CHAPTER 5.0 RESULTS AND DISCUSSION

The present study was focused to evaluate the effect of oral administration of curcumin against high fructose diet-fed model of Insulin Resistance (IR) on biochemical and oxidative stress parameters, their association with potential changes at metabolic and molecular level, and multivariate analysis to determine the most contributing factors in adult male Wistar rats. Rats were fed with high fructose diet (Group 2) to induce insulin resistance and curcumin was co-administered orally (Group 4) for a minimum period of eight weeks. In group 2 rats, the level of glucose, insulin, low density lipoprotein (LDL), total cholesterol (TC), triglyceride (TG), urea, uric acid, creatinine, very low density lipoprotein (VLDL), nitric oxide (NO) were significantly (p≤0.05) increased and the level of albumin, high density lipoprotein (HDL) and total protein were observed to be decreased significantly (p≤0.05) when compared with group 1 control rats. The analysis of liver and kidney tissue homogenate showed a significant (p≤0.05) decrease in antioxidants such as catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GSH), superoxide dismutase (SOD), vitamin C and E, hexokinase and a significant (p≤0.05) increase in glucose-6-phosphatase and fructose-1, 6-bisphosphatase, lipid peroxides such as hydroperoxides and TBARS in group 2 rats. These metabolic alterations with high fructose diet (Group 2) were significantly (p≤0.05) improved in rats co-administered with curcumin (Group 4). Docking study was carried out to determine the binding efficiency of curcumin as agonist of PPARγ which shows high affinity towards the receptor. Gene expression studies revealed that Endothelin-1 (ET-1), Peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α), Pancreatic duodenal homeobox-1 (PDX-1), Forkhead Box containing protein-O1 (FOXO1) genes influence IR through alteration of metabolic enzymes in group 2 and group 4 rats. With this observation, it is evident that the interaction of PPARγ increases insulin sensitivity which regulates the transcription of genes that are associated with insulin resistance. Histological analysis of liver and kidney also revealed that the administration of curcumin protects the organs from the abnormal changes caused by the high
fructose diet. Overall, these results suggest that the co-administration of curcumin along with fructose effectively prevented the metabolic abnormalities and oxidative stress in high fructose diet-induced IR in rats.

5.1 Effect of curcumin on food, water intake and body weight

Body weight is tightly associated with food intake and determined by a balance between energy intake and energy expenses which contribute in the metabolic changes. Insulin is an anabolic hormone that regulate storage of glucose and fat. The association between insulin action or insulin sensitivity and body weight has actually suggests that insulin resistance develops as an adaptive physiological mechanism to prevent additional weight gain. Decreased sensitivity to insulin in adipose tissue will lead to a tendency to reduce fat deposition, resulting in elevated fatty acid levels and triglyceride in muscle and liver and whole body insulin resistance (Dokken and Tsao, 2007).

Fig.5.1 Effect of curcumin on food intake of control and experimental group of rats

Values are represented as mean ± SD, n = 6, p ≤ 0.05, comparisons are made between the groups
The effect of curcumin on food, water intake and body weight changes between control and experimental group of rats are shown in figures 5.1, 5.2 and 5.3. Though the results showed that the level of food and water intake was not statistically significant between control and experimental groups, physiologically the food and water intake was reduced in group 2 rats during the initial phase of the experimental period. The body weight showed a significant ($p \leq 0.05$) reduction in group 2 compared to all other group of rats. The results suggests, that the administration of high fructose diet contributed to the reduction in body weight which indicates the induction of insulin resistance in rats.

Fig. 5.2 Effect of curcumin on Water intake of control and experimental group of rats

Values are represented as mean ± SD, n = 6, $p \leq 0.05$, comparisons are made between the groups
Fig. 5.3 Effect of curcumin on body weight of control and experimental group of rats

Values are represented as mean ± SD, n = 6, p ≤ 0.05, comparisons are made between *Group 1 vs Group 2 and #Group 2 vs Group 4.

Previous studies showed that high fat / fructose diet did not affect the food and water intake among the experimental groups at any time during study (Wilkes et al. 1998; Rizkalla, 2014; Lopez-Rodriguez et al. 2015). In diabetic rats, a decrease in body weight could be due to dehydration induced by increase in the blood glucose level and also due to catabolism of fats and proteins. Elevated catabolic reactions are most important cause for the reduction in weight in diabetic rats (Kamalakkannan et al. 2006; Farkhad et al. 2012). Recent studies suggested that appearance of transient metabolic disorder with fructose beverage-intake has not led to weight gain and does not affect the food and water intake (Lozano et al. 2016). Similarly a 63% (w/w) glucose or fructose diet cause insulin resistance which influences the reduction of body weight (Tillman et al. 2014; Liu et al. 2015) and administration of curcumin control the changes in body weight, food and water intake by inhibiting the intracellular lipid accumulation that activate the lipolysis thereby reduces the lipogenesis and regulate the energy metabolism of the tissues (Koo et al. 2008; Kim and Kim,
2010; Maithilikarpagaselvi et al. 2016). These studies correlates with our results and showed that there is no significant changes in food or water intake and the changes in the body weight caused by high fructose diet has been maintained by the co-administration of curcumin in the experimental group of rats, thereby ensures the protective role of curcumin.

