**Synthesis of Zinc-flavonol complex**

The Zinc-morin complex (C_{30}H_{20}O_{14}Zn) obtained was brown in colour and the yield was 96% (Scheme1). The spectral data of Zinc-morin complex are presented as obtained. (Figure 6) IR (KBr, ν cm⁻¹) 3327, 1678, (Figure 7) EI Mass (m/z): 667.20, (Figure 8) \(^1\)H NMR (DMSO, 500 MHz): δ 8.71 (d, J=7.5 Hz, 2H), 8.23 (d, J=8.0 Hz, 1H), 7.42-7.87(m, 6H), (Figure 9) \(^13\)C NMR (DMSO, 125.75 MHz), δ 178.72, 154.38, 149.03, 133.81, 133.48, 128.96, 128.82, 127.13, 125.21, 124.63, 119.21, 119.00.

**FTIR - spectroscopy**

The α-hydroxy carbonyl group of flavonol was reported as the preferential site for the binding of the metal ions (AL-Kindy et al., 2011). The peaks observed at 1678 and 3327 (ν cm⁻¹) correspond to the presence of carbonyl and hydroxyl groups in the ligand respectively. The non existence of the peak at 1678 (ν cm⁻¹) in the spectrum of Zinc-flavonol complex indicates the co-ordination of the metal to C (4)=O residue of flavonol. Likewise, the deprotonation of the –OH group in C (3) of the flavonol during the complexation resulted in the non-existence of the corresponding vibration in the spectra obtained for Zinc-flavonol complex.

**Mass spectra**

The molecular mass determined for the zinc-morin complex by mass spectra was found to be 667.20 g/mol. The mass of the complex implies
that the stoichiometry of the complex involves the binding of one zinc ion and two ligands. Thus, the mass spectra studies suggest that the molar fraction of zinc and morin in the complex is in the ratio of 1:2. Similar observations have also been reported earlier (Hernandez Hernandez et al., 1986; Cornard et al., 1995; Zhang and Mei, 2011).

Proton NMR studies

The Proton NMR studies carried out on a Bruker AM500 instrument using DMSO as solvent showed nine proton peaks for the zinc-flavonol complex as against the ten proton peaks obtained for the flavonol alone, indicating the deprotonation of the α-hydroxy moiety bound to C(3) of the flavonol during the complex formation. Thus, the proton NMR studies provide additional information that the complexation involves the binding of zinc ion with α-hydroxyl keto group of the flavonol.

13C NMR studies

The 13C NMR of the metallo-complex was recorded in Bruker AM500 NMR spectrophotometer at 125.75 MHz, using DMSO as the solvent. The peak at δ 178.72 indicates the involvement of the carbonyl group in the complex formation.

pH–Potentiometric Titrations and Job’s plot measurement

To further understand the formation of the complex formed by zinc ion and Morin molecule, pH titration experiments were performed (Figure10)
following the previously reported method (Choi et al., 2011; Braymer et al., 2011). The stability constants of the formed zinc-Morin complex was determined are listed below in Eqns. (a) – (d). Potentiometric titrations of Zn-Morin were consistent with the pattern of equilibrium described previously for Vanadium-3-hydroxy flavone (Pillai et al., 2013). The analysis of the results was consistent with the following equilibria (Eqs. a, b, c, d):

\[
\begin{align*}
H^+ + M^- & \rightleftharpoons HM & \log K_\alpha = 9.21 \\
\text{Zn}^{2+} + M^- & \rightleftharpoons \text{Zn}(M)^+ & \log K_1 = 9.58 \\
\text{Zn}(M)^+ + M^- & \rightleftharpoons \text{Zn}(M)_2 & \log K_2 = 8.32 \\
\text{Zn}^{2+} + 2(M) & \rightleftharpoons \text{Zn}(M)_2 & \log \beta = 17.80
\end{align*}
\]

To analyze the complexation ratio between ligand and the Zn\(^{2+}\) ion, the Job’s plot measurement was carried out by altering the concentration of both ligand and the Zn\(^{2+}\) ion (Figure11). The maximum point appears at the mole fraction of 0.65, close to the typical ligand mole fraction (0.66) for a 2:1 ligand to- metal complex (Jung et al., 2009).

The prevalence of type 2 diabetes has been increasing rapidly over the last decade and is now considered as a global epidemic metabolic disorder. Insulin resistance is a major risk factor for the onset and progression of type 2 diabetes. During last decades, several hypotheses have been proposed to explain the pathogenesis of T2DM, and much attention has been given to the lipid toxicity and chronic low grade inflammation as major causes on insulin resistance (DeFronzo, 2010). A number of strategies have
been proposed to improve insulin sensitivity, because early treatment and prevention play a pivotal role in reducing the population burden of diabetes. Lifestyle changes such as exercise, diet management and weight control are often recommended, but have been difficult to maintain over a long term.

Although several drugs are available for the treatment of diabetes, adverse effects and drug resistance are of great concern. As an alternative, a greater number of people are seeking novel interventions to treat diabetes. Recently, Zn complexes have been attracting attention due to their potential antidiabetic activity. Because, zinc is known to be as one of the important essential trace elements involved in the physiology of insulin and found in many metalloproteins and metalloenzymes, the insulinomimetic effect of the zinc(II), has attracted keen interest among researchers in this field (Jurowski et al., 2014).

Zinc is primarily absorbed in the small intestine. However, it is known that binding form and other dietary ingredients can influence the bioavailability of zinc. However, there are differences in bioavailability between various zinc sources. Most of them are quite low, for example: the absolute bioavailability for zinc oxide is 22%, for zinc sulphate 23% and for zinc acetate 19% (Goswami et al., 2005). In contrast to inorganic Zn, the bioavailability of organic Zn sources is high. However, bioavailability of organically bound Zn seems to be influenced by the type of complex being used. Several types of zinc(II) complexes have been synthesized for the purpose of improving absorption from the gastrointestinal system or reducing
the toxicity of zinc(II) compounds, and these new complexes have been shown to have significant insulinomimetic and blood glucose-lowering activities. Hence, in the present study we have designed, synthesized and characterized a zinc complex using bioorganic ligand, morin and studied its antidiabetic properties in high fat diet – low dose STZ-induced experimental type 2 diabetes in rats.

