mania, restlessness, agitation, emotional liability, insomnia and confusion [183-184]. MAOIs have pronounced (sometimes fatal) interactions with a wide variety of medications and over the counter drugs. If taken with foods that contain high amount tyramine (e.g. cheese, cured meats or yeast extracts), they may induce a potentially lethal hypertensive crisis. At lower doses the person bothered only by headache due to an increase in blood pressure [185]. The use of SSRIs during pregnancy is associated with an increased risk of spontaneous abortion of about 1.7-fold and associated with pre-term birth and low birth weight [96, 186]. Studies have shown that, therapy with antidepressants is correlated with an increased risk of suicidal behaviour.

and thinking (suicidality) in those aged under 25 [96]. Sexual side effects are also common with the use of SSRIs, such as loss of sexual drive, failure to reach orgasm and erectile dysfunction. These effects are usually reversible, these sexual side effects can, in rare cases, last for months or years after the drug has been completely withdrawn [97]. Antidepressant medication use increases the risk of hyperglycemia and diabetes mellitus. The incidence of diagnosed diabetes is higher among antidepressant users than nonusers [98-99].

2.4. Diabetes

Diabetes mellitus, commonly referred to as diabetes, is a chronic disease in which the body cannot regulate the amount of sugar in the blood. Insulin is a peptide hormone produced by the pancreatic β-cells to control blood sugar. Diabetes can be caused by low insulin, resistance to insulin, or both. A sugar also called glucose enters the blood stream and act as source of fuel for the body. An organ called the pancreas contains β-cells makes insulin. An important role of insulin is to transport glucose from the blood stream into muscle, fat and liver cells, where it can be stored or used as fuel [39-40]. The disease burden of diabetes is very high and rising in every country, indicated by global rise in the prevalence of obesity and unhealthy lifestyles. Literature showed that global prevalence of 382 million people with diabetes in 2013, expected to rise 592 million by 2035 [187].

There are two types of diabetes namely type 1 and type 2. Type 1 diabetes is a chronic disease in which there is a high level of glucose in the blood.

Type 1 diabetes can occur at any age and often diagnosed in children, adolescents or young adults. With type 1 diabetes, pancreatic β-cells did not produce insulin. In absence of insulin, glucose builds up in the blood stream instead of moving into cytoplasm. This build up of glucose in the blood is called hyperglycemia. The body is unable to utilize the glucose as fuel and leads to the symptoms of type 1 diabetes. The exact cause of type 1 diabetes is not known; however, most likely it is considered as an autoimmune disorder. In this condition, immune system mistakenly attacks and destroys healthy tissue. Any infection can also trigger the body to mistakenly attack the cells in the pancreas that makes the hormone insulin. The tendency to develop autoimmune diseases, including type 1 diabetes, can run down through families [39, 188-189].
The symptoms may be the first signs of type 1 diabetes include being very thirsty, feeling hungry, feeling tired all the time, having blurry eyesight, feeling numbness or tingling in feet, losing weight without trying, urinating more often as well as urinating at night or bedwetting in children who were dry overnight before the symptom. The serious warning symptoms of type 1 diabetes occurs when blood sugar is very high, which include deep and rapid breathing, dry skin and mouth, flushed face, fruity breath odour, nausea or vomiting and stomach pain [39].

Type 2 diabetes is much more common and most often occurs in adulthood, but because of high obesity rates, teens and young adults are also diagnosed with this disease. Type 2 diabetes is a chronic metabolic disease in which there is a high level of sugar in the blood. Insulin is a peptide hormone produced by the pancreatic β-cells. The location of pancreas is below and behind the stomach. Insulin is required to transport blood glucose into cells. Inside the cells, glucose is utilized and stored as fuel for later use. When type 2 diabetes occur fat, liver and muscle cells do not respond correctly to the hormone insulin. This is called insulin resistance. As the result of insulin resistance, blood glucose does not influx in these cells to be stored for energy, and thus a high level of glucose builds up in the blood, which is termed as hyperglycemia. Family history and genes may also play a role in the pathogenesis of type 2 diabetes. Low physical activity, poor diet and excess body weight around the waist increases the chance of getting this disease. People with type 2 diabetes often have no symptoms at first or they may not have symptoms for many years. Early symptoms of diabetes has been considered as bladder, kidney, skin or other infections that are more frequent or heal slowly, fatigue, hunger, increased thirst, increased urination, blurred vision, erectile dysfunction, pain or numbness in the feet or hands [39, 190-193].

2.5. Diabetes and central nervous system

Prolonged hyperglycemia often associated with the number of complications such as diabetic neuropathy, retinopathy, nephropathy, cardiomyopathy etc. Diabetic neuropathy is damage to nerves in the body that occurs due to high blood sugar level [41]. In the central nervous system, diabetes exacerbates depression, phobias, anorexia [42-43] and reduces complex reasoning skills [44]. Clinically, patients with diabetes are at increased risk of developing depression and cognitive impairment as compared to the general population [44-45]. Further, diabetes accelerates the progression from mild cognitive impairment to severe dementia.
Diabetes induces oxidative stress and inflammation in the hippocampal neurons resulting in neurodegeneration [14, 46].

In clinical settings, there was a close relationship between diabetes and depression [45]. Genesis of cognitive deficits in diabetic patients is very complex. A meta-analysis was conducted on 42 published studies inclusive of 21,351 adults and found that the prevalence of co-morbid depression was higher in diabetic women (28%) than in diabetic men (18%), in uncontrolled studies (30%) than in controlled studies (21%), in clinical samples (32%) than in community (20%) samples and when assessed by self-report questionnaires (31%) than in diagnostic interviews (11%) [45, 195]. The, worldwide estimations of depression prevalence among individuals with diabetes mellitus appear to vary with the type of diabetes and developed and developing nations. Depression is highly prevalent among people with diabetes and the prevalence rate varied greatly by demographic condition and diabetes types [196]. In U.S., data from the 2006 Behavioral Risk Factor Surveillance System by telephone survey of U.S. adults aged 18 and older, found that age adjusted depression rate was 8.3%, ranging from a low of 2.0% to a high of 28.8% among the 50 states [196]. In follow-up study it was found that about 45% of all diabetes patients had undiagnosed depression [197].

Glycated hemoglobin (HbA1c) is a form of hemoglobin that is measured primarily to observe the average plasma glucose level over prolonged periods of time. It is formed by non-enzymatic glycation pathway by exposure of hemoglobin's to plasma glucose. Clinical study reported co-morbidity of depression and diabetes in terms of HbA1c levels (Figure 4) [45].

Both diabetes and depression are the risk factor for neurocognitive impairment. How depression exacerbates diabetes has been covered in our earlier section. Study conducted in India revealed that 48% of diabetic patients with co-morbid depression showed cognitive impairment [198]. The basic pathophysiology of diabetes and depression with cognitive dysfunction has been shown in Figure 5. Animal models also revealed the persistence of depression and neurocognitive impairment during chronic diabetes. In experimental studies, STZ induced diabetic animals showed depressive like behaviour in Porsolt's forced swim test [47-48] and cognitive deficit in Morris water maze task [49].
Figure 4: Comparison of unadjusted mean HbA1c over time among depressed and non-depressed adults with diabetes, reprinted from [45], Copyright © 2015, with permission from Elsevier.

