3 Chapter 3 Metformin monotherapy: a potential strategy against diabetes comorbid depression in rats

3.1 Introduction

Depression and diabetes share common pathophysiological mechanisms (hyperglycemia, autonomic dysregulation, neurohormonal dysregulation, inflammation, and oxidative stress); an existence of bidirectional relationship has been proposed (Semenkovich et al., 2015). Considering the involvement of multiple mechanisms, it can be hypothesized that targeting more than one mechanism would be a suitable strategy to mitigate both depression and diabetes. As hyperglycemia is a major contributor to diabetes comorbid depression, this part of the thesis explores the potential of metformin monotherapy to identify a low dose combination therapy.

Apart from its pleiotropic effect on carbohydrate, protein, and fat metabolism (Huang et al., 2014), metformin has recently attracted much attention because of its beneficial effects on the central nervous system (Moreira, 2014, Huang et al., 2014). Metformin can cross the blood-brain barrier and have specific pharmacological effects on the central nervous system (Łabuzek et al., 2010). It decreases inflammation in the brain, protects against apoptotic cell death of neurons, stimulates neurogenesis, and alters neurotransmitter levels in cellular and animal models of neurodegeneration and epilepsy (Wang et al., 2012, Ma et al., 2007, El-Mir et al., 2008, Shivavedi et al., 2017). This part of the thesis evaluated the efficacy of metformin against hyperglycemia, inflammation, and depression markers in rats with diabetes comorbid depression.

3.2 Materials and methods

3.2.1 Materials and reagents

Some of the materials and reagents used in the following experimental protocol were obtained from the sources mentioned previously (see section 2.2.2). The sources and details of the remaining materials, equipment, and reagents are incorporated at designated places.

3.2.2 Induction of diabetes and comorbid depressive-like behavior

In overnight fasted rats, diabetes was induced by intraperitoneal injections of nicotinamide (SD Fine-Chemical Ltd., India) and streptozotocin (Sigma, India) as described previously (see section 2.2.3). The comorbid depressive-like behavior in diabetic rats was induced as described previously in this thesis (see section 2.2.4).

3.2.3 Experimental design

Rats were randomly allocated into seven experimental groups of six animals each (Table 2). Out of seven groups, four groups of rats were named as nondiabetic control, depressed control, diabetic control, and diabetic comorbid depressed control groups. The control animals received distilled water only at 1 mL/kg perorally (p.o.). The remaining three groups of diabetic comorbid depressed rats were named as metformin (5, 25, and 125 mg/kg) treatment groups.

Table 2: Experimental design of monotherapy of metformin against diabetes comorbid depression in rats

Group	Glycemic Status	Treatment (p.o.) (Day 1 to Day 11)	Comorbid Depression (Day 1, 5, 7, and 10)	N
Nondiabetic control	Nondiabetic	Distilled water (1 mL/kg)	No intermittent foot-shock	6
Depressed control	Nondiabetic	Distilled water (1 mL/kg)	Intermittent foot-shock	6
Diabetic control	Diabetic	Distilled water (1 mL/kg)	No intermittent foot-shock	6
DCD control	Diabetic	Distilled water (1 mL/kg)	Intermittent foot-shock	6
DCD + Metformin	Diabetic	Metformin (5 mg/kg)	Intermittent foot-shock	6
		Metformin (25 mg/kg)		6
		Metformin (125 mg/kg)		6

DCD: Diabetes comorbid depression; N: Number of rats in a group

Metformin (Sigma Aldrich, USA) was dissolved in distilled water for oral administration. In this experiment, the effect of eleven repeated doses of metformin (5, 25, and 125 mg/kg/day/p.o.) was examined in diabetic comorbid depressed rats. On 1st, 5th, 7th, and 10th experimental day, the animals were subjected to intermittent foot-shocks to produce depressive-like symptoms in diabetic rats.