**Dosage fixation studies**

Figure 12 shows the dose-dependent hypoglycemic activity of zinc-morin complex in HFD-STZ diabetic rats. Graded doses of zinc-morin complex (5, 10, 20 and 50mg/kg per day) were administered to diabetic groups of rats for 30 days and the fasting blood glucose levels were monitored periodically. However, there was no significant variation in the hypoglycemic effects of all the doses. Hence, 5mg/kg/rat/day zinc-morin complex was fixed as the dosage for further studies to evaluate the antidiabetic potential in HFD-STZ diabetic rats.

The changes in body weight in control and experimental groups of rats are shown in Figure 13. Diabetic group of rats showed significant reduction in body weight when compared with control group of rats. There was a significant improvement in body weight in diabetic rats after oral administration of zinc-morin complex as well as metformin for 30 days. Induction of diabetes with HFD-STZ is associated with the characteristic loss of body weight, which is due to increased muscle wasting and catabolism of
tissue proteins, which was also observed in the present study (Swanston-Flat et al., 1990). A significant increase in the body weight observed in diabetic rats treated with zinc-morin complex as well as metformin indicates the maintenance of glucose homeostasis by the complex and controlling muscle wasting. However, there was no significant difference between control and control rats treated with zinc-morin complex.

Table 5 shows the levels of blood glucose, glycosylated hemoglobin, plasma insulin, and urine sugar in control and experimental groups of rats. In diabetes, fasting hyperglycemia is primarily due to the excessive release of glucose from liver by gluconeogenesis and glycogenolysis. Liver plays a vital role in the maintenance of normal blood glucose levels and is the primary organ responsible for endogenous glucose production. In fasting condition, liver produces glucose whereas in postprandial state it stores excess glucose in the form of glycogen (Sheehan, 2004). Insulin controls hepatic glucose production by regulating the key enzymes involved in gluconeogenesis and glycogen metabolism (Barthel and Schmoll, 2003). Dysregulation of carbohydrate metabolic enzymes in diabetes due to insulin deficiency or its resistance result in increase of the hepatic glucose production and decreases the storage of glucose as glycogen (Edgerton et al., 2002; Girard, 2006; Roden, 2008). The increased levels of glucose, glycosylated hemoglobin and decreased plasma insulin level in diabetic rats were restored to near normal levels in zinc-morin complex as
well as metformin treated diabetic rats. Urine sugar present in diabetic rats was absent in zinc-flavonol complex treated diabetic rats (Ilouz et al., 2002).

Under physiological conditions, HbA1c is formed by non-enzymatic, irreversible covalent bonding of glucose with hemoglobin in the circulation. In diabetes, the level of glycosylated hemoglobin is elevated because of increased glycation of hemoglobin due to persistent hyperglycemia. HbA1c level strongly correlates with the level of ambient glycemia during a 2 to 3 month period and is a more accurate and reliable measure than fasting blood glucose level. HbA1c levels are also used to predict the diabetic risk of individuals. The observed increase in the level of glycosylated hemoglobin in diabetic rats was significantly decreased upon treatment with zinc-morin complex. This is due to improved glucose homeostasis which is evident from FBG and plasma insulin thereby reducing the intensity of glycation of hemoglobin in diabetic rats (Luijf et al., 2011; Zhuang et al., 2012).

Figure 14 shows the blood glucose levels of control and experimental groups of rats after an oral glucose load. The oral glucose tolerance test (OGTT) is a measure of effective glucose utilization by the system and generally aids in the early diagnosis of diabetes (Alberti and Zimmet, 1998). Impaired glucose tolerance (IGT) due to pancreatic dysfunction results in the defective utilization of glucose by the tissues and increased hepatic gluconeogenesis. Diabetic rats showed significantly elevated fasting blood glucose levels compared with control rats. There is no
significant difference between control and drug control rats. After the oral glucose load, blood glucose levels peaked at 60 min in diabetic rats and did not return to basal levels over the next 60 min. Treatment of HFD-STZ diabetic rats with zinc-morin complex as well as metformin resulted in a significant decrease in blood glucose concentrations (at 0 (fasting), 30 and 60 min) when compared with untreated HFD-STZ diabetic rats. In addition, blood glucose levels returned to basal levels 120 min after the oral glucose load in zinc-morin complex as well as metformin treated HFD-STZ diabetic rats. The result of the OGTT exemplifies the positive impact of zinc-morin complex in glucose homeostasis (Sendrayaperumal et al., 2014).

Many studies have reported that the rats fed with highfat diet (HFD) develop insulin resistance but not frank hyperglycemia or diabetes (Srinivasan et al., 2005; Zhang et al., 2009). To investigate the effect of zinc-morin complex on insulin sensitivity, insulin (0.75 IU/kg) was injected intraperitoneally to the control as well as experimental rats and blood was collected at different time intervals 0, 30, 60, 90 and 120 min for the measurement of glucose concentration figure 15. The glucose concentration in diabetic rats was not reduced even after 120 min. However, in control rats as well as control rats treated with zinc-morin complex, glucose concentrations declined rapidly at 60 min and the decline was slower over the next 60 min. Similarly, the glucose concentrations were rapidly declined up to 60 min in diabetic rats treated with zinc-morin complex as well as metformin and the decline continued slowly over the next 60 min. Zinc-morin complex might
improve glucose response and insulin resistance associated with type 2 diabetes by alleviating metabolic dysregulation of free fatty acids, suppressing oxidative stress, up-regulating glucose uptake at peripheral tissues, and/or down-regulating inflammatory gene expression in liver.

Figure 16 shows HOMA IR of control and experimental group of rats. HOMA IR was higher in diabetic rats indicating the state of insulin resistance. However, there was no significant difference in the HOMA IR value of control rats and control rats treated with zinc-morin complex. Oral administration of zinc-morin complex as well as metformin treatment improves insulin sensitivity, which is evident from the results of fasting blood glucose, plasma insulin and HOMA-IR. Zinc-morin complex administration augments insulin stimulated glucose uptake into peripheral tissues.