Figure 5: The possible mechanistic contribution of cognitive impairment seen in diabetes mellitus, stress and depression. Hyperglycemia, hypoglycaemia, depression, dyslipidemia and
abnormal insulin action have been implicated as major causes of cognitive impairment in diabetic patients, but many other factors, such as those shown in the Figure, are also involved. APOE, apolipoprotein E.Reprinted from [100], Copyright © 2015, with permission from John Wiley and Sons.

Many signaling pathway has been involved in the pathogenesis of diabetic neuropathy. ROSI, a PPARγ agonist is known to improve neuronal insulin receptor functioning in rat hippocampus during insulin resistance (Figure 6) [199]. Further, dysfunctioning of neuronal PPARγ receptor was observed in cognitive dysfunction [56].

**Figure 6:** An overview on neuronal intracellular signaling of insulin receptor. IRS1, insulin receptor substrate 1; IRS2, insulin receptor substrate 2; PI3K, phosphatidylinositol 3 kinase; GLUT4, glucose transporter type 4; INSG1, insulin induced gene 1; PPARγ, peroxisome proliferator activated receptor-γ; MAPK1, mitogen activated protein kinase 1; PKB, protein kinase B; BCL2, B-cell lymphoma 2.

During neuronal activity, insulin binds to the α-subunit of the IR and activates the tyrosine kinase residue of the β-subunit (TrkB) with subsequent activation of intracellular signaling cascades. Activation of the IR→Shc (Src homology collagen peptide)→MAPK pathway induces gene expression, that are required for cellular glucose homeostasis and synapse growth [121, 200]. IR has a direct effect on modulation of neurotransmission which influence
cognitive processes via insulin receptor substrate 1/2 (IRS1/2) → PI3K (phosphatidylinositol 3-kinase) → cyclic phosphodiesterase 3β (cPD3B) pathway [121]. Activation of IR → IRS1/2 → PI3K → PDK (phosphoinositide dependent kinase) → protein kinase B (Akt/PKB) pathway suppresses the induction of apoptosis [201-202] and induces translocation of GLUT4 in the hippocampal neurons [203]. Insulin-like growth factor 1 receptor (ILGF 1r) is also known to stimulate IRS1/2 → PI3K → Akt/PKB and Shc → MAPK pathways resulting in memory consolidation [204]. IR mediated translocation of GLUT4 in hippocampal neurons rapidly increases glucose uptake and utilization during neuronal activity, which is associated with hippocampal dependent learning and memory formation [17]. Conversely, cellular and metabolic alterations in the hippocampus caused by alteration in insulin signaling cascade is associated with cognitive deficit [205]. Experimental evidence suggests that, a decrease in the insulin receptor signaling cascade during diabetes [121]. Study revealed that both protein and mRNA levels of BDNF were severely reduced after STZ treatment. A recent study reported that downregulation of PPARγ levels in the hippocampus of diabetic mice [57] is associated with depressive like behaviour in forced swim test [58]. Further, downregulation of IR expression in hypothalamus induces depressive like behaviour in rats [59]. Diabetic neuropathies in brain are associated with severe deficiency of BDNF with depressive behaviour [60].

There is a correlation between BDNF, diabetes and Shh signaling. Diabetes induces oxidative stress and inflammation in hippocampus [14]. Oxidative stress is a process by which exposure of reactive oxygen intermediates, such as hydrogen peroxide ($H_2O_2$), superoxide anion ($O_2^−$), NO and hydroxyl ($OH^−$) radical’s damage the proteins and nucleic acids. The levels reactive oxygen species are neutralized by antioxidant defense system in the body [206]. NO induces inflammation of hippocampal neurons [30]. NO derived from iNOS contribute to the depressive like behaviors in mice due to neurodegenerative effects in the cerebral cortex [20]. Diabetes reduces coetaneous Shh signaling and associated with delayed wound healing potential in children. Dysregulation of Shh signaling was associated with impaired NO function and wound healing. Activation of Shh pathway is known to upregulates two important factors, namely BDNF and vascular endothelial growth factor. Shh signaling attenuates the effect of oxidative stress on cortical neurons and has potential role in neurodegenerative disorders [206].
Shh signaling is known to modulate cellular and neurochemical homeostasis in the adult nigrostriatal circuit. It has been reported that, the interruption of Shh signaling emerging from dopaminergic neurons of the mesostriatal circuit causes progressive adult onset degeneration in cholinergic, dopaminergic and fast spiking GABAergic neurons. Further, the imbalance between cholinergic and dopaminergic transmission leads to motor deficits indicative of Parkinson’s disease [207].

ACh is necessary for proper functioning of cholinergic neurons in hippocampus and regulates the process of learning and memory [30, 208]. ChAT and AChE are responsible for the synthesis and metabolism of ACh. Evidence suggests that ChAT, a specific marker for functional state of cholinergic neurons [61], activity is reduced during STZ administration resulting in cognitive deficit [62-63]. It was reported that, STZ increases AChE activity in hippocampus resulting in cognitive deficit [64]. Previous study suggest that STZ-induced diabetes significantly downregulated the expression of mAChR1 in hippocampus termed cholinergic dysfunction [64]. Chronic diabetes resulted in motor control activity deficit [65]. Upregulation of mAChR4 in the striatum inhibits dopaminergic-D1 receptor-induced locomotor stimulation in mice [29]. Autophagic dysfunction has also been observed in diabetes mice [66].

2.6. Drug therapy

Antidiabetic drugs are used to treat diabetes by lowering the level of blood glucose. Apart from insulin, exenatide, pramlintide and liraglutide, all other drugs are administered orally and thus called oral antihyperglycemic agents. Type 1 diabetes is caused by the lack of insulin hence insulin injection is required to control type 1 diabetes. Type 2 diabetes is caused by insulin resistance [209]. The major categories of drugs used to control type 2 diabetes have been reported in Table 2 [209-210].

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Class</th>
<th>Drugs</th>
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<td>1.</td>
<td>Sulfonylureas</td>
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<td>Gliclazide, Gliquidone</td>
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</tbody>
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Sulfonylureas are act by stimulating or depolarizing pancreatic β-cells to facilitate insulin release. Biguanides acts on the liver cells to reduce gluconeogenesis and attenuate insulin resistance via increasing 5'AMP-activated protein kinase signalling. Thiazolidinediones is known to reduce insulin resistance by PPARγ gene transcription. Alpha glucosidase inhibitors delay the digestion of starch from the intestine and restrict intestinal absorption. These agents are effective only in the earliest stages of glucose intolerance, but might be helpful in combination with other oral antihyperglycemics. Meglitinides analogues depolarize the pancreas to produce insulin and are often called short acting secretagogues. Meglitidines act on the potassium channels as sulfonylureas but at different binding site. Dipeptidyl peptidase-4 (DPP4) inhibitors increase the concentration of incretin and glucagon like peptide-1 (GLP 1) in blood and thus induce insulin release [209, 211-212].

In view of pathogenesis in type 2 diabetes mellitus and brain disease, antidiabetic medications also known to positively modulate the metabolism of neuronal cell, which could be of clinical importance for the treatment of neurological disorders [85]. Type 2 diabetes mellitus affects neuronal cells by affecting neuronal metabolism, neuronal viability and behaviour. GLP 1 receptor (GLP 1r) agonists, biguanides and thiazolidinediones can attenuate hyperglycemia periphery and counteract the CNS complications of type 2 diabetes (Figure 7).