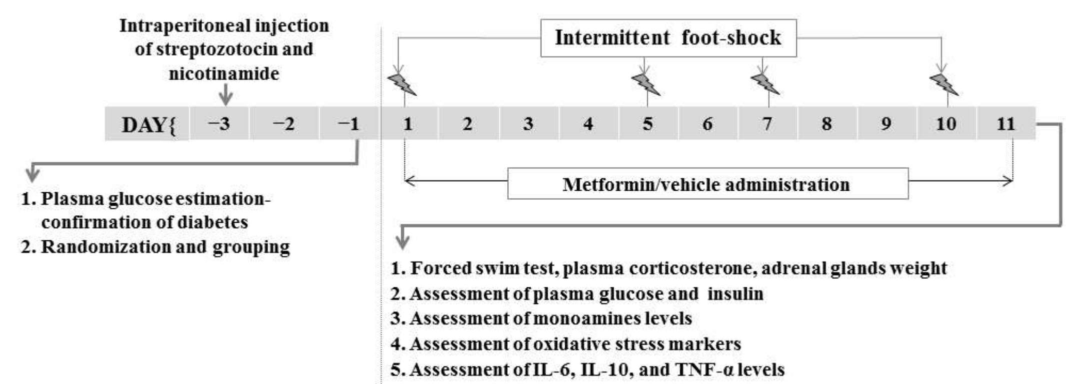

Figure 3.1: Schematic representation of experimental protocol.

Metformin was administered 1 h before initiation of intermittent foot-shock stress. After completion of experiment, blood and brain samples were collected to estimate plasma glucose, insulin, and corticosterone levels, and prefrontal brain concentrations of monoamines (NE and 5-HT) and inflammatory cytokines (IL-6, IL-10, and TNF-α). The timeline of the tasks executed during the experimentation has been represented in Figure 3.1.

3.2.4 Forced swim test

On the 11th day, the immobility period as a marker of depression-like behavior was estimated as described previously (see section 2.2.6).

3.2.5 Blood and brain sample collection

After performing forced swim test, blood samples and prefrontal cortex of all animals were collected and processed as described previously (see section 2.2.7).

3.2.6 Estimation of plasma glucose, insulin, and corticosterone levels

Fasting plasma glucose level (mg/dL) was estimated using biochemical enzyme (glucose oxidase-peroxidase) test kit (Accurex Biomedical Pvt. Ltd., India) at 505 nm wavelength. Plasma insulin level was estimated using Enzyme-Linked Immunosorbent Assay (ELISA) test kit (Mercel Expert Assays, USA) and corticosterone ELISA kit (KinesisDx, USA). All estimations were performed using microplate reader (iMark™- Bio-Rad Laboratories, California, USA) according to instructions guidebook of respective enzyme test kits.

3.2.7 Estimation of monoamines and inflammatory cytokines

The levels of monoamines, i.e. norepinephrine and serotonin were measured using rat Elisa kits according to the manufacturer's guidelines (Labor Diagnostika Nord GmbH & Co, KG, Nordhorn, Germany). The proinflammatory cytokines such as TNF-α and IL-6 (Krishgen Biosystem, USA) and the anti-inflammatory cytokines such as IL-10 (Krishgen Biosystem, USA) in the prefrontal cortex were measured using specific ELISA kits.

3.2.8 Estimation of oxidative stress markers

We estimated the markers of oxidative stress such as lipid peroxidation, SOD content, and catalase activity in 10% prefrontal cortex homogenate using methods described previously (see section 2.2.10).

3.2.9 Statistical analysis

Results were reported as mean ± standard error of mean (SEM). Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. GraphPad Prism version 7.03 software (GraphPad Software, Inc. CA, USA) was used for statistical analysis. Statistical significance was determined at $P < 0.05$.

3.3 Results

3.3.1 Metformin against comorbid depression

We examined the antidepressive-like activity of metformin at 5, 25, and 125 mg/kg dose in the DCD rats, summarized in Figure 3.2.

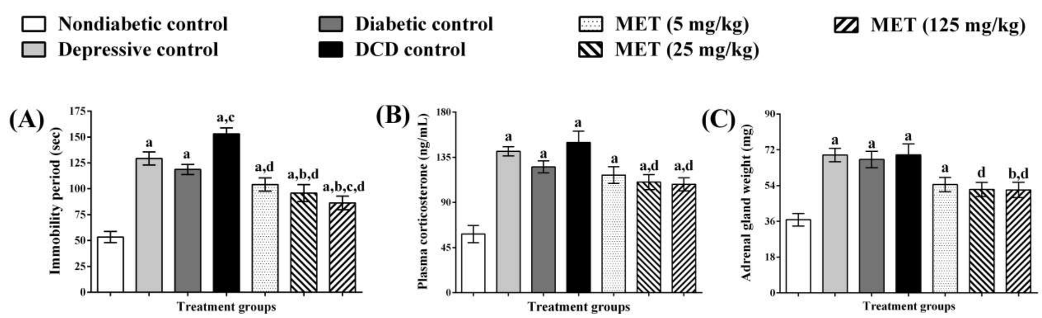

Figure 3.2: Metformin against comorbid depression

Effects of metformin on immobility period, plasma corticosterone, and adrenal gland weights in diabetes comorbid depressed (DCD) rats. $^{a}P < 0.05$ versus nondiabetic control group, $^{b}P < 0.05$ versus depressed control group, $^{c}P < 0.05$ versus diabetic control group and $^{d}P < 0.05$ versus DCD control group.