Table 6 depicts the plasma protein, blood urea and serum creatinine levels in control and experimental groups of rats. Hyperglycemia induces protein catabolism and elevation in the serum levels of urea and creatinine which are considered as significant markers of renal dysfunction. The antidiabetic property of zinc-morin complex may account for the observed increase in the levels of plasma proteins as well as improved body weight in diabetic rats treated with the complex indicate the improved nitrogen balance. Urea is the main end product of protein catabolism in the body.

During the diabetic conditions, the prominent deprivation of both hepatic as well as plasma proteins leads to the excessive accumulation of urea
in the systemic circulation than its excretion. In diabetes, due to the elevated concentration of glucose, damages occur in tissues like kidney causing impairment in renal function resulting in the accumulation of nitrogenous wastes in circulation (Almdal and Vilstrup, 1988). This in turn elevates urea and creatinine levels in blood which acts as a biochemical diagnostic markers for assessing renal function. The administration of zinc-morin complex and metformin to diabetic rats normalized the blood urea and creatinine levels indicating the recovered renal function, which is due to improved glycemic control. The increased levels of urea and creatinine and reduced levels of plasma protein in diabetic rats were significantly improved in zinc-morin complex treated diabetic rats. Whereas, control rats treated with zinc-morin complex showed no significant difference in plasma protein, blood urea and creatinine levels compared to control rats.

The activities of AST, ALT and ALP in the serum of control and experimental groups of rats are depicted in table 7. One of the most sensitive and dramatic indicators of hepatocyte injury is the release of intracellular enzymes, such as transaminases and serum alkaline phosphatase in the circulation. The enzyme ALP is located in the cytoplasm and will be released into circulation during cellular damage (Sallie et al., 1991). In addition, the soluble enzymes ALT and AST are released when injury involves organelles such as mitochondria (Kumar et al., 2003). Several researchers reported that the elevated activities of these enzymes were indicative of cellular damage and loss of the functional integrity of the cell membranes (Saraswat et al.,
The increased activities of hepatic marker enzymes AST, ALT and ALP in diabetic rats were significantly reduced upon treatment with zinc-morin complex as well as treated with metformin. There was no significant alteration in the activities of these enzymes in control rats treated with zinc-morin complex when compared to control rats.

The levels of plasma adipokines such as adiponectin, leptin and TNF-α are presented in table 8. Subjects with T2DM exhibited higher serum levels of pro-inflammatory cytokines such as TNF-α. In addition, leptin and adiponectin are remarkably effective in improving hyperglycemia in some models of T2DM (Coppari and Bjorbaek, 2012). Adiponectin activates fatty acid oxidation and improves insulin sensitivity and glucose uptake in skeletal muscle and liver FFAs. Plasma adiponectin levels were decreased, whereas the concentrations of leptin and inflammatory cytokines such as TNF-α were significantly increased in diabetic rats compared with control rats. The levels of these adipokines were significantly improved in diabetic rats treated with zinc-morin complex as well as metformin. Further, there was no significant difference in adipokines level in control rats treated with zinc-morin complex when compared with control rats.

Table 9 show the total cholesterol, triglycerides and free fatty acid concentrations in plasma of control and experimental diabetic rats. The levels of these lipid components were significantly increased in plasma of diabetic rats whereas diabetic rats treated with zinc-morin complex as well as metformin showed significantly decreased levels of these lipid contents.
However, there was no significant alteration in the plasma levels of total cholesterol, TG and free fatty acid concentrations in control rats treated with the complex compared to control rats.

Table 10 shows the levels of serum HDL, LDL and VLDL-cholesterol in control and experimental groups of rats. The levels of LDL and VLDL-cholesterol were significantly increased, whereas the HDL-cholesterol was markedly decreased in rats induced with fat-fed STZ induced diabetic rats when compared with control rats. The diabetic rats treated with zinc-morin and metformin for 30 days, the levels of LDL and VLDL-cholesterol were significantly reduced, whereas the HDL-cholesterol was significantly increased. When compared to control rats, there was no significant difference in the serum lipoprotein cholesterol levels in control rats treated with zinc-morin complex.

Adipose tissue is biologically active and undertakes a range of metabolic as well as endocrine functions. FFAs released from adipocytes provide the body's main source of fuel in the post-absorptive state. Plasma FFA levels reflect a balance between release from the intravascular lipolysis of triglyceride-rich lipoproteins and lipolysis of adipose tissue triglyceride stores and uptake of FFA predominantly re-esterified in adipose tissue and liver and oxidized in muscle, heart, liver, and other tissues. Although the majority of circulating FFAs arise from outside the intra-abdominal region, FFAs released from adipose tissues serve as a marker for insulin resistance (Jensen, 2006). Several studies also suggested that impaired blood lipids are
characteristic of type 2 diabetes with insulin resistance, especially circulating FFAs (Krauss, 2004; Guilherme et al., 2008; Karpe et al., 2011).

Increased free fatty acid (FFA) flux from adipose tissue to nonadipose tissues especially liver resulting from abnormalities of fat metabolism, participates in increased synthesis and secretion of VLDL. High plasma concentrations of triglyceride-rich lipoprotein, VLDL lead to an increase in the release of FFAs and generation of remnants as a result of lipolysis by LPL. The increased VLDL level down regulates LDL receptor (Madsen and Kahn, 2012) resulting in increased proportion of LDL cholesterol particles (Chatrath et al., 2012), decreased HDL cholesterol level and elevated peripheral TG concentrations (Kotronen et al., 2007). FFAs and remnants of triglyceride-rich lipoproteins contribute to increase the hepatocyte fatty acid pool, thereby setting up a vicious cycle and further driving VLDL production. In the present study, the increased levels of circulatory FFAs and triglycerides in diabetic rats were significantly reduced upon treatment with zinc-morin complex indicating the beneficial effects of the complex in preventing the secondary complications of diabetes.

Blood glucose levels are primarily maintained by the regulation of insulin secretion from the pancreatic β- cells. Zinc-morin complex improves glucose homeostasis in HFD-STZ induced diabetic rats, which is evident from OGTT and ITT, liver as well as muscle glycogen content and other basic biochemical parameters. The observed improvement in the glycemic status of the diabetic rats upon treatment with zinc-morin complex may be due to the
protection of β-cells from hyperglycemia induced oxidative stress or β-cell proliferation or increases the β-cell secretion. Hence, the effect of zinc-morin complex on β-cells and its role on hyperglycemia mediated oxidative damage of β-cells was studied in rat pancreas and RINm5F pancreatic β-cells.