These drugs also have ameliorative effects on the central nervous system diseases [213-215]. The mechanisms of actions of these drugs in the brain pathology are being to be investigated. They might have direct effect on brain cells or act indirectly by modulating whole body metabolism [85].

Study demonstrated that ROSI possesses antidepressant like activity in behavioral models [216]. Glyburide potentiates the effect of antidepressants in the forced swimming test [217].
Chronic administration of metformin and milnacipran reduces the co-morbidity of depression and diabetes in patients [218]. GLP 1 is known to exert neuroprotective effects against cognitive deficits in individuals with depressive disorders [219]. The randomized controlled trials summarized current and ongoing research on the management of depression in patients with diabetes mellitus revealed that, there is no single treatment that consistently leads to better therapeutic outcomes in patients with co-morbid depression and diabetes [220-221].

Metformin is known to induce neuroprotection against cytotoxic stress and improved insulin sensitivity in neuronal cell lines [222-223]. Metformin also induces neurogenesis in the rodent brain and improved spatial memory formation [224]. Results of clinical study showed that patients with type 2 diabetes mellitus with Alzheimer’s disease receiving metformin had lower rate of cognitive dysfunction than patients not receiving metformin [213]. In a randomized double blind trial ROSI, a thiazolidinedione, or glyburide, a sulfonylurea, was combined with metformin, showed improvement in glycemic control as well as working memory in type 2 diabetic patients after 24 weeks [225]. In randomized controlled trial study, subjects were administered with either repaglinide or glibenclamide as oral hypoglycemic agents for 1 year, cognitive function test showed greater decline in cognitive performance in the glibenclamide group [226]. However, a population based case control trial showed that patients with type 2 diabetes mellitus receiving metformin had a slightly higher risk of developing Alzheimer’s disease than those who did not receive the metformin [227].

Figure 7: Summary of the effects of antidiabetics on the peripheral and central nervous system. Reprinted from [85], Copyright © 2015, with permission from Elsevier.
Thiazolidinediones act via PPAR\(\gamma\) receptor. Its regulation depends on transcriptional coactivators peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1\(\alpha\)). Downregulation of PGC-1\(\alpha\) leads to mitochondrial dysfunctions and associated with increased oxidative stress that are commonly observed in type 2 diabetic patients and neurological disorders [228-230]. GLP 1r activation increases the cellular insulin sensitivity by amplifying insulin signaling [231]. Further, GLP 1r agonists are known to counteract neuroinflammation, enhance neurosynaptic transmission and memory performance [232-233]. The potential cellular targets of major antidiabetic drugs in the central nervous system have been reported in Figure 8.

**Figure 8:** The potential cellular targets of major antidiabetic drugs in the central nervous system. Drugs used to treat type 2 diabetes mellitus have various targets in the central
nervous system, including neuronal cells (1), glial cells including both microglia and astroglia (2) and neuronal precursor cells (3). These drugs exert neuroprotective effects by acting at the level of gene transcription, mitochondria and cell signalling cascades. These drugs block the effect of chronic diabetes on microglia. Evidence suggests that these drugs can stimulate neurogenesis in the epithelium and contribute to tissue regeneration and repair. Reprinted from [85], Copyright © 2015, with permission from Elsevier.

Thiazolidinediones, like ROSI and pioglitazone are the potent insulin sensitizers in type 2 diabetes mellitus that effectively reduces hyperglycemia as well as fatty acids [234]. Clinical trials report showed that ROSI improves memory performance in Alzheimer’s patients [235]. Another study revealed that ROSI did not improve cognitive performance in patients with Alzheimer’s disease [236]. Cognitive impairment has been observed in patients receiving insulin therapy [237-238]. There have been reports that agents ameliorating incretin effects will have protective effects on neurons and could be effective in preventing dementia [239-240].

Many antidiabetic drugs are known to induce hypoglycaemia. Besides, hypoglycaemia is associated cognitive impairment [100]. Sulfonylurea causes an average of 2-4 kg weight gain and hypoglycaemia. Adverse effects of repaglinide includes, upper respiratory infection, dizziness, athralgia, back pain, diarrhea and hypoglycaemia [241-242]. Sitagliptin is associated with constipation, influenza, nasopharyngitis, upper respiratory infection, headache and cough. Acarbose elicited flatulence, abdominal pain, diarrhea, dyspepsia and nausea [241]. It has been reported that patients receiving chronic pioglitazone therapy showed increased incidence of bladder cancer compared to general population [101-102]. Chronic ROSI therapy is associated with an increased incidence of myocardial infarction and heart failure in type 2 diabetic patients [103].

2.7. Animal models of depression

The chronic unpredictable mild stress model (CUMS; also referred to as chronic variable or intermittent stress), is a widely used rodent model of depression, which consists of the repeated exposure to an array of unpredictable and mild stressors over a sustained period of time (ranging from 10 days to 8 weeks). The CUMS model was originally developed by Paul Willner in the late 1980s based on both clinical and preclinical research regarding the
etiology of depression [8-9]. In humans, chronic exposure to uncontrollable and unpredictable life stressors is often said to be a major participant in the development of depressive disorders [243-244]. Based on this knowledge, earlier studies demonstrated that exposure of rodents to severe stressors resulted in a reduction in physical activity and of their consumption of rewarding, palatable substances namely sucrose [9]. This reduction in sucrose consumption was believed to be similar to the impairments in reward processing, which is commonly anhedonia, a core symptom of major depression. Therefore, the chronic mild stress model was developed, which consisted of repeated exposure of rodents to a series of unpredictable “microstressors”, making the more reliable model for depression [245]. The endpoint of this model focused exclusively on sucrose intake and preference as well as decreased physical activity, which was also used as an endpoint, which was believed to relate with the deficit in hedonic impact in these rodents. The validity of this model was evaluated by the fact that this reduction in hedonic impact was reversible by chronic treatment with antidepressant agents, which mimicked the time course required for clinical effectiveness [246-247]. One major argument regarding the reductions in sucrose intake was simply a reflection of the reduced body weight and food consumption that are typically concurrent with chronic mild stress exposure [248]. However, subsequent studies controlled the body weight changes and demonstrated deficits in sucrose preference (as opposed to intake) argued against this proposition [249-250].

During the last two decades, there has been an explosion of behavioural research, which has extended the behavioural endpoints of this model with other models of depression beyond hedonic processing and reward salience. For example, exposure of animals to CUMS enhances immobility in the forced swim and learned helplessness test, decreases the frequency of male sexual and aggressive behaviors, reduces self-care and increases rapid eye movement sleep latency [251]. Thus, despite a few anomalous findings from some laboratories, and regardless of some continuing controversy about the reliability of this model from laboratory to laboratory, the CUMS model has been widely accepted as a valid model of depression in rodents.