Immobility periods of depressed control, diabetic control, and DCD control rats were significantly higher ($P < 0.05$) as compared to the nondiabetic control rats. As compared to diabetic control rats the immobility period was higher ($P > 0.05$) in depressed control rats. Furthermore, it is apparent from the results that metformin (5, 25, and 125 mg/kg) showed statistically significant ($P < 0.05$) reductions in immobility period as compared to depressed control and DCD control rats (Figure 3.2A). Moreover, the plasma corticosterone levels were significantly higher ($P < 0.05$) in depressed control, diabetic control, and DCD control rats compared with nondiabetic control rats. In depressed control rats, the plasma corticosterone level was higher ($P > 0.05$) as compared to diabetic control rats. Pretreatment with middle (25 mg/kg) and high dose (125 mg/kg) of metformin caused significant (P < 0.05) decrease in plasma corticosterone levels as compared to DCD control rats (Figure 3.2B). In addition to corticosterone levels, depressed control, diabetic control, and DCD control rats showed significantly (P > 0.05) higher adrenal gland weights as compared to nondiabetic control rats. Pretreatment with middle (25 mg/kg) and high dose (125 mg/kg) of metformin caused significant (P < 0.05) reduction in adrenal gland weights as compared to DCD control rats (Figure 3.2C).

3.3.2 Metformin against hyperglycemia and hypoinsulinemia

Results of metformin (5, 25, and 125 mg/kg) treatment against hyperglycemia and hypoinsulinemia are summarized in Figure 3.3.

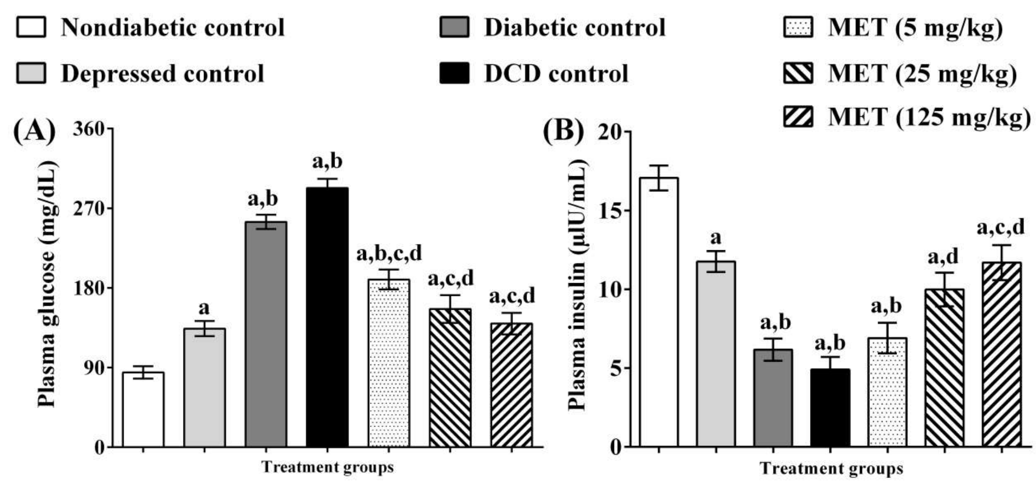

Figure 3.3: Metformin against hyperglycemia and hypoinsulinemia

Effects of metformin on plasma glucose (A) and insulin levels (B) in diabetes comorbid depressed (DCD) rats. Data represent mean ± SEM, n = 6. ^{a}P < 0.05 versus nondiabetic control group, ^{b}P < 0.05 versus depressed control group, ^{c}P < 0.05 versus diabetic control group, and ^{d}P < 0.05 versus DCD control group.