The effect of zinc-morin on the viability of RINm5F pancreatic β-cells was determined by MTT colorimetric assay. This assay is based on the conversion of a yellow color MTT into insoluble, purple colored formazan crystals by mitochondrial reductases, which determines the mitochondrial activity. Since for most cell populations the total mitochondrial activity is related to the number of viable cells, this assay is widely used to measure the in vitro cytotoxic effect of drugs on cell lines. There was more than 90% viability up to 50 μM of zinc-morin complex (Figure 17) and no notable change was observed in insulin secretion from the RINm5F pancreatic beta cells treated with zinc-morin complex even at the maximum of 200 μM concentration (Figure 18).

Oxidative stress is a biological entity accountable for several pathological conditions including diabetes mellitus. Hyperglycemia induced insulin resistance in type 2 diabetes leads to chronic oxidative stress, resulting in oxidative damage to vital organs. The consequences of an oxidative environment are the development of insulin resistance, β-cell dysfunction, impaired glucose tolerance, and mitochondrial dysfunction, which leads to diabetes mellitus (Rains and Jain 2011). Type 2 diabetes mellitus is the most common form of diabetes, which is associated with insulin resistance. As a
result, there is an increased formation of anti glycation end products (AGEs) and lipid peroxidation products that exacerbate intracellular oxidative stress resulting in a loss of molecular integrity, disruption in cellular signaling and homeostasis, followed by inflammation and tissue injury. Having evolved in an oxygen environment, most cells, including pancreatic $\beta$-cells, have acquired complex mechanisms to defend ROS toxicity. However, the reduced antioxidant capacity potentially makes pancreatic $\beta$-cells sensitive to ROS mediated signal transduction and cellular response. Thus, the maintenance of $\beta$-cell function and their protection against oxidative stress mediated tissue damage might delay the onset of diabetes as well as the progression of its complications (Bierhaus et al. 1998; Basta et al. 2004).

Although tissues have their own antioxidant defense systems, the defense can be exogenously strengthened by supplementing with dietary antioxidants such as vitamin E, vitamin C, and beta-carotene. Several studies reported that treatment with antioxidants reduces the development of pathological complications aroused out of oxidative stress in diabetes (Sadi et al., 2008; Pazdro and Burgess, 2010). Zinc plays a relevant role in antioxidant defense in patients with type 2 diabetes mellitus. This mineral may act by different protection mechanisms by notably being an essential cofactor for more than 300 enzymes, such as superoxide dismutase. This mineral also facilitates reduction and neutralization of free radicals (Cruz et al., 2013). The free radical scavenging potential of natural products can be assessed by several assays. Among them, enzymatic and non-enzymatic antioxidants
assays are routinely practiced for the assessment of antioxidant properties of different zinc complexes, as they are easy, affordable and reliable.

Oxidative stress may also play an important role in cellular injury from hyperglycemia. High glucose levels can stimulate free radical production and reactive oxygen species formation. However, in diabetes due to prolonged exposure of pancreatic β-cells to supraphysiological concentrations of glucose, the production of ROS remains higher, which directly contribute to the increase of oxidative stress (Robertson, 2004; Robertson and Harmon, 2006). Persistent accumulation of surplus ROS aggravates the development of oxidative stress in the pancreatic β-cells (Harmon et al., 1999; Olofsson et al., 2007; Robertson et al., 2007). Pancreatic β-cells are more vulnerable to oxidative stress because these cells have the lowest levels of intrinsic antioxidant defenses compared with other metabolic tissues such as liver, kidney, skeletal muscles, and adipose tissues (Lenzen, 2008). Pancreatic β-cells contain relatively feeble activities of major antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (Grankvist et al., 1981). The low levels of SOD, catalase, and GPx are responsible for the astound sensitivity of pancreatic β-cells towards oxidative stress (Lenzen et al., 1996). Chronic oxidative stress in β-cells, leads to defective insulin synthesis, insulin secretion and induction of early cell death (Poitout and Robertson, 2008).

Table 11, 12 depicts the activities of enzymatic and non-enzymatic antioxidants such as SOD, Catalase and GPx and Vitamin C, Vitamin E and
GSH in control and experimental groups of rats. The activities were significantly diminished in the diabetic group of rats. Oral treatment of zinc-morin complex as well as metformin attenuated the altered activities of these enzymatic and non-enzymatic antioxidants to near normalcy in diabetic rats. Control rats treated with zinc-morin complex did not show any significant difference in the levels of activity of these enzymes when compared to the control rats.

The levels of lipid peroxides and hydroperoxides, the levels of activities of antioxidant enzymes in pancreatic tissues were shown in Table 13 and Table 14 respectively. The decreased activities of antioxidant enzymes, SOD, catalase, GPx and GST level, and increased oxidative stress marker in the pancreatic tissues of diabetic group of rats were significantly altered after oral administration of zinc-morin complex. The increased levels of non-enzymatic antioxidant, lipid peroxides and hydroperoxides in pancreas of diabetic rats (Table 13) were also significantly altered upon oral administration of zinc-morin complex (Sendrayaperumal and Subramanian, 2014). Further, zinc-morin complex protects the RINm5F pancreatic β-cells which were chronically exposed to highglucose concentration (Figure 19). These findings indicate the promising role of zinc-morin complex in maintaining the antioxidant status of pancreatic β-cells of the experimental diabetic rats and this might be due to abolition of ROS by its antioxidant potential.
Chronic inflammation is strongly associated with type 2 diabetes and insulin resistance (Uysal et al. 1997). Inflammation causes insulin resistance via inhibiting the signaling downstream of insulin receptor. Hyperglycemia mediated oxidative stress leads to the superactivation of stress-sensitive signaling pathways including NF-kB. The deterioration of pancreatic β-cells due to chronic oxidative stress results in the activation of transcription factor, NF-kB and release of the proinflammatory cytokines. Studies have suggested the role for pro-inflammatory cytokines in regulating insulin sensitivity. It has been reported that individuals with T2DM exhibited higher levels of pro-inflammatory cytokines such as TNF-α, IL-1 β, and IL-6 (Pickup and Crook 1998).

