Preface

Diabetes mellitus is the most common chronic metabolic disorder with a prevalence of 8.3% globally. According to World Health Organization, diabetes mellitus will become the seventh leading cause of mortality worldwide in 2030. There are mainly three types of diabetes mellitus, which are type 1 (insulin dependent diabetes mellitus), type 2 (non-insulin dependent diabetes mellitus), and gestational diabetes mellitus (hyperglycemia during pregnancy). According to the global statistics of diabetes mellitus in 2013, about 382 million people are afflicted with diabetes mellitus and type 2 diabetes mellitus contributes up to 90% of cases. A recent report revealed that depression is a common comorbid condition frequently observed in type 2 diabetic patients, one out of four patients experience depressive disorders. There is a bidirectional relationship between depression and type 2 diabetes mellitus. A great body of literature supports the fact that both depression and type 2 diabetes mellitus share several common pathophysiological mechanisms. The pathophysiology of depression in diabetic patient is multifactorial, which includes functional insulin-resistance, inflammation, oxidative stress, and decreased activity of norepinephrine (NE), serotonin (5-HT), and decreased brain-derived neurotrophic factor. The comorbid depression increases both micro- and macro-vascular complications, the major cause of multi-organ damage and mortality. Previous preclinical studies evidenced an increased risk of development of depression due to decreased functional activity of central neurotransmitters, such as NE and 5-HT in diabetic rats. Inflammation and immune activation have been implicated in the pathogenesis of both diabetes and depression. It has been suggested that proinflammatory cytokines such as IL-1β, TNF-α, and IL-6 have the potential to interact with insulin sensitivity and pancreatic β-cell function and induce diabetes. Depression is associated with overactivation of hypothalamic-pituitary-

adrenal (HPA) axis and overproduction of glucocorticoids leading to aberrant glucose homeostasis. A recent cross-sectional study has indicated a relationship between inflammation and depression in newly diagnosed diabetic individuals. Furthermore, vitamin C (ascorbic acid) deficiency and stressful events contribute in the development of depressive disorders. Despite vast improvement in our understanding on diabetes comorbid depression, there is an unmet need to develop therapeutic strategies to treat both diabetes mellitus and comorbid depression. Earlier studies have reported a lower circulating ascorbic acid levels in patients with diabetes mellitus. Ascorbic acid supplementation has been shown to produce antidiabetic activity and antidepressant activity. On the other hand, metformin is a potent oral hypoglycemic agent now recommended as the first-line therapy for type 2 diabetes mellitus. In addition to its hypoglycemic activity, metformin has been shown to elicit marked antioxidant activity, weight loss activity, hepatoprotective activity, anti-inflammatory activity, antidepressant-like activity, and antiepileptic activity. The pleiotropic pharmacological activities of metformin and ascorbic acid make them suitable for the treatment of diabetes mellitus and comorbid depression, which involves a myriad of pathophysiological characteristics. However, till date, no studies have been conducted to assess the therapeutic potential of metformin and ascorbic acid against diabetes comorbid depression in rats.

Considering all the pathophysiological factors, it can be hypothesized that a combination strategy, which can abrogate hyperglycemia, inflammation, oxidative stress, and imbalance in neurotransmitter levels, would be a possible option for treating diabetes mellitus and comorbid depression. In the present study, we explored the potential benefits of metformin and ascorbic acid in a rat model of diabetes comorbid depression that primarily focuses on a clinical situation where occurrence of diabetes

mellitus leads to depression. Experiments were designed to investigate the effects of ascorbic acid, metformin, and their combination on the markers of depression (immobility period in forced swim test, plasma corticosterone levels, and adrenal hyperplasia), markers of diabetes mellitus (plasma glucose and insulin levels), brain monoamines (levels of NE and 5-HT in the brain), oxidative stress (lipid peroxidation (LPO), superoxide dismutase (SOD), and catalase activity (CAT) in the brain), inflammatory processes (levels of proinflammatory (TNF-α and IL-6)/anti-inflammatory (IL-10) cytokines in the brain), apoptotic proteins (caspase 3 and caspase 9), mitochondrial health, and brain-derived neurotrophic factor (BDNF). We used streptozotocin (dissolved in 0.1 M citrate buffer, pH 4.5) and nicotinamide (dissolved in normal saline) to induce type 2 diabetes mellitus. Briefly, overnight fasted rats received a single intraperitoneal (i.p.) injection of streptozotocin (65 mg/kg) 15 min after a single dose of nicotinamide (120 mg/kg, i.p.). A group of rats received only the vehicles (0.1 M citrate buffer and normal saline) and considered as nondiabetic control group. All the animals were allowed free access to commercial food pellets and 10% sucrose solution to minimize hypoglycemic shock induced by streptozotocin. After 72 hr of streptozotocin injection, blood glucose levels were measured using glucose strips through a tail-nick blood sample. Rats with ≥250 mg/dL blood glucose were considered diabetic and selected for induction of comorbid depression. Comorbid depression was induced in diabetic rats by five inescapable foot-shocks (2 mA, 2 ms duration) at 10 s intervals on days 1, 5, 7, and 10 after 1 hr of vehicle or drug treatment. Diabetic rats received foot-shocks in a black box (24 x 29 x 40 cm) with a grid floor. Nondiabetic control rats were placed in the black box but did not receive foot-shocks.