As compared to the nondiabetic control rats, significant (P < 0.05) increase in plasma glucose levels was observed in depressed control, diabetic control, and DCD control rats. In diabetic control rats, the levels of plasma glucose were significantly (P < 0.05) higher as compared to depressed control rats. Pretreatment with metformin at 5, 25, and 125 mg/kg dose caused a significant (P < 0.05) reduction in plasma glucose levels as compared to depressed control, diabetic control, and DCD control rats (Figure 3.3A). On the other hand, plasma insulin levels were found to be reduced significantly (P < 0.05) in depressed control, diabetic control, and DCD control rats as compared to nondiabetic control rats. Moreover, diabetic control rats showed lower (P < 0.05) levels of plasma corticosterone as compared to depressed control rats. Interestingly, middle (25 mg/kg) and high dose (125 mg/kg) of metformin caused significant (P < 0.05) increase in plasma insulin levels as compared to diabetes control and DCD control rats (Figure 3.3B).

3.3.3 Metformin against reduction in monoamine levels

The effects of metformin pretreatment on monoamines (serotonin and norepinephrine) levels in DCD rats are summarised in Figure 3.4.

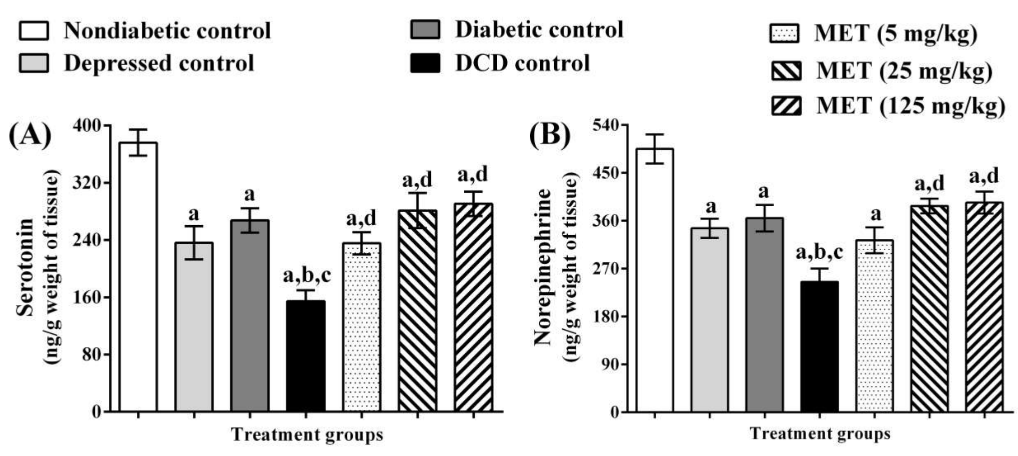

Figure 3.4: Metformin against reduction in monoamine levels

Effects of metformin on norepinephrine (A) and serotonin (B) in diabetes comorbid depressed (DCD) rats. Data represent mean ± SEM, n = 6. [a]P < 0.05 versus nondiabetic control group, [b]P < 0.05 versus depressed control group, $^{c}P < 0.05$ versus diabetic control group, and $^{d}P < 0.05$ versus DCD control group.

Due to intermittent foot-shocks in depressed control rats, streptozotocin-nicotinamide induced hyperglycemia in diabetes control rats, and both the situations in DCD control rats, the levels of serotonin and norepinephrine levels were significantly ($P < 0.05$) decreased as compared to nondiabetic control rats. Pretreatment with metformin at dose 5, 25, or 125 mg/kg in DCD rats showed significant increase ($P < 0.05$) in serotonin levels as compared to DCD control rats (Figure 3.4A). The middle (25 mg/kg) and high dose (125 mg/kg) of metformin caused significant ($P > 0.05$) increase in levels of norepinephrine as compared to DCD control rats (Figure 3.4B).

3.3.4 Metformin against inflammatory cytokines in prefrontal cortex

The levels of anti-inflammatory (IL-10) and pro-inflammatory (IL-6 and TNF-α) cytokines were estimated as a marker of inflammation in DCD rats, results depicted in Figure 3.5.