IL-1 β is an important cytokine mediator of pancreatic islet injury and is secreted by activated macrophages and neutrophils (Barshes et al. 2005). IL-1 β causes a decrease in glucose stimulated insulin biosynthesis and secretion and in high doses, apoptosis of the pancreatic islets. IL-1β also leads to down-regulation of GLUT2 and glucokinase mRNA expression, enzymes that transport and phosphorylate glucose in the pancreatic islet, respectively (Cnop et al. 2005). IL-1 β lead to cell membrane damage, DNA strand breaks, although this event may be mediated by NO (Kaneto et al. 1995). The levels of inflammatory markers in pancreatic tissues of control and experimental groups of rats were shown in table 15. Oral administration of zinc-morin complex as well as metformin to HFD-STZ induced diabetic rats decreases the levels of NF-kB, IL-1 β, and NO indicating the anti-inflammatory activity
of zinc-morin complex (Sendrayaperumal and Subramanian, 2014). This suggests that zinc-morin complex protects the β-cell integrity by averting inflammatory responses mediated by chronic oxidative stress and this might be abolition of ROS thereby inhibition of NF-κB and its target genes.

Plate 1 A-E represents the photomicrographs of hematoxylin eosin staining of pancreatic tissues of control and experimental groups of rats. Normal histological features of both exocrine and endocrine part were shown in the section of pancreatic tissue of control rats Plate 1A. Also the section of pancreatic tissue of control rats treated with zinc-morin complex showing normal islets Plate 1B. Plate 1C portrays the section pancreatic tissues of diabetic group of rats showing the degenerative changes of islets. It is characterized by reduction in the number and size of islets. Thereafter the central areas of most pancreatic islets are completely empty when compared to those of control groups. Plate 1D demonstrates the section of pancreatic tissues of diabetic group of rats treated with presenting with less marked degeneration of islets with significant number of granulated cells than that of diabetic group of rats. Moreover, several islets are well granulated when compared to the pancreatic islets of diabetic rats indicates the improved functioning of cells, which was reflected in the increased plasma insulin level. Likewise, the pancreatic tissues of diabetic rats treated with metformin shows similar pattern of islet structure with well granulated cells (Plate 1E).

The ultrastructural changes in the pancreatic β-cells of control and experimental groups of rats are shown in Plate 2A–E. Plate 2A represents the
electron micrograph of pancreatic β-cell of control group of rats showing normal cellular organelles such as mitochondria, endoplasmic reticulum, Golgi complex and large number of secretory granules containing insulin distributed in the cytoplasm. The electron micrograph of pancreatic β-cell of diabetic group of rats (Plate 2C) revealed the degeneration of β-cell with loss of nuclear envelope and vacuolization with ballooning appearance of mitochondria as well as dilation of the rough endoplasmic reticulum. A marked decrease in the number of secretory granules with insulin was observed in the cells of the diabetic group of rats. The electron micrograph (Plate 2D) apparently shows the pancreatic β-cell protective nature of zinc-morin complex in diabetic group of rats by means of moderate increase in secretory granules with insulin, organized nuclear structure, less swelling of mitochondria and no vacuolarization of cytoplasmic region of β-cells. The cells of normal rats treated with zinc-morin complex (Plate 2B) showed similar pattern of ultra structure of cells of normal control rats. Diabetic rats treated with metformin shows the normal insulin secreting cells (Plate 2E).

Plate 3A shows the islets with positive insulin-immunoreactivity representing the existence of normal insulin secreting cells in the pancreas of normal rats also normal rats treated with zinc-morin complex shows the normal insulin secreting cells (Plate 3B). Diabetic rats showed low amount of insulin positive cells (Plate 3C). Zinc-morin complex treated diabetic groups showed positive insulin-immune reactivity for the presence of insulin with regular brown insulin granules (Plate 3D). In addition, the section of
pancreatic tissue of diabetic rats treated with metformin showing normal insulin granules (Plate 3E).

The histological, ultrastructural and immunohistochemical observations made on the pancreatic tissues substantiate the claim that zinc complex protects the pancreatic β-cells from hyperglycemia mediated oxidative stress. The amelioration of histological as well as ultrastructural changes in the diabetic rats treated with the complex could be due to the antioxidant potential of the complex thereby protecting the pancreatic tissues. It has been reported that zinc may counteract the deleterious effects of oxidative stress, which contributes to reduce insulin resistance, and may also protect pancreatic β-cells from glucolipotoxicity (Tamaki and Fujitani 2014).

The improved antioxidant status, inflammatory responses in pancreatic tissues of experimental diabetic rats upon zinc-morin complex treatment is responsible for the improvement of pancreatic islets, insulin content in β-cells and RINm5F pancreatic beta cell line (Figure 20 and 21) and glucose stimulated insulin secretion (GSIS), which is reflected in the plasma insulin level and thus partly responsible for the improved glycemic status in diabetic rats. The findings of the study exemplifies that the pancreatic β-cell protective role of zinc-morin complex against glucotoxicity mediated oxidative damage might be due to the antioxidant potential of the complex.
Liver being the major site for fatty acid synthesis and for the storage and release of carbohydrates, has a crucial role in the control of whole body metabolism of energy nutrients. It is the primary site of insulin action, and is innately linked to the progression of systemic insulin resistance. Any alteration in the liver metabolism impairs the suppression of hepatic glucose production leading to the distortion of normal glucose homeostasis (Postic et al., 2004). Hepatic insulin resistance has been suggested to be the primary event leading to diabetes and the ensuing development of peripheral tissue insulin resistance (Perry et al., 2014). Further, cumulative deposition of the lipids within the liver impedes the insulin signaling and cause insulin resistance (Charbonneau and Marette, 2010). In addition, the contribution of oxidative stress to insulin resistance also contributes to the development of T2D (Henriksen, 2011).