Another variable that comes into light is the duration of CUMS. The original CUMS paradigm [247] was developed as an 8-week paradigm that would allow 3 weeks of initial stress exposure prior to the onset of antidepressant treatment and then continue for the next 5
weeks. The rationale behind this outcome from a clinical standpoint is that, the depressive behaviour must be established before the onset of antidepressant therapy. However, recent studies demonstrates that many of the effects of CUMS, especially the robust and reliable effects, are present as early as 10 days following the onset of CUMS exposure and are nearly all present following 3 weeks of CUMS [245]. For example, unpredictable mild foot shock stress for 21 days induced significant hyperglycaemia, glucose intolerance, hypercorticosteronemia, gastric ulcerations, male sexual dysfunction, immunosuppression, cognitive deficits and mental depression in rats [10]. Exposure to 21 days of CUMS significantly reduced brain-pancreas relative protein, accompanied by an increase in levels of blood sugar with hypoinsulinemia [11].

An important variable that comes into light from literature is the precise post-CUMS time interval when measurements should ideally be considered. Most studies conducted the CUMS model performed their tissue extraction on the day following the last day of CUMS, a point at which residual effects of CUMS appeared, but acute effects of stress exposure would be absent given the lag time since the last stress exposure. However, when studies employed a longer rest period following CUMS exposure (such as 1 week following CUMS termination) most of these effects were not present [252-253]. These findings are encouraging in the sense that, they suggest the recovery of functions following the cessation of stress exposures, they also highlighted the importance of consistency in experimental methodology and the importance of standardizing time points of analysis. In addition, termination of animals for longer time intervals, such as 1 or 2 weeks post-CUMS or longer, would provide important information on how long-lasting the effects of CUMS and/or antidepressant therapy are present on specific outcome measures. Whether effects are transient or long-lasting is important in extrapolating the adverse effects of chronic stressors and therapeutic effect of antidepressants from the animal model to the human situation [245].

Apart from CUMS, tail suspension and forced swim test models, other models have also been described. Behavioural changes after neonatal clomipramine treatment produces changes in adult rats that resemble endogenous depression in man. Not only clomipramine, but also other psychotropic drugs induced changes in the behaviour of adult rats after treatment in neonatal age. However, the specificity of this procedure to evaluate potential antidepressant compounds remains to be established [254]. A selective inhibition of mouse-killing behaviour
in rats by antidepressants has also been investigated as a test model. The test can be use to evaluate antidepressants such as tricyclics and monoamine oxidase inhibitors [255]. In this test neuroleptics and benzodiazepines are active in doses which impair motor performance [256]. Major drawback of this test was might be the large numbers of mice are required to be sacrifice by rats during trails. Antidepressants like drugs block the re-uptake of biogenic amines into nervous tissue. In this way, the toxic effects of norepinephrine are potentiated. Hence the test for antidepressant activity based on potentiation of norepinephrine toxicity was investigated [256]. The critical assessment of this method was that, several antidepressants block not only the uptake of noradrenaline, but also of dopamine and of serotonin, which might interfere with the potency and nature of drugs. It has been reported that apomorphine induces emesis in man and in other species, like dogs. Treatment of rodents with apomorphine causes compulsive gnawing behaviour instead of vomiting. The compulsive gnawing behaviour in mice by apomorphine is due to stimulation of dopaminergic system. Centrally acting anticholinergics shift the balance between acetylcholine and dopamine resulting in potentiation of the apomorphine effect. Therefore, many drugs with psychotropic activity are known to possess apomorphine-synergistic effect. This enhancement was also found after the administration of tricyclic antidepressants. Critical assessment of this test was that, not only antidepressants, but also centrally acting anticholinergics and antihistaminics are active during the test [256-257]. However, the test has the advantage of its simplicity without any pretraining of the rodents.

2.8. Animal models of diabetes

Animal models have been extensively used in diabetes research. In the 1880s, von Mering was working on the absorption of fat from the intestine when Minkowski removed the pancreas of a dog. The animal developed polydipsia and polyuria, and was found to have diabetes mellitus. Thereafter, many experiments on rabbits and dogs followed, although history has given the special place to Marjorie, one of the dogs used by Banting and Best in their experiments on the isolation and purification of the peptide insulin in the 1920s. Marjorie was the most famous experimental animal in history, only to be superseded by the Dolly sheep in recent years [258]. Recently, most experiments are carried out in rodents [259-260], although some studies are still conducted on larger animals [258, 261].
Several toxins including STZ and alloxan induce hyperglycaemia in rats and mice. Since the initial findings in 1943 of alloxan induced β-cell toxicity in rabbits, this compound has long been used for inducing diabetes. Alloxan is a uric acid derivative and is highly unstable in water at neutral pH, but stable at pH 3. Alloxan acts by selectively destroying the pancreatic beta cells leading to hypoinsulinemia, hyperglycaemia and ketosis [262]. Alloxan causes diabetes in many rodent and non-rodent animals and is most preferably used in case of rabbit because of the ineffectiveness of STZ in rabbits for induction of diabetes and development of well characterized diabetic complications [262-264]. However, guineapig and musk shrew have been reported to be resistant to the action of alloxan due to unknown mechanisms [262].

Because of low stability of alloxan, relatively very shorter half-life (less than 1 min) and acidic nature of the solution, intravenous route of administration is preferred. The alloxan treated animal’s exhibit severe hyperglycaemia, glucosuria, hyperlipidemia, polyphagia, polydypsia and other symptoms of diabetes and also develop various complications such as neuropathy, cardiomyopathy, as well as marked retinopathy etc. Alloxan use is disadvantageous as the percentage incidence of diabetes is quite variable among the experimental groups. In addition, the incidence of ketosis and resulting mortality is very high. Because of these drawbacks, alloxan is now almost replaced by STZ for induction of diabetes in laboratory animals [261].

Streptozotocin is an antibiotic derived from Streptomyces achrromogenes and is structurally to the glucosamine derivative of nitrosourea. Rakieten et al. first demonstrated the diabetogenic property of STZ in dogs and rats [50]. Like alloxan, it induces hyperglycaemia by its direct cytotoxic action on the pancreatic β-cells [51-52]. Its nitrosourea moiety is responsible for destruction of pancreatic β-cells, while deoxyglucose moiety facilitates transport across the cellular membrane. Like alloxan, the participation of free radicals generation and resulting in alteration of endogenous scavengers of these radicals has been reported in STZ diabetogenecity. In addition, STZ causes alkylation or breakage of DNA strands and subsequent increase in the activity of poly-ADP-ribose synthetase, an enzyme depleting nicotinamide adenine dinucleotide in β-cells, resulting in energy deprivation and death of β-cells. These hypotheses have been confirmed by different studies in which the administration of various chemicals such as free radical scavengers and poly ADP-ribose synthase inhibitors, concomitantly or before STZ injection prevent or lessen the severity of the induction of diabetes [51, 265-266]. STZ is a preferred chemical agent to induce hyperglycaemia.
experimental diabetes because it has some advantages over alloxan such as, relatively longer half-life (15 min), sustained hyperglycaemia for longer duration and the development of well characterized diabetic complications with limited incidence of ketosis as well as mortality [51]. Alloxan and STZ diabetic animals are widely used for screening the compounds including natural products for their insulinomimetic, insulinotropic, hypoglycaemic, antihyperglycaemic activities [261]. It has been reported that STZ induces both type 1 diabetes and type 2 diabetes [267-268]. Experimental evidence suggests that, high doses of STZ induce rapid and complete insulin deficiency resembling type 1 diabetes. However, multiple lower doses of STZ, which cause partial destruction of β-cells, can be used to produce type 2 diabetes [269]. In STZ treated mice, changes in spinal terminals of calcitonin gene-related peptide in sensory neurons were observed 4 weeks after diabetes and progressively worsened with time (6-7 weeks) [53]. With increasing duration of diabetes from 7 to 9 weeks, there is a loss in cutaneous C-fiber innervations [270] and decrease in motor nerve conduction velocity, sensory nerve conduction velocity as well as hypoalgesia [271-272]. Besides, STZ induced chronic diabetes showed depressive-like behaviour when submitted to the forced swim test, which is a predictive animal model of depression. It has been reported that, depressive like behaviour in diabetic animals was due to alteration of monoamine levels in brain as well as oxidative stress and inflammation. In addition, STZ induced diabetic animals showed a significant hypolocomotion with respect to control animals [54]. In another study, STZ induced diabetic animals showed cognitive dysfunction in a spatial version of the Morris water maze test. It has been suggested that STZ exacerbates cognitive ability in animals by down-regulating the expressions of BDNF and cAMP responsive element binding protein and by inducing hippocampus neuronal apoptosis [55].