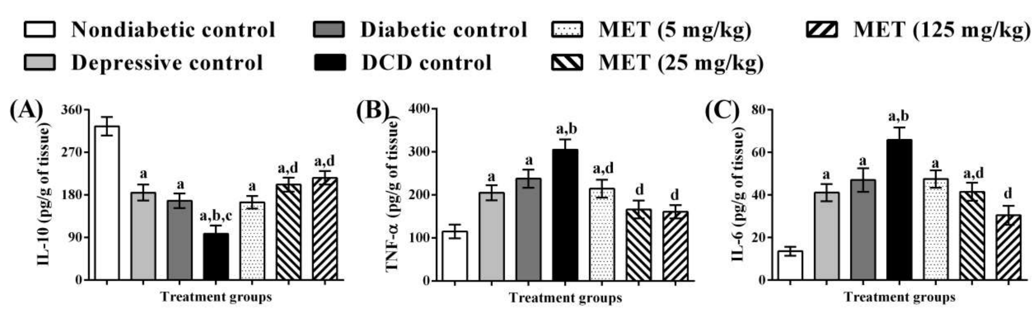

Figure 3.5: Metformin against inflammatory cytokines in prefrontal cortex

Effects of metformin on proinflammatory cytokines TNF-α levels (A), IL-6 levels (B), and anti-inflammatory cytokines IL-10 levels (C) in diabetes comorbid depressed (DCD) rats. Data represent mean ± SEM, n = 6. $^{a}P < 0.05$ versus nondiabetic control group, $^{b}P < 0.05$ versus depressed control group, $^{c}P < 0.05$ versus diabetic control group, and $^{d}P < 0.05$ versus DCD control group.

As compared to nondiabetic control rats, the anti-inflammatory cytokine (IL-10) levels were significantly ($P < 0.05$) decreased and the levels pro-inflammatory cytokines (TNF-α and IL-6) were significantly ($P < 0.05$) increased in depressed control, diabetes control, and DCD control rats. Diabetes control rats showed lower ($P > 0.05$) levels of IL-10, and higher ($P > 0.05$) levels of IL-6 and TNF-α compared with depressed control rats. The middle (25 mg/kg) and high dose (125 mg/kg) of metformin caused significant ($P < 0.05$) increase in the levels of anti-inflammatory cytokines (IL-10) as compared to DCD control rats (Figure 3.5A). Moreover, metformin (5, 25, and 125 mg/kg) treated DCD rats showed statistically significant ($P < 0.05$) decrease in levels of TNF-α as compared to the DCD control rats. (Figure 3.5B). Pretreatment with middle (25 mg/kg) and high dose (125 mg/kg) of metformin caused significant ($P < 0.05$) decrease in levels of IL-6 as compared to the DCD control rats (Figure 3.5C).

3.3.5 Metformin against oxidative stress markers in prefrontal cortex

The anti-oxidative enzymes i.e. superoxide dismutase content, catalase activity, and lipid peroxidation were estimated as marker of oxidative stress in DCD rats, results depicted in Figure 3.6.

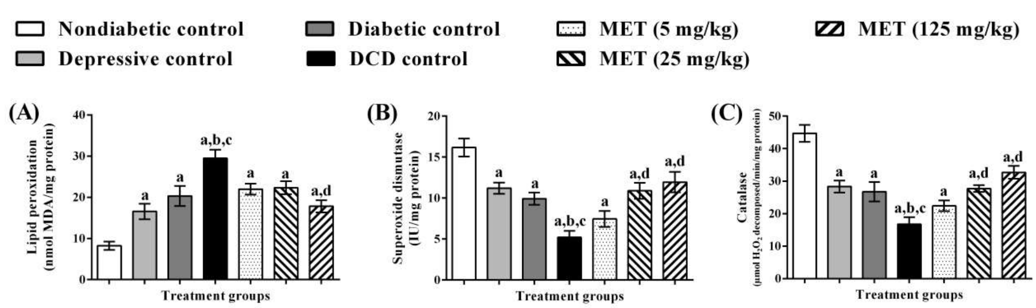

Figure 3.6: Metformin against oxidative stress markers in prefrontal cortex

Effects of low dose of metformin on superoxide dismutase (IU/mg protein) (A), catalase (μmol H_2O_2 decomposed/min/mg protein) (B), and lipid peroxidation (nmol MDA/mg protein) (C) in diabetes comorbid depressed (DCD) rats. Data represent mean ± SEM, n = 6. $^aP < 0.05$ versus nondiabetic control group, $^bP < 0.05$ versus depressed control group, $^cP < 0.05$ versus diabetic control group, and $^dP < 0.05$ versus DCD control group.