Insulin regulates liver glucose, in part, by suppressing hepatic gluconeogenesis and glycogenolysis and facilitating hepatic glycogen synthesis. Hexokinase is an insulin-dependent enzyme and its activity in diabetic rats is almost entirely inhibited or inactivated due to insulin resistance (Suhail and Rizvi, 1989). This impairment results in the rate of glucose oxidation via glycolysis, which ultimately leads to hyperglycemia. The markedly decreased level of insulin observed in the high fat diet-STZ induced diabetic animals ultimately leads to the impairment in the activity of hexokinase. Table 16 represent the effect of zinc-morin complex supplementation on the carbohydrate metabolizing enzymes in the liver of
control and experimental group of rats. The activities of hexokinase, pyruvatekinase were significantly decreased whereas, the activities of lactate dehydrogenase were significantly increased in diabetic rats when compared to normal control rats. Oral administration of zinc-morin complex to high fat diet-STZ induced diabetic rats resulted in a significant reversal in the activity of hexokinase. The decreased fasting blood glucose levels observed in zinc-morin complex administered diabetic rats may be due to increased hexokinase activity in the hepatic tissue thereby increasing the oxidation of glucose leading to controlled glucose homeostasis.

Pyruvate kinase (PK) is a universally expressed enzyme that catalyses the conversion of phosphoenol pyruvate to pyruvate with the release of ATP. The reduction in PK levels in diabetic condition alters the glucose metabolism and ATP production, which might be promptly responsible for the reduced rate of glycolysis and augmented gluconeogenesis (Chaneton et al., 2012). Oral administration of zinc-morin complex to the diabetic rats showed increase in the PK activity. Lactate dehydrogenase (LDH) is a terminal glycolytic enzyme which facilitates the inter conversion of pyruvate to lactate to yield energy under anaerobic conditions (Denko, 2008). Elevated levels of LDH is observed in experimental diabetic animals which is attributed to impaired glucose-stimulated insulin secretion (Rajeswarareddy et al., 2012). Thus, the normal glucose metabolism and insulin secretion in the β-cells are disturbed with the increased activity of LDH. Upon treatment with zinc-morin complex to diabetic rats showed a significant reduction in the
LDH activity, probably due to the regulation of NAD+/NADH ratio by the oxidation of glucose.

Glucose-6-phosphatase, a gluconeogenic enzyme, catalyzes the dephosphorylation of glucose-6-phosphate to glucose (Saltiel and Kahn, 2001). Fructose-1, 6-bisphosphatase is another gluconeogenic enzyme that catalyzes the dephosphorylation of fructose-1,6-bisphosphate to fructose-6-phosphate. Phosphate serves as a site for the regulation of gluconeogenesis (Mahendran et al., 2014). The activities of glucose-6-phosphatase and fructose-6-phosphatase were significantly increased in diabetic rats when compared to normal control rats whereas the activities of glucose-6-phosphate dehydrogenase were significantly decreased. Upon treatment with the zinc-morin complex, the activities of glucose-6-phosphatase, fructose-1, 6-diphosphatase were found to be decreased. This might be due to increased insulin secretion and sensitivity, which is responsible for the suppression of the activities of gluconeogenic enzymes.

Glycogen is the primary intracellular storable form of glucose and its quantity in various tissues is a direct manifestation of insulin activity as insulin supports intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase (Flückiger-Isler and Walter, 1993). Glycogen synthase is a vital enzyme, which catalyzes the transfer of glucose from UDP-glucose to glycogen. Glycogen phosphorylase is a rate-limiting enzyme of glycogenolysis and is regulated by phosphorylation and by allosteric binding of AMP, ATP, glucose-6-phosphate and glucose (Parolin et
Activities of glycogen synthase and glycogen phosphorylase were shown in Table 18. During diabetic conditions, the glycogen levels, glycogen synthase activity and responsiveness to insulin signaling are reduced and glycogen phosphorylase activity is significantly increased. The levels of glycogen content in liver tissue of control and experimental groups of rats are shown in Figure 22. Glycogen, a branched polymer of glucose residues, is the primary intracellular storable form of glucose. The quantity of glycogen in various tissues is a direct manifestation of insulin activity as insulin supports intracellular glycogen deposition by stimulating the activities of glycogen synthase and inhibiting glycogen phosphorylase (Ferrer et al., 2003). The decreased glycogen content in liver observed in diabetic rats was improved upon treatment with complex and metformin indicating the improved insulin sensitivity in the liver tissue. No significant difference in glycogen level was observed in control rats treated with the zinc-morin complex.

Zinc-morin complex administration alleviates hepatic steatosis which is evidenced in the histological studies (Plate 4A-4E). Plate 4A shows the section of hepatic tissue of control rats exhibiting a concentric arrangement of the hepatocytes with sinusoidal cards around the central vein and portal tracts. The portal tracts show portal triad with portal vein, hepatic artery and bile duct which was similar to the control rats treated with zinc-morin complex (Plate 4B). Plate 4C portrays the section of hepatic tissues of diabetic group of rats exhibiting marked necrotic lesion, distorted
arrangement of hepatocytes, periportal fatty infiltration with focal necrosis of hepatocytes, congestion of sinusoids around central vein regions, granular degeneration, microvesicular vacuolization, focal necrosis, hyperemia in the sinusoids and portal tract inflammation. Treatment with zinc-morin complex showed normal hepatocyte arrangement around the central vein with minimal necrosis, declined fat accumulation (Plate 4D). Similarly, the hepatic tissues of diabetic rats treated with metformin shows normal appearance of hepatocyte arrangement and marginal histological damage (Plate 4E).

Plate 5A-E represents the ultrastructural observations in the hepatocytes of control and experimental group of rats. Plate 5A represents the electron micrograph of hepatocyte of control group of rats showing the normal cellular organelles, mitochondria, rough endoplasmic reticulum, golgi complex, nucleus with intact nuclear membrane and nuclear chromatin which was similar to the control rats treated with zinc-morin complex (Plate 5B) The electron micrograph of hepatocytes of HFD-STZ induced diabetic group revealed decrease of organelle regeneration, small nuclei with dense peripheral chromatin, cytoplasmic vacuole and swollen mitochondria, degranulation of rough endoplasmic reticulum, fat accumulation. The hepatocytes also contained many different sized lipid droplets (Plate 5C), Whereas, the hepatocytes of zinc-morin complex treated group showed relatively fewer lipid droplets when compared to the diabetic group with normal appearance of the nuclear membrane and chromatin, intact rough endoplasmic reticulum and mitochondria (Plate 5D). The electron micrograph
of the metformin treated diabetic group of rats also showed a similar pattern (Plate 5E).