Apart from chemically induced hyperglycemia, other animal models of type 2 diabetes has also been known and divided into following major categories. (1) Spontaneous or genetically derived diabetic animals, which includes both obese and non-obese diabetic models. Obese type-2 diabetic models include ob/ob mouse, db/db mouse, KK mouse, KK/Ay mouse, NZO mouse, NONcNZO10 mouse, TSOD mouse, M16 mouse, Zucker fatty rat, ZDF rat, SHR/N-cp rat, JCR/LA-cp rat, OLETF rat, Obese rhesus monkey. Non-obese type 2 diabetic models include, Cohen diabetic rat, GK rats, Torri rat Non obese C57BL/6, ALS/Lt mouse. (2) Surgical diabetes by partial pancreatectomized animals. (3) Transgenic/knock-out diabetic animals such as transgenic or knockout mice involving genes of insulin and insulin receptor (31)
and its components of downstream insulin signaling e.g. IRS-1, IRS-2, GLUT4 and others like PPAR$\gamma$ tissue specific knockout mouse, glucokinase or GLUT2 gene knockout mice, human islet amyloid polypeptide overexpressed rat (HIP rat) [261].

2.9. Stinging nettle (Urtica dioica)

2.9.1. Description

*Urtica dioica* (UD) is indigenous to Asia and Africa, but also found in all temperate regions of the world including Australia, Europe and America. It is mainly found in the North Western Himalayas from Kashmir to Kumaon at the altitudes of 2100-3200 m. Vernacular names of the plant are Bichu Butti (Hindi and Punjabi), Vrishchhiyaa shaaka (Sanskrit), Anjuraa (Unani) and Shisuun in (Kumaon). UD is an herbaceous plant and commonly known as stinging nettle (Figure 9) belonging to the family Urticaceae. Leaves are opposite, oblong or ovate and toothed. The colour of upper surface is dark green and underside paler or light green. Flowers are small, incomplete and green [273].

![Figure 9: Parts of stinging nettle plant. *Urtica dioica* whole plant (A), leaf upper surface (B), leaf lower surface (C) and stem with trichomes (D).](image)

Both leaves and stems are covered with glandular trichomes that contain ACh, formic acid, 5HT and histamine. The plants cause intense skin irritation if touched [104].
section of nettle leaf has a layer of upper and lower epidermis, underside of the leaf contain more stomata. Stinging nettle leaf contains glandular and non glandular trichomes. Mesophyll occupied by 5 to 6 row of spongy parenchyma embedded with rosette and cluster type of calcium oxalate crystals [105].

2.9.2. Phytochemical Studies

A large number of chemical compounds belonging to different chemical classes including fatty acids, phenylpropanes, coumarins, phenolics, flavonoids, lignans, ceramides, terpenes, lectins, triterpenes and sterols have been isolated from the herb of UD [273-276]. Flowers of UD contains p-hydroxybenzoic acid, gentisic acid, protocatechuic acid, vanillic acid, quinic acid, caffeic acid, ferulic acid, 5-O-caffeoylquinic acid, esculetin, scopoletin, chrysoeriol, kaempferol, isorhamnetin, kaempferol 3-O-glucoside, quercetin 3-O-glucoside, quercitrin, rutin and amentoflavon. UD leaves are known to contain cholineacetyltransferase, 5HT, ACh, p-hydroxybenzoic acid, gentisic acid, protocatechuic acid, quinic acid, caffeic acid, ferulic acid, 5-O-caffeoylquinic acid, esculetin, scopoletin, chrysoeriol, rutin and amentoflavon. UD root contains p-hydroxybenzoic acid, quinic acid, p-coumaric acid, caffeic acid, ferulic acid, 5-O-caffeoylquinic acid, esculetin, scopoletin, secoisolariresinol, chrysoeriol and rutin. UD stem contains p-hydroxybenzoic acid, quinic acid, p-coumaric acid, caffeic acid, ferulic acid, 5-O-caffeoylquinic acid, esculetin, scopoletin, chrysoeriol, quercetin 3-O-glucoside and rutin (Figure 10) [277-280].

**Figure 10:** Structure of the some chemical constituents present on stinging nettle.
2.9.3. Pharmacology

Stinging nettle extract has been known to decrease the level of blood glucose. Administration of alcoholic and aqueous extract of UD leaves repaired pancreatic tissue damage induced by STZ in rats [106]. A polyherbal mixture containing UD, Cinnamomum zeylanicum, Nigella sativa colucynthis, Citrullus Juglans regia, Olea europaea, Vaccinimum arctostaphylos, Trigonella foenum, Allium sativum, Punica granatum, Salvia officinalis and Teucrium polium reduced fasting blood glucose level, water intake, urine output and hyperlipidemia in diabetic rats [281]. Kadan et al., demonstrated that the antihyperglycemic effect of hydroalcoholic
extracts of UD was mediated through GLUT4 membrane translocation in L6-GLUT4myc cells [282]. UD extract significantly reduced serum glucose, insulin, low-density lipoprotein (LDL) and leptin, and LDL/HDL (high-density lipoprotein) ratio and insulin resistance in fructose induced diabetic rats [107]. The aqueous extract of UD leaves significantly attenuated blood glucose level during oral glucose tolerance test in rodents [283].

In a randomized double blind placebo controlled clinical trial, UD is reported to have glycemic control in type 2 diabetic patients by lowering the levels of fasting and postprandial blood glucose [108]. Earlier studies reported that administration of diabetic patients with UD significantly increased antioxidant capacity and reduced inflammatory stress and glycated hemoglobin [109-110].

It has been demonstrated that UD extract compensate astrocytes loss and granule cell density in the dentate gyrus, which can attenuate cognitive impairment in diabetic rat [284]. Nettle supplementation has been known to reverse the brain injury in rats caused by N-methyl-D-aspartate (NMDA) receptor. Nettle supplementation reduced the level of free radicals and DNA binding activity of nuclear factor kappa-B. Nettle supplementation has also been known to possess antiapoptotic effect, thereby promoting cell survival in the brain. These data demonstrated that nettle supplementation improves antioxidant capacities, downregulates inflammatory transcription factors and ameliorate learning performance in passive avoidance step through task [285-286]. Hydroalcoholic extract of stinging nettle significantly lowered the activity of cytochrome P450, lactate dehydrogenase, nicotinamide adenine dinucleotide, cytochrome P450 reductase, total sulfhydryl groups, nonprotein sulfhydryl groups and protein bound sulfhydryl groups. UD extract effectively improved glutathione S-transferase, DT-diaphorase, superoxide dismutase and catalase activity in forestomach and superoxide dismutase and catalase activity the lungs [287].