5.2 Effect of curcumin on glucose level during the experimental period

The effect of curcumin on plasma glucose level was observed at regular intervals over the experimental period of 8 weeks to understand the changes in the levels between the control and experimental group of rats.

The level of plasma blood glucose in control and experimental group rats are shown in figure 5.4. The data showed that the level of blood glucose was gradually increased in a significant \((p \leq 0.05)\) manner from the 4th week in group 2 rats and reached up to 3 fold increase as an indication of induction of diabetes mellitus when compared with group 1 control rats. However, the co-administration of curcumin along with fructose in group 4 rats significantly \((p \leq 0.05)\) protected the increase in the glucose level as a indication of prevention or delay in the onset of disease when compared with group 2 rats.

Previous studies showed that high fat diet induced insulin resistance with elevated glucose and insulin levels in plasma (Higashida et al. 2013; Liu et al. 2015; Wu et al. 2015) due to reduced uptake of glucose by the insulin sensitive tissues. High consumption of fructose increased plasma glucose and insulin concentration as fructose metabolism was not regulated by the liver. Fructose is rapidly converted to fatty acid and triglyceride which is a substrate for the lipogenesis. Thus fructose significantly contributes on insulin-mediated glucose metabolism, the development of insulin resistance and Type 2 DM (T2DM) (Toop and gentili, 2016). Earlier studies showed that curcumin reduces glucose level in rats (Zhang et al. 2013; Nabavi et al. 2015; Kato et al. 2016).
Fig 5.4 Effect of curcumin on Glucose level in blood of control and experimental group of rats during the experimental period

Values are represented as mean ± SD, n = 6, p≤ 0.05. Comparisons are made between *Group 1 vs Group 2 and #Group 2 vs Group 4.

In our study the co-administration of curcumin along with high fructose diet fed rats maintained the normal level of glucose till the end of the experimental period and thus demonstrated that curcumin has the antihyperglycemic effect which also improved insulin sensitivity to maintain the homeostasis of glucose level in the body.

5.3 Effect of curcumin on blood glucose

The most commonly used basal parameter or test to diagnose and monitor diabetes is fasting blood glucose. Glucose gives energy for the human / animal body and for many cells and its regulation involves many hormones such as insulin and glucagon (Aronoff et al. 2004; Xu et al. 2011). Glucose homeostasis is contributed by three main processes such as secretion of insulin, glucose
uptake by tissue and glucose production by liver (Kawahito et al. 2009; Goodwin, 2010). The liver plays a major role in maintaining the blood glucose level in the body by balancing the uptake and storage of glucose via glycogenesis and the release of glucose via glycogenolysis and gluconeogenesis (Nordlie et al, 1999; Xin et al, 2012; Fernandez-Novell et al. 2014). Glucose on the other hand, in abundance contributes to insulin resistance when the cell does not respond normally to the insulin.

The effect of curcumin on the level of blood glucose in control and experimental groups are given in figure 5.5. The blood glucose was significantly ($p \leq 0.05$) increased in group 2 fructose administered insulin resistance induced rats when compared with group 1 control rats. However, the co-administration of curcumin along with fructose (group 4) showed significant ($p \leq 0.05$) reduction in the level of glucose when compared with group 2 rats.

Previous studies showed that high fructose diet induce hyperglycemia, hyperinsulinemia, insulin resistance, hepatic steatosis and oxidative stress in mice (Martyn et al. 2008; Ren et al. 2015) and metabolic syndrome (MS) in rats (Shahataa et al. 2016). High consumption of fructose produces more glucose which leads to defects in the carbohydrate metabolism thereby contributing to the insulin resistance (Madhavan et al. 2015; Baena et al. 2016; Toop and Gentili 2016). These reports are in accordance to the present study in which the level of glucose was significantly higher in group 2, high fructose diet administered rats. The high percentage of fructose diet may mediated the uncontrolled production of glucose which peripherally remain increased as previously reported by Basiano et al. (2005). Similarly the raise in blood glucose level may be due to high quantity of fructose turned in to glucose in liver which can be deposited as glycogen or converted into glucose and released to blood (Tappy and Kim, 2010). However, the co-administration of curcumin along with fructose in group 4 rats showed significant ($p \leq 0.05$) reduction of glucose when compared with group 2 rats. Previous studies demonstrated that treatment with curcumin for 60 and 75 days reduced hyperglycemia in high fat diet induced in
rat models (Shapiro and Bruck, 2005; Mohamed et al. 2009; EI-Moselhy et al. 2011). These reports correlates with our study which showed decreased level of plasma glucose in rats administered curcumin along with high fructose diet (group 4). This suggests or exhibited the protective or preventive effect of co-administration of curcumin on insulin resistance with increased glucose uptake and decreased hepatic production of glucose.

