6 Chapter 6: General discussion and conclusions

The main outcome of the current study is that combination therapy of metformin and ascorbic acid causes significant improvement in diabetes comorbid depression in rats. The combination therapy was able to abrogate the physiopathologies such as behavior alteration, reduced monoamine levels, oxidative stress, inflammation, and hyperglycemia associated with comorbid depression in diabetes rats. The forced swim test is broadly used for evaluating the potential anti-depressant drugs. The basic principle of forced swim test is that, when animal is allowed to forcefully swim into the restricted area where rat could not be able to escape itself, and despaired to the present condition and immobile position is observed. This immobile position is considered as a depression-like condition, used to evaluate the several anti-depressant drugs (de Morais et al., 2014). In our current study, we observed that monotherapy of ascorbic acid, metformin, and the combination therapy significantly reduces the immobility period in DCD rats after eleven daily treatments. It is important to highlight that as compared to the ascorbic acid monotherapy, metformin monotherapy highly reduces the immobility period at the same dose. In addition, the combination therapy has higher reduction of immobility period compared with monotherapies of metformin and ascorbic acid. Depression arises due to hyperactivation of HPA axis which leads to increased release of cortisol to blood circulation (Glynn et al., 2013; Juruena, 2014). Similarly, we observed higher levels of corticosterone levels in the plasma of diabetes comorbid depressed rats as compared to nondiabetic control rats. Likewise, treatment with the combination therapy significant reduction in the corticosterone levels was observed may be due to normalization of HPA axis.

As a model for type 2 diabetes mellitus, it is well established that the administration of nicotinamide with streptozotocin results in pancreatic beta cell toxicity with similar clinical sign of diabetes within three days. The combined administration of streptozotocin with nicotinamide produced a non-insulin dependent diabetes mellitus, characterized by stable hyperglycemia and reduced pancreatic insulin in rats. It is important to highlight that streptozotocin produces destruction of β-cells but prior administration of nicotinamide protects the β-cells through preservation of NAD pool. This nicotinamide on one-side required for a precursor for NAD synthesis and on other-side this is an inhibitor of poly(ADP-ribose) synthetase. Poly(ADP-ribose) synthetase catalyzes chromatin-bound polymerization of an ADP-ribose moiety of NAD, and evidence has accumulated that this enzyme plays major role in NAD degradation in mammalian cells. In our study, we observed that in diabetes comorbid depressed rats the level of glucose was significantly higher, which is sign of diabetes. There are many diabetic complication arises due to hyperglycemic condition which causes the depression. The hyperglycemic condition ultimately results in increased oxidative stress, inflammation, and decreased monoamine levels observed in diabetes comorbid depressed rats. In this context, our data showed that in diabetes comorbid depressed rat higher levels of lipid peroxidation, and lower superoxide dismutase, and catalase enzymes was observed in the prefrontal cortex. As many literatures depicted that prefrontal cortex dysfunction occurs in clinically depressed patients (Baxter et al., 1989; George et al., 1994) and preclinical depression-like animals (Ates et al., 2014; Gellén et al., 2017). There is a monoamine theory for the depression where decreased levels of monoamines (serotonin and norepinephrine) have been occurred in prefrontal cortex (Hasler, 2010; Krishnan and

Nestler, 2011). Many antidepressants including selective serotonin reuptake inhibitor (fluoxetine) and serotonin-norepinephrine reuptake inhibitors (venlafaxine) are used to treat depressive symptoms in diabetic patients (Gill et al., 2010). Overall, many diabetic complications arise due to antidepressant medication which is a risk factor for type 2 diabetes (Barnard et al., 2013). Here, it is interesting to observe that while the use of metformin with ascorbic acid significantly increased the serotonin and norepinephrine levels in prefrontal cortex of the diabetes comorbid depressed rats. Supporting to our data, it is important to note that neurons are more prone to the oxidative damage (Salim, 2017). Several line of evidence showed that in diabetes comorbid depression condition the balance between reactive oxygen species and endogenous antioxidative activity is disturbed (Menezes Zanoveli et al., 2016). Similarly, we also observed in our experiment that the oxidative stress in prefrontal cortex of diabetes comorbid depressed rats suggesting that prefrontal cortex directly involved in development of depression. In this connection, increased oxidative stress and inflammation is responsible for the development of depression-like symptoms (Patki et al., 2013). The proinflammatory cytokines (IL-6 and TNF-α) are elevated in brain area which is responsible for the depression (Liu et al., 2012). On the other hand, anti-inflammatory cytokines IL-10 levels were reduced (Voorhees et al., 2013). Similarly, in our study, we observed that higher levels of TNF-α and IL-6 and lower levels of IL-10 levels in streptozotocin induced diabetes and foot-shock produced depression-like symptoms in rats.