UD leaves extract showed significant hepatoprotective activity against carbon tetrachloride induced liver toxicity in rats by decreasing lipid peroxidation and increasing antioxidant defense system [288]. Treatment of UD seeds extract restored the aflatoxin induced imbalance between malondialdehyde and antioxidant system in rat liver [289]. Methanolic extract of UD showed defensive role against cisplatin induced toxicity in tumor bearing mice by decreasing alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, lactate dehydrogenase, creatinine, protein oxidation and lipid peroxidation, as well as
increasing catalase, reduced glutathione, glutathione peroxidase, superoxide dismutase and glutathione S-transferase status resulted in nephroprotective, hepatoprotective and antioxidant activity [290]. UD seeds extract showed protective effect against ischemia/reperfusion induced morphological changes in rat liver [291].

Tourniquets are used to provide bloodless field for many surgical procedure by minimizing the blood loss. They might induce ischemia/reperfusion injury locally or systematically. In one experiment tourniquet application in rats was used to induce oxidative stress in muscle tissues and the effect of UD extract was evaluated. Results of this study demonstrated that UD extract has a potential to restore ischemic muscle tissues via antioxidant mechanism [292]. UD extract is also known to possess hypotensive, natriuretic and diuretic effects in rats which were comparable to furosemide [293]. In a Langendorff perfused rat heart experiment, aqueous extract of stinging nettle exerts vasoconstriction effect on aorta via stimulation of alpha1 adrenergic receptors. Further, stinging nettle strongly induced bradycardia which might be responsible for hypotensive action [294].

Methanolic extract of stinging nettle dose dependently inhibited the carrageenan induced paw edema as well as acetic acid-induced abdominal twitches in mice; the highest effective dose of stinging nettle was 400 mg/kg [295]. In another experiment, aqueous extract of stinging nettle dose dependently inhibited (50, 100 and 200 mg/kg) the acetic acid induced writhing in mice [296]. Activation of nuclear factor kappa B is increased in several inflammatory diseases and responsible for the upregulation of many pro-inflammatory genes. Results of the earlier study demonstrated that UD leaves extract have potential to inhibit cytokines production as well as inflammation and arthritis by blocking nuclear factor kappa B pathway [293].

Aqueous extract of UD showed immunomodulatory and antiinflammatory effects on the murine splenocytes and murine peritoneal macrophages. It was demonstrated that UD extract activated the proliferation of T-lymphocytes and decreased nitric oxide production in lipopolysaccharide induced macrophages without affecting the cell viability [297].

A randomized double blind clinical trial was performed to evaluate the effect of stinging nettle plant in the symptoms of benign prostatic hyperplasia. Nettle supplementation significantly relived the clinical symptoms in patients with benign prostate hyperplasia.
compared to placebo without any side effect [298]. Further, in a prospective, randomized
double blind, placebo controlled, crossover study UD administration significantly reduced the
symptoms of benign prostatic hyperplasia [299-300]. UD extract in combination with Sabal
extract showed clinically relevant benefit in patients with lower urinary tract symptoms
[301]. A protein fraction from the aerial parts of stinging nettle elicited antimitagenic and
antioxidant activity in cell lines of human hepatoma HepG2 cells [302]. Extract of stinging
nettle attenuated testosterone induced prostatic hyperplasia in rodents [278]. The 20%
methanolic extract of UD significantly inhibited benign prostatic hyperplasia induced by
implanting urogenital sinus into the ventral prostate gland of mouse [303].

The crude UD extract exhibited significant antimicrobial activity against Gram-positive
bacteria [304]. UD extract exhibited promising antibacterial activity against fast growing, non
pathogenic Mycobacterium semegnatis bacteria in disk diffusion method [305]. In earlier
study, UD extract also showed promising effect against C. michiganensis and Xanthomonas
vesicatoria bacteria [306]. Aqueous extract of UD showed antifungal effect against A.
alternate and R. solani [307]. N-acetylglucosamine, the specific lectin from stinging nettle is
the potent and selective inhibitor of cytomegalovirus and human immunodeficiency virus
replication in vitro [308].

The LD $\text{S}_{0}$ value of UD leaf extract was observed as 3.625 g/kg in mice. Higher dose (>750
mg/kg) was associated with hypothermia and reduced muscular tone. In toxicity studies, 50
mg/kg of hydroalcoholic extract of UD was administered orally to rabbits for 10 days.
Results of this study revealed that UD extract caused occasional diarrhoea and reduced 40%
bodyweight [273]. However, UD extract did not show significant toxicity after long term use
in clinical trials [309-310].

2.10. St. John's wort (Hypericum perforatum)

2.10.1. Description

Saint John's wort also known as Hypericum perforatum is a flowering plant of the genus
Hypericum belonging to the family Hypericaceae (Figure 11). Common name of Hypericum
perforatum derived from its traditional flowering as well as harvesting on 24 June St. John's
day. St. John's wort is also known as Johns wort, Goat weed, Rosin rose, Klamath weed and
Tipton weed. Hypericum perforatum is a branching shrubby herb. The branches and stems of

(37)

Hypericum perforatum are densely covered by oblong and smooth margined leaves. Leaves are green in colour with black spots which is visible against light. Leaves exerts distinct and balsamic odour with astringent and bitter taste. The aerial part of mature plant produces several dozen of five petaled yellow colour flowers. The edges of petals are covered through black dots. Crushed flowers of St. John's wort produce a blood red colour pigment. The species is native of Europe but has also spread to temperate regions of Asia, Africa, Australia and North and South America. Due to photosensitivity reaction, Hypericum perforatum is listed as a noxious weed in the United States [67-68].

**Figure 11:** Parts of Saint John's wort plant. St. John's wort whole plant (A) and flower (B).

2.10.2. Phytochemical Studies

The most common chemical constituent naphthodianthrones include hypericin, isohypericin pseudohypericin and protohypericin [311]. Flavonoids content of Hypericum perforatum ranging from 7.0% in stems to 12.0% in flowers and leaves. Kaempferol, quercetin, luteolin, hyperside, isoquercitin, rutin, amentoflavone, hyperin, myricetin, rutin and miquelianin are the important flavonoids of this plant [312-315]. Extracts of Hypericum perforatum contains lipophilic compounds including hyperforin, adhyperforin and furohyperforin. Essential oils of Hypericum perforatum consist of mono and sesquiterpenes i.e. 2-methyl-octane, pinene, terpineol, myrecene, caryophyllene, geranil and limonene. Additional components of Hypericum perforatum contain chlorogenic acid, caffeic acid, p-coumaric acid, nicotinic acid, myristic acid, palmitic acid, stearic acid, carotenoids, pectin, choline, long-chain alcohols and
hydrocarbons. Several amino acids like cysteine, leucine, glutamine, lysine and γ-aminobutyric acid have also been isolated from *Hypericum perforatum* extract [67, 316-317].