As compared to nondiabetic control rats, the superoxide dismutase content and catalase activity were significantly (P < 0.05) decreased, and lipid peroxidation was significantly (P < 0.05) increased in depressed control, diabetes control, and DCD control rats. Similarly, diabetic control rats showed higher (P > 0.05) levels of lipid peroxidation and lower (P > 0.05) levels of superoxide dismutase content and catalase activity compared with depressed control rats. Pretreatment with middle (25 mg/kg) and high dose (125 mg/kg) of metformin caused significant (P < 0.05) increase in the levels of superoxide dismutase content (Figure 3.6A) and catalase activity (Figure 3.6B), and significant (P < 0.05) decrease in the levels of lipid peroxidation at high dose (125 mg/kg) of metformin compared to DCD control rats (Figure 3.6C).

3.4 Discussion

The present data demonstrates that metformin has a protective role against diabetes comorbid depression in rats. Metformin is a widely used antihyperglycemic agent. Besides its control over glucose metabolism, metformin has also been reported to enhance spatial memory, promotes neurogenesis, and protects neuronal cell from oxidative damage (Pintana et al., 2012, Correia et al., 2008). In this part of the thesis, the protective effect of metformin against diabetes comorbid depression and its possible ameliorating effect on inflammation and monoamine levels in diabetic comorbid depressed rats were evaluated.

Behavioral despair swim test also known as forced swim test is a reliable screening test for antidepressant action of drugs (Petit-Demouliere et al., 2005).

Duration of immobility during the test indicates behavioral despair in rats which reflects depressive-like behavior in rats (Petit-Demouliere et al., 2005). It is evident in the present study that DCD rats showed longer duration of immobility whereas, DCD rats treated with metformin showed significant shorter duration of immobility which indicates antidepressive-like activity. The possible reasons for antidepressive-like activity of metformin are evidenced through decrease in corticosterone, reduction in inflammation, and increase in monoamine levels in the prefrontal cortex of metformin treated DCD rats. In the present study, chronic hyperglycemia and intermittent foot-shock represent two independent stressors. Involvement of these two independent stressors activates hypothalamic-pituitary-adrenal (HPA)-axis which inturn increses the release of corticosterone from adrenal gland (Krugers and Hoogenraad, 2009). It is evident in the present study that DCD rats showed higher corticosterone levels whereas, DCD rats treated with metformin have lower corticosterone levels. It has been reported that long term exposure of corticosterone induces depressive-like behaviors in experimental animals (van Donkelaar et al., 2014). Hence, it can be assumed that antidepressive-like activity of metformin may be related to its plasma glucose and corticosterone lowering effect. Long-term exposure of corticosterone also induces adrenal hyperplasia and corticosterone steroidogenesis leads to increase in adrenal gland weight (Ulrich-Lai et al., 2006). In the present study, DCD rats showed higher adrenal gland weight whereas metformin treated rats showed lesser gland weight due to its corticosterone lowering effect. It has also been reported that long-term exposure of stress and corticosterone decreases the monoamine levels in prefrontal cortex (Luine et al., 1993, Ahmad et al., 2010). Decreased levels of monoamines in prefrontal cortex has been associated with the development of depression (Krishnan and Nestler, 2008). In the present study, metformin treated DCD rats have higher levels of monoamines than DCD rats. Metformin has also been reported to promote neurogenesis and protects neuronal cell from oxidative damage in type 2 diabetes (Correia et al., 2008). These findings suggests that the antidepressive-like activity of metformin possibly due to its inhibition on chronic hyperglycemia induced corticosterone levels and its protective action against neuronal cell damage from oxidative damage.

Chronic hyperglycemia induced oxidative stress plays a major role in increased expression of proinflammatory mediators like TNF-α and IL-6 (Nguyen et al., 2012, Esposito et al., 2002). It has been reported that increase in inflammatory mediators will result in development of depression (Miller and Raison, 2016, Dowlati et al., 2010). Decrease in anti-inflammatory mediators like IL-10 has also been implicated in the development of depression (Dhabhar et al., 2009). In the present study, metformin treated rats showed decreased TNF-α and IL-6 and increased IL-10 levels suggesting that the antidepressive-like activity of metformin is related to its activity against inflammatory mediators.

3.5 Conclusions

Metformin showed potent activity against hyperglycemia and reduction in monoamine levels. A submaximal activity was observed against depressive-like behavior and oxidative stress. Considering oxidative stress as the major contributor to diabetes comorbid depression, the combination with other antioxidant (ascorbic acid) will be more beneficial in treating diabetes comorbid depression. The middle dose (25 mg/kg) of metformin was considered for the combination therapy.