The PAS staining of the diabetic liver showed more glycogen content when compared to the control section as well drug control section (Plate 5F, Plate 5G and Plate 5H). However, glycogen content was found to be depleted in hepatic tissues upon treatment with zinc-morin complex and metformin (Plate 5I and Plate 5J). Plate 6A and Plate 6B shows normal architecture of the hepatic tissue. Elongated cells near the blood sinusoids with dark cytoplasm, distorted nuclei and few lipid droplets and more number of mast cells in the periportal area were found in the toluidine blue- stained liver section of diabetic rats (Plate 6C) which was reduced to a larger extent in the zinc-morin complex as well as metformin treated liver sections (Plate 6D and Plate 6E).

Plate 6F-J represents the masson trichrome staining of hepatic tissue sections in which the diabetic liver showed an increase in the connective tissue and the stellate cells transformed into proliferative, fibrogenic and contractile. Further, hepatocyte with dark basophyl nuclei and acidophyl cytoplasm around the necrotic foci was also observed (Plate 6H). Whereas zinc-morin complex treated liver (Plate 6I) showed decreased connective tissue with more number of resting presinusoidal stellate cells which was similar to the diabetic rats treated with metformin (Plate 6J).
In the present study, zinc-morin complex administration alleviated the hepatic-steatosis which is evidenced from the histological observations where, the liver section from the control rats showed normal appearance of liver cells, whereas the liver section from rats induced with HFD-STZ showed marked fatty infiltration of hepatocytes with diffuse steatosis that significantly turned milder on treatment with zinc-morin complex. Further, the dilation of hepatic sinusoids and kupffer cell hyperplasia observed in the diabetic liver was also reversed upon zinc-morin complex administration. The Masson-Trichrome staining showed the action of zinc-morin complex on collagen fibre pattern in the liver sections of the diabetic rats which was similar to that of resveratrol in STZ induced diabetic liver (Bagul et al., 2012).

Since glycogen deposition from glucose in the hepatocytes of diabetic animals is defected, glycogen depletion is one of the main criteria for histopathological evaluation in the current study. As the glycogen synthase, phosphatase lowering effect of zinc-morin complex in diabetic rats is previously elucidated (Sendrayaperumal and Subramanian, 2014), the PAS staining of the diabetic liver showed more glycogen content when compared to the control liver sections, most of which depleted on zinc-morin complex as well as metformin treatment. Elongated cells near the blood sinusoids with dark cytoplasm, distorted nuclei and few lipid droplets and more number of mast cells in the periportal area were found in the toluidine blue- stained liver section of diabetic rats which was reduced to a larger extent in the zinc-morin complex as well as metformin treated liver sections.
Skeletal muscle accounts for approximately 75% of whole body insulin-stimulated glucose uptake, defects in this tissue play a major role in the glucose homeostasis in patients with T2DM (DeFronzo and Tripathy, 2009). The skeletal muscle stimulated by insulin takes glucose from the blood, and utilizes it to produce energy and/or store it as glycogen (Jensen et al., 2011). Although the pathways of glucose metabolism (glucose oxidation, and glycogenesis) in skeletal muscle cells are influenced by insulin, the most important effect insulin in skeletal muscle is the induction of glucose uptake by muscle cells. Skeletal muscle is the major site of insulin-stimulated glucose disposal and has also been suggested to be the primary tissue responsible for insulin resistance in the postabsorptive state. In skeletal muscle, insulin resistance manifests itself primarily by decreased insulin stimulation of glycogen synthesis, although this is due to a defect in muscle glucose transport rather than to defects in hexokinase activity (Sesti, 2006).

The effect of zinc-morin complex on skeletal muscle glucose uptake in diabetic rats was studied by the method described by Yamamoto et al. (2008) using 2-deoxyglucose (2DG), which is transported into cells, where it is phosphorylated and accumulated as 2-deoxy-d-glucose-6-phosphate (DG6P). This method was based on the assay of DG6P, which is monitored by the resazurin–diaphorase amplified detection of NADPH produced during the oxidation of DG6P by glucose-6-phosphate dehydrogenase (G6PDH). This amplifying detection system could detect the fluorescence intensity induced by uptake of 2DG into skeletal muscle cells.
Oral administration of zinc-morin complex significantly improved the uptake of 2DG into the muscles of experimental diabetic rats (Figure 23). This enhanced muscle glucose uptake is also evidenced from the improved glycogen content in skeletal muscles of diabetic rats treated with zinc-morin complex (Figure 24). Therefore, in order to assess the mechanism behind the action of zinc-morin complex on skeletal muscle glucose uptake and storage, the effect of zinc-morin complex on rat L6 myotubes was studied.

For differentiation of myoblast into myotubes, the myoblasts were seeded into appropriate culture plates and maintained in DMEM containing 2% fetal bovine serum for 6-7 days with subsequent media change for every 48 h. Rat L6 myoblast cells morphologically differentiated in terms of alignment, elongation, and fusion of mononucleated myoblasts into multinucleated myotubes. The effect of zinc-morin complex on the viability of L6 myotubes was evaluated by MTT assay at multiple morin concentrations (1.56, 3.125, 6.25, 12.5, 25, 50, 100 and 200 µM). There was more than 90% viability at concentrations of zinc-morin complex up to 200 µM, when compared with control survival (Figure 25).

Zinc-morin complex stimulates muscle glucose uptake in a dose and time dependent manner. As shown in Figure 26, zinc-morin complex enhanced glucose uptake as percentage compared to control cells, 103.48%, 114.72%, 101.56%, 116.72%, 128.24%, 144.41%, 151.64% and 156.06% at concentrations, 1.56, 3.125, 6.25, 12.5, 25, 50, 100 and 200 µM respectively. Dose dependant significant glucose uptake was observed at doses 25, 50, 100
and 200 µM after 3 h incubation, when compared to control. However, zinc-morin complex at concentrations 100 and 200 µM did not show any difference when compared to the effect of insulin (100 nM). This data indicates that morin may stimulate metabolic effects in skeletal muscle cells.