**Figure 12:** Structure of the some chemical constituents present on St. John’s wort.
rutin

hyperoside

amentoflavone

myricetin

p-coumaric acid

glutamine

stearic acid

palmitic acid

cysteine

myristic acid

2-methyl-octane

limonene

pinene

nicotinic acid

lysine

leucine

choline

2.10.3. Pharmacology

The herb of St. John's wort has been used from centuries to treat various ailments. In Europe, St. John's wort is commonly prescribed for the treatment of depression and in United States, it is available as over the counter herbal supplement [69]. Different controlled clinical trials showed its effectiveness in the treatment of mild-to-moderate depression [70]. Randomised and double blind controlled trials in 5489 patients demonstrated that Hypericum extract is superior to placebo in patients with depressive disorder and their effectiveness is similar to that of standard antidepressants with fewer side effects [71]. Evidence suggests that the antidepressant activity of Hypericum extract is mediated through inhibition of the reuptake of synaptosomal neurotransmitters such as 5HT, dopamine, NE [318] and modulation of neuronal excitability via GABAergic mechanisms [319].

Earlier study suggested that hypericin is the main active constituent of Hypericum extract, stimulates capillary blood flow, resulting in antidepressant effect [67]. Hypericin is also known to strongly inhibits the enzyme monoamine oxidases (MAO) [320-321]. MAO enzyme is involved in the metabolism and degradation of amine neurotransmitters in the synapse. Hypericin also modulate the levels of dopamine via sigma receptors. Hypericin modulate neuronal action potential by antagonizing benzodiazepine, gamma amino butyric acid A, gamma amino butyric acid B (GABA-B), adenosine and inositol triphosphate receptors [67]. Later studies suggest that, hypericin can not by itself completely responsible for the antidepressant effect. Follow-up study demonstrated that hyperforin is responsible for antidepressant action [322]. Hyperforin is a powerful reuptake inhibitor of 5HT, dopamine, NE, GABA and L-glutamate in synaptic cleft with ICs0 values between 0.05-0.1 μg/ml [323-326]. Hyperforin alleviates the symptoms of depression by blocking the the reuptake of 5HT from the synaptic cleft [327].

Hypericum extract improves hippocampus dependent spatial memory [72], proliferation of progenitor cells and dendritic spine in hippocampal neurons, and restored the synaptic plasticity [328]. Recent study reported that St. John's Wort reduces Alzheimer's pathology by facilitating blood brain barrier ABCC1 transporter protein expression and microglia activation [74]. St. John's wort attenuated object recognition memory impairment caused by chronic restraint stress in rodents [72]. It has been reported that Hypericum extract is a better
alternative for the management of depression associated with cognitive impairment than other antidepressants known to possess anticholinergic side effects such as sedation and delirium. Hypericum extract also increased the retrieval of memory in passive avoidance task [75]. Hypericum extract significantly attenuated scopolamine and sodium nitrite-induced impaired cognition in active avoidance task [329].

St. John's Wort and its active constituent hyperforin protect rat as well as human islets against cytokine mediated β-cell injury in type 1 diabetes [76]. Hypericum administration significantly reduced hyperglycemia in STZ treated diabetic rats. It also reduced depression and anxiety in type 2 diabetes and might be the potential candidate for management of comorbidities caused by depression, anxiety and diabetes [48]. It has been demonstrated that Hypericum extract restored psychiatric illness such as depressive moods, cognitive deficits, locomotion and sleeping disturbances associated with STZ induced diabetes [330]. Extract of Hypericum perforatum is known to modulate antioxidant status and cholinergic system, thereby ameliorate acquisition and retrieval processes in passive avoidance task [331]. In addition, Hypericum extract significantly induced insulin release in rats with nicotinamide-STZ induced type 2 diabetes [332]. In contrast, Hypericum extract significantly inhibits the differentiation of adipocyte and induces insulin resistance in mature 3T3-L1 adipocytes [333].

Hydroalcoholic extract of Hypericum perforatum showed analgesic effect against acetic acid induced abdominal constriction in mice via opioid receptor activation [334]. It has been reported that Hypericum extract attenuates physical withdrawal signs in opium dependence [335] comparable to clonidine [336].

In a randomized, double blind and vehicle controlled study, Hypericum cream significantly attenuated UV induced erythema and this effect was mediated by the constituent hyperforin, a powerful free radical scavenger [337]. The mechanisms for antiinflammatory response of Hypericum was found to be due to downregulation of interleukin-6 (IL6), cyclooxygenase-2 and inducible nitric oxide synthase expressions [67]. Hypericum extract is also known to possess cardioprotective [338], wound healing [339], anticancer [67] and antihyperlipidemic [340] activities. An herbal mixture containing Urtica dioica and Hypericum perforatum
effectively reduced the redness, distortion, swelling and ankylosis of the joint in muramyl dipeptide/collagen induced arthritis in rats [341].

The most common adverse effects associated with the normal dose of St. John’s Wort are gastrointestinal symptoms, dizziness, confusion, allergic reactions, lethargy, restlessness and dryness of the mouth. St. John’s wort also induced neuropathy and mania. Hypericin, an active constituent of St. John’s Wort has elicited phototoxic dermititis in high doses. Normal doses of St. John’s Wort taken for treatment of depression did not show any significant phototoxic effects [67]. It has been widely accepted that, St. John’s wort extract induces P-glycoprotein along with a series of drug metabolizing enzymes cytochrome P450s (CYPs) including CYP3A4 and CYP2C9, and participates in drug-drug interaction [342-344].

2.11. Fluoxetine (FLX)

The systematic (IUPAC) name of FLX is (RS) N-methyl-3-phenyl-3-[4-(trifluoro-methyl) phenoxy] propan-1-amine.

The U.S. Food and Drug Administration have approved the FLX for treatment of depressive disorder in December 1987. FLX is a bicyclic derivative of phenyl propylamine. FLX is the most widely used SSRI and prescribed for a variety of neurological disorders including mood and eating disorders, depression in elderly and obsessive compulsive disorders. FLX seems to facilitate the serotonergic transmission in central nervous system via downregulation of presynaptic autoreceptors. The dosage of 20 mg/day has been found to be effective for neurological disorders. The oral bioavailability of FLX is 72% and C<sub>max</sub> (15-55 µg/L) reached in 6 to 8 hours. FLX is highly (94%) bound to plasma proteins, mostly α<sub>1</sub>-glycoprotein and albumin. Volume of distribution of FLX is 12-43 L/kg. Its elimination half-life is 1-4 days. Its clearance is 36-50 L/h (urine and faeces). FLX is metabolised to norfluoxetine via O-demethylation. Norfluoxetine has potency and selectivity similar to that of parent compound [345-347].
In a meta-analysis, FLX was administered to 2635 adult and 960 geriatric patients with depressive disorder. In all age of depressive patients, FLX showed significant improvement relative to placebo. Improvement rate was largest for adult patients receiving FLX (35% greater than placebo) and the remission rate was 30.1% [83]. In randomized controlled trials, FLX exerted neurocognitive improvement in patients with moderate depression [77]. FLX attenuated impaired cognitive behaviour in depressed rodents as well [78]. FLX increased neurotrophins (vascular endothelial growth factor and brain derived neurotrophic factor) levels in hippocampus and modulated adult neurogenesis and depressive-like behaviour in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxin induced brain lesions [79].