In order to investigate that the increased inflammation and oxidative stress causes depression-like symptom, we measured the brain derived neurotrophic factor. Several literatures showed that the depression-like symptoms are associated with

reduced levels of BDNF (Dwivedi, 2009; Phillips, 2017). To support our data, we investigated the BDNF protein through western blot and DNA damage through real-time polymerase chain reaction in the prefrontal cortex of diabetes depressed rats. BDNF plays an important role in neuronal differentiation, survival, and growth (Numakawa et al., 2014; Phillips, 2017). Similarly in our study, as in hyperglycemic condition and depression-like symptoms, the level of BDNF has been reduced, which suggest that the increased oxidative stress and inflammation in prefrontal cortex directly led to depression. As expected, increased oxidative stress and inflammation caused cellular damage and mitochondrial dysfunction. This ultimately leads to increase in free radical generation and activates intrinsic pathway of caspase activity. The alterations in mitochondrial membrane potential cause release of cytochrome c, which converts the procaspase 9 into active caspase 9 (Chandra and Tang, 2003). The caspase 9 activates caspase 3 activities, which leads to the apoptosis pathways.

It is worthwhile to note the protective effect of combination therapy of metformin and ascorbic acid against steptozotocin induced diabetes and intermittent foot-shock induced depression-like symptoms. The combination therapy was found to be effective against hyperglycemia, hyperactivation of HPA axis, oxidative stress, depressive-like behavior, and reduced monoaminergic transmission. Taken together, the present findings suggest that diabetes comorbid depressed patients could be benefitted by the combination therapy of metformin and ascorbic acid.

6.1 Summary of major findings

Depression is frequently associated with comorbid condition with diabetes. The comorbid depression increases both micro- and macro-vascular complications, the

major cause of multi-organ damage and mortality. 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. The present research work was designed to evaluate the combination therapy of metformin and ascorbic acid in diabetes comorbid depressant rats. Additionally, we have observed antidepressant-like activity of the individual treated metformin and ascorbic acid in diabetes comorbid depressant rats to explore the potential role of this treatment in the management of diabetes comorbid depression. Major findings of the present research work are as follows:

- Ascorbic acid therapy was found highly efficacious in reducing oxidative stress but submaximal efficacy was observed against hyperglycemia and inflammatory response.

- Metformin was found efficacious in reducing anti-depressant markers, oxidative stress, hyperglycemia, and pro-inflammatory cytokines.

- Metformin was found efficacious in increasing monoamine levels and anti-inflammatory cytokines.

- Combination therapy of metformin and ascorbic acid was found efficacious in reducing anti-depressant markers, oxidative stress, hyperglycemia, and pro-inflammatory cytokines than monotherapy of metformin and ascorbic acid.

- Combination therapy of metformin and ascorbic acid was found efficacious in increasing mitochondrial membrane potential and BDNF

expression in the prefrontal cortex than monotherapy of metformin and ascorbic acid.

- Combination therapy of metformin and ascorbic acid was found efficacious in reducing mitochondrial reactive oxygen species, caspase -9 and -3 expressions, and NF-κB activity in the prefrontal cortex than monotherapy of metformin and ascorbic acid.

6.2 Scope for further work

The strategy followed in this thesis was based on a consideration that diabetes leads to the development of depression. However, the relationship between diabetes and depression is bidirectional. So, evaluation of metformin and ascorbic acid combination potential in other direction, i.e., depression induced diabetes using chronic unpredictable stress model would provide complete insight in understanding the molecular mechanism of combination therapy of metformin and ascorbic acid. Furthermore, electrophysiological investigation (Nayak and Kerr, 2015) of the effects of the combination therapy in the hippocampus would provide better insight into the mechanisms involved in the pathophysiology of depression in diabetes and beneficial effects of the combination therapy.

7 Chapter 7: References