Plasma glucose and plasma insulin levels were estimated to assess the antidiabetic efficacy of the combination therapy (metformin and ascorbic acid at 25 mg/kg, p.o.) and

monotherapy of both metformin (25 mg/kg, p.o.) and ascorbic acid (25 mg/kg, p.o.) after 11 days of administration. The combination treatment showed a significant ($P < 0.05$) decrease in the levels of plasma glucose compared with diabetes comorbid depressed (DCD) control group. The combination therapy caused a significant increase in plasma insulin levels compared with DCD control rats, metformin monotherapy, and ascorbic acid monotherapy. The combination therapy produced an additive synergistic effect, with significant reductions in plasma corticosterone levels compared with DCD controls, metformin monotherapy, and ascorbic acid monotherapy. The presence of comorbid depression was assessed on day 11 by estimating immobility period through forced swim test. The combination therapy showed an additive synergistic effect, with significant decrease in immobility period compared with DCD control rats, metformin monotherapy, and ascorbic acid monotherapy. Oxidative stress in the brain, due to diabetes and comorbid depression, was assessed by estimating lipid peroxidation, superoxide dismutase content, and catalase activity in the prefrontal cortex. The combination therapy showed significantly lower LPO compared with DCD control rats and metformin monotherapy. The combination therapy showed significantly higher levels of SOD content compared with the DCD control rats and metformin monotherapy. The combination therapy showed significantly higher levels of CAT activity as compared to the DCD control rats and metformin monotherapy. The alterations in levels of brain monoamines are the pathophysiological hallmark of depression. We estimated the levels of NE and 5-HT in the prefrontal cortex, which is one of the major brain structures implicated in depression. Rats administered with the combination showed significantly higher levels of NE compared with DCD control rats and ascorbic acid monotherapy. The combination therapy showed significantly higher levels of 5-HT as compared to DCD control rats, metformin monotherapy, and ascorbic

acid monotherapy. Diabetes and depression have been implicated in the activation of inflammatory processes with increase in proinflammatory cytokines in the brain. Treatment with the combination showed significantly lower levels of TNF-α compared with DCD control rats and ascorbic acid monotherapy. The combination therapy showed significantly lower levels of IL-6 compared with DCD control rats, metformin monotherapy, and ascorbic acid monotherapy. Mitochondrial function, a key indicator of cell health, can be assessed by monitoring changes in mitochondrial membrane potential. The combination therapy showed significantly higher mitochondrial membrane potential compared with DCD control rats. Caspases are intracellular proteases that are responsible for the disassembly of cells into apoptotic bodies during apoptosis. Caspase 9 activates caspase 3 by proteolytic cleavage and caspase 3 then cleaves vital cellular proteins which lead to apoptosis. The combination therapy showed significantly lower levels of caspase 3 and 9 compared with DCD control rats. Brain-derived neurotrophic factor is a neurotrophin essential for growth, differentiation, plasticity, and survival of neurons. BDNF is also required for processes such as energy metabolism, behavior, mental health, learning, memory, stress, pain, and apoptosis. BDNF is implicated in various neuronal disorders such as Alzheimer's disease, Huntington's disease, depression, and bipolar disorder. The combination therapy showed significantly higher levels of BDNF protein and gene expression compared with DCD control rats.

Ascorbic acid monotherapy was found highly efficacious in reducing oxidative stress but submaximal efficacy was observed against hyperglycemia and inflammatory response. Metformin monotherapy was found highly efficacious in reducing hyperglycemia and increasing monoamine levels but submaximal efficacy was observed against oxidative stress. Metformin and ascorbic acid combination therapy

could be a potential strategy against diabetes comorbid depression. Restoration of monoamine levels by the combination therapy is possibly through alterations in caspase activity and BDNF level.