Study revealed that, clinical dose of FLX for the treatment of depression symptoms induced hypoglycaemia in type 1 diabetic patient and prompted a progressive reduction of the insulin dose. The insulin requirement was reduced during FLX therapy while the level of HbA1c remained stable. However, FLX withdrawal increased insulin requirements progressively to the patients usual dose [348]. FLX treatment reduced the level of HbA1c during co-mobidity depression and diabetes in rodents. FLX ameliorated spatial learning in Morris water maze task, and showed hypoglycaemic and antidepressant effects in a model of CUMS in rats with diabetes mellitus [80]. In contrast, FLX treatment reduced glucose mediated insulin secretion. This decreased pancreatic β-cell function concomitant with increased oxidative stress, which further contributes to decreased mitochondrial electron-transport chain enzyme activity in pancreas. This study revealed that FLX administration during depressive episodes increases the onset type 2 diabetes by causing oxidative stress in β-cells [349]. Another study suggested that, FLX inhibits insulin secretion, induces unfolded protein response and apoptotic process, triggers β-cell death and promotes insulin resistance in murine islets or Min6 β-cell line [350]. An analysis of published case reports further revealed the association between antidepressants therapy and glucose dysregulation. In these reports, FLX was associated with hypoglycaemia [351].
FLX is also prescribed for variety of pathological conditions including mood and eating disorders [81], obsessive compulsive disorders [82], depression in the elderly [83] and dysthymia [84].

FLX may precipitate side effects including nervousness, dryness of mouth, nausea, sore throat, drowsiness, weakness, tremor, akathisia, anorexia, weight loss, changes in sexual drive, excessive sweating, hives, rashes, fever, joint pain, swelling of the throat, face, tongue, eyes, lips, hands, ankles, feet and lower legs, difficulty in swallowing or breathing, confusion, fast or irregular heartbeat, severe muscle stiffness, hallucinations and seizures [352-355]. The discontinuation of FLX was associated with dizziness, insomnia, fatigue, light headedness, agitation, nausea and sensory disturbances. These symptoms last for around three weeks and disappeared by restarting FLX dose or any other antidepressant having similar pharmacological profile [356].

FLX inhibits many drug metabolizing isozymes of cytochrome P450 group. FLX is the potent inhibitor of CYP2D6 and mild to moderate inhibitor of CYP1A2, CYP2C9, CYP2C19, and CYP3A4 [357]. Its use is avoided in patients receiving other medications such as monoamine oxidase inhibitors, methamphetamine, tricyclic antidepressants, buspirone, triptans, serotonin-norepinephrine-reuptake inhibitors and other SSRIs due exacerbation of FLX side effects [358-359].

2.12. Rosiglitazone (ROSI)

The systematic (IUPAC) name of ROSI is (RS)-5-[4-(2-[methyl(pyridin-2-yl) amino] ethoxy) benzyl] thiazolidine-2,4-dione.

![Rosiglitazone](image)

ROSI is thiazolidinedione class of drug, a potential oral antidiabetic drug for therapy of type 2 diabetes mellitus. The dosage of 4 mg/day has been found to be effective for type 2 diabetes. The oral bioavailability of ROSI is 99% and $C_{\text{max}}$ (427.68 ng/ml) reached in 1 hours. ROSI is highly (99.8%) bound to plasma proteins mostly $\alpha_1$-glycoprotein. Volume of
distribution of ROSI is 17.6 L/kg. CYP2C8 and CYP2C9 are responsible for metabolism of ROSI in liver by N-demethylation and hydroxylation. Its elimination half-life is 4.45 hrs. Its clearance is renal (64%) as well as fecal (23%) [86-87].

ROSI is the selective agonist for nuclear PPARγ receptor. It also increases the gene expression of PPARγ receptor. ROSI bind to PPARγ, which activates insulin receptor gene expression that regulate carbohydrate, protein and lipid metabolism. ROSI require insulin for their action. ROSI increases glucose transport influx in adipose tissue and muscle by enhancing synthesis and translocation of glucose transporters [88-89].

In a trial, individuals (5,269) with impaired fasting glucose and/or impaired glucose tolerance were administered with ROSI. After three years, 982 participants had oral glucose tolerance tests at baseline. ROSI significantly improved β-cells functions and attenuated insulin resistance in type 2 diabetic patients [360]. In animal model, ROSI showed central anti-diabetic action against D-glucose fed and STZ-induced diabetes [90]. In another investigation, ROSI improved hyperglycemia and insulin resistance in high fat diet and STZ-nicotinamide induced type 2 diabetes in mice [361]. Earlier report demonstrated that, ROSI administration significantly decreased fasting blood glucose and pancreatic levels of tumor necrosis factor alpha (TNF-α), interferon gamma and NO in cyclosporin A and multiple lower doses of STZ induced diabetic mice. In the similar experiment, the level of insulin in serum was increased after ROSI treatment. ROSI treatment induced regeneration of pancreatic islets and attenuated the effect of CD4 and CD8 T-cells in pancreas. ROSI also showed anti-inflammatory effect in autoimmune disease mediated diabetes [362]. Study revealed that ROSI administration attenuated lipopolysaccharide induced IL-1β and interferon gamma secretion from peritoneal cells and enhanced islet engraftment [363].

Studies suggested that insulin resistance induces cognitive deficit in individuals with type 2 diabetes mellitus. ROSI is known to protect cognitive impairment in individuals with type 2 diabetes [91]. Study revealed that ROSI improves cognition and memory performance in patients with mild Alzheimer disease and animal models of Alzheimer disease [92]. ROSI improved hippocampus dependent cognitive impairment in some Alzheimer disease patients and attenuated cognitive deficits in a mouse model amyloidosis [364]. Clinical report suggests that, ROSI does not ameliorate cognition when used as adjunctive therapy with AChE inhibitors in patients with mild to moderate Alzheimer's disease [365]. Further,
administration of ROSI induced cognitive impairment in some patients with type 2 diabetes [366].

Earlier cross sectional studies suggested that insulin resistance is associated with affective disorders. It has been documented that, insulin sensitizing drug ROSI exhibited significant declines in depression severity associated with insulin resistance [93]. ROSI is known to have antidepressant activity in both human and rodents. ROSI upregulated fibroblast growth factor 2 expression in neurotrophic factor-α1 dependent manner in the hippocampus of stressed mice. Mice administered with ROSI showed increased hippocampal neurogenesis in depressed mice [94]. ROSI improved glucose tolerance and normalized hyperglycemia in db/db mice. ROSI significantly increased mobility time in forced swim test in db/db mice, suggested antidepressant like effect. In the open field task, ROSI did not modulate locomotor activity of db/db mice [367]. ROSI significantly reduced hypercorticosteronemia in animal model of depression, thereby improved mobility in forced swim and tail suspension test [216]. ROSI exhibited antiinflammatory activity in the hippocampus by significantly inhibiting the expression of TNFα, CD40 and microglial activation [368].

ROSI may precipitate side effects including runny nose and other cold symptoms, headache, back pain, sore throat, pain in the jaw, arm, neck, back, or stomach, lightheadedness, chest pain, anorexia, nausea, vomiting, dark urine, changes in vision, yellowing of the skin or eyes, vision loss, dizziness, pale skin, hoarseness, swelling of the eyes, lips, face, tongue or throat, hives, difficulty in swallowing or breathing, itching, blisters, fever, bone fractures, hypoglycaemia, hepatotoxicity, myocardial infarction and death [89, 346, 369-372].