Peroxisome proliferator-activated receptor gamma (PPARγ) has been the focus of intense research because ligands for this receptor have emerged as potent insulin sensitizers used in the treatment of type 2 diabetes. There have been described three PPAR isotypes α, δ and γ which have an integrated role in controlling the expression of genes playing key roles in the storage and mobilization of lipids, in glucose metabolism, in morphogenesis and inflammatory response. Recent advances include the discovery of novel genes that are regulated by PPARγ, which helps to explain how activation of this adipocyte predominant transcription factor regulates glucose and lipid homeostasis (Rangwala and Lazar, 2006). Increased levels of circulating free fatty acids and lipid accumulation in non-adipose tissue have been implicated in the development of insulin resistance. This situation is improved by PPARγ ligands, which promotes fatty acid storage in fat deposits and regulates the expression of adipocyte-secreted hormones that impacts on glucose homeostasis. So the net result of the pleiotropic effects of PPARγ ligands is improvement of insulin sensitivity. The role that PPARγ play in the regulation of gene expression of multiple diseases including obesity, diabetes and cancer highlights the gene isolation transformation role (Janani and RanjithaKumari, 2015).
PPARγ has been associated with several genes that affect insulin action. TNFα, a pro-inflammatory cytokine that is expressed by adipocytes, has been associated with insulin resistance and diminished insulin signal transduction. PPARγ agonists inhibited expression of TNFα in adipose tissue of obese rodents and TNFα-induced insulin resistance (Miles et al., 1997). They also ablated the actions of TNFα in adipocytes in vitro. Activation of PPARγ has been shown to increase expression of c-CBL-associated protein in cultured adipocytes. This protein, which appears to play a positive role in the insulin signaling pathway, contains a functional PPRE within the 50 regulatory region of its gene. Expression of IRS-2, a protein with a proven role in insulin signal transduction in insulin-sensitive tissue, was also increased in cultured adipocytes and human adipose tissue incubated with PPARγ agonists (Smith et al., 2001).

Adenosine monophosphate-activated protein kinase (AMPK) is another key ligand dependent nuclear receptor that boosts the efficacy of exercise endurance by regulating skeletal muscle fuel utilization. AMPK is a major cellular energy sensor and master regulator of metabolic homeostasis. AMPK is a heterotrimeric serine/threonine kinase composed of a catalytic α subunit and two regulatory β and γ subunits. Adenosine monophosphate (AMP) is a key monitor of the cellular energy status. The major molecular sensor for AMP level in cells is AMPK (Cantó and Auwerx, 2010). When ATP consumption is high and glucose levels are low, AMP levels increase. Elevated AMP binds and allosterically modifies AMPK, rendering it a better
substrate for the upstream activating kinases and a less likely target for protein phosphatases. AMPK is thought to be regulated by factors that change the ratio of AMP: ATP such as exercise or contraction, hypoxia, heat shock and metabolic toxicity (Chambers et al., 2009).

In order to determine whether zinc-morin complex stimulates muscle glucose uptake by increasing the translocation of GLUT4 to the membrane either via insulin mediated PI-3kinase or exercise mimetics, PPARγ and AMPK, myotubes were treated with zinc-morin complex in the presence or absence of LY294002 (PI-3 kinase inhibitor), Compound C (AMPK inhibitor) as well as GW9662 (PPARγ antagonist). Figure 27 shows that there was no significant change in the glucose uptake stimulatory activity of zinc-morin complex in the presence Compound C whereas; the role of zinc-morin complex in muscle glucose uptake was significantly reversed in the presence of GW9662 and LY294002 (Reiterer et al., 2004; Nakayama et al., 2008). Plate 7 shows the distribution of membrane GLUT4 in control as well as zinc-morin complex treated myotubes. The distribution of membrane GLUT4 in zinc-morin complex treated myotubes was not altered in the presence of Compound C as like in the absence of these antagonists. However, in the presence of GW9662 and LY294002 the distribution of membrane GLUT4 was significantly altered.

Further, the levels of Akt, phospho-Akt, PPARγ, AMPK and phospho-AMPK in myotubes were determined to elucidate the mechanism of action of the zinc-morin complex. As shown in Plate 8, the level of
PPARγ and phospho-Akt treated cells was significantly increased, when compared to control cells. However, there was no significant difference in the level of phospho-AMPK treated myotubes. This indicates that either zinc-morin complex enhances rat skeletal muscle glucose uptake through PPARγ and PI-3 kinase but not via AMPK signaling pathways.

Insulin resistance is a common pathological state in which target tissues fail to respond properly to normal levels of circulating insulin. Pancreatic β-cells first compensate for peripheral insulin resistance by increasing insulin secretion to maintain euglycaemia. Thereafter, Impaired Glucose Tolerance (IGT) can develop, leading to overt clinical type 2 diabetes. Free fatty acids (FFA) play an important role in the establishment of insulin resistance (Boden, 2003). Indeed, chronic elevation in plasma FFA levels is commonly associated with impaired insulin-mediated glucose uptake in skeletal muscles and often coexists with obesity and type 2 diabetes. However, a dysregulation of fatty acid metabolism lead to accumulation of lipids and its metabolites in skeletal muscle and liver (Savage et al., 2007).

Accumulation of these metabolic products activates a serine/theronine cascade leading to phosphorylation of serine/theronine sites of the Insulin receptor substrate-1 (IRS-1), which in turn reduces the ability of IRS-1 to activate PI-3K and Akt. As a consequence, the glucose transport activity and other endogenous events downstream of insulin receptor signaling pathways are diminished (Yu et al., 2002). PPARγ is themolecular target to treat T2DM which is downregulated during tissue insulin resistance.
(Larsen et al., 2003). Zinc-morin complex significantly improved the glucose uptake in FFA-induced insulin resistant rat L6 myotubes (Figure 28) which represents that zinc-morin complex enhance glucose uptake in skeletal muscles upon chronically exposed to free fatty acids and this is due to the activation of PPARγ and PI3K signaling pathway by zinc-morin complex (Sakurai et al., 2002; Maxel et al., 2015). Further, zinc-morin complex improves insulin sensitivity in FFA treated rat L6 myotubes(Figure 29) thereby reducing the IRS-1 serine (307) phosphorylation (Plate 9).