**Fig. 5.5 Effect of curcumin on Glucose in blood of control and experimental group of rats**

![Graph showing glucose levels in different groups](image)

Values are represented as mean ± SD, n = 6, p< 0.05, comparisons are made between * Group 1 vs Group 2; # Group 2 vs Group 4

**5.4 Effect of curcumin on insulin**

Insulin drives the body to utilize carbohydrates as a source of energy and to spare its fat reserves. Insulin is the principal hormone of glucose homeostasis; it stimulates glucose influx into muscle, glycogen synthesis in the liver and muscle, and fat deposition in adipocytes (Wilcox, 2005). The insulin released by the pancreatic β-cells is the hormone responsible for glucose homeostasis. The inappropriate utilization of insulin leads to insulin resistance, which is
characterised by the inability of cells to respond to normal levels of circulating insulin, thus leading to the occurrence of the disease (Coman et al. 2012).

The effect of curcumin on insulin level in blood of control and experimental rats are depicted in Figure 5.6. The results showed that the level of insulin was significantly \((p \leq 0.05)\) increased in group 2 rats when compared to group 1 control rats. Co-administration of curcumin with fructose (Group 4) significantly \((p \leq 0.05)\) maintained the insulin level at normal level when compared to group 2 fructose administered insulin resistance induced rats.

**Fig. 5.6 Effect of curcumin on insulin in blood of control and experimental group of rats**

![Graph showing insulin levels in different groups](image)

Values are represented as mean ± SD, \(n = 6\), \(p \leq 0.05\), comparisons are made between * Group 1 vs Group 2; # Group 2 vs Group 4.

Diet high in fructose induces insulin resistances and reduces insulin sensitivity associated with impairment in the action of hepatic insulin and also glucose disposal from the body. Fructose diet is independent of insulin and does not stimulate insulin secretion but in case of high consumption of fructose in diet for long term induces insulin resistance and hyperinsulinemia (Elliott et al. 2002; Chandramohan and pari, 2014). Previous studies showed that the level of fasting serum insulin and glucose was increased in high fructose and fat diets in wistar
rats (Shapiro et al. 2005; Jiao et al. 2008; Padiya et al. 2011). High consumption of fructose diet leads to glucose intolerance, increased insulin level and loss of insulin sensitivity and play an important role in the development of type 2 diabetes (Noshahr et al. 2015). These reports correlates with the present study that group 2 rats showed an increase in the level of insulin in serum. Similarly, curcumin sensitizes insulin secretion from pancreatic tissues and reduce insulin resistance by increasing the insulin sensitivity (Seo et al. 2008). This correlates with the present study that co-administration of curcumin along with high fructose diet decreased the serum insulin level which highlights the anti-hyperglycemic effect of curcumin which alternates the insulin sensitivity and thus enhanced transport of blood glucose to the peripheral tissue promoting glucose transport and its utilization.

5.5 Effect of curcumin on metabolizing enzymes

Insulin resistance causes the alteration in the metabolic enzymes to produce increased level of triglycerides for fatty acid synthesis, which increased hepatic glucose production and overt fasting hyperglycemia in diabetes. Glucose-6-phosphatase and Fructose-1, 6-bisphosphatase are the essential gluconeogenic enzymes, involved in the dephosphorylation of glucose 6-phosphate to glucose in the liver and it regulate the gluconeogenesis (Kanchana et al. 2011). The increased activity of hexokinase suggests that enhanced lipid metabolism during diabetes is shifted towards carbohydrate metabolism and it enhances the utilization of glucose at peripheral sites.

Table 5.1 and 5.2 shows the influence of curcumin on metabolizing enzymes in liver and kidney of control and experimental group of rats. The activities of hexokinase was significantly ($p\leq 0.05$) decreased whereas glucose 6-phosphatase and fructose 1,6-bisphosphatase were significantly increased in group 2 insulin resistance induced rats when compared to group 1 control rats. Co-administration of curcumin along with high fructose diet (Group 4 rats)
prevented these alterations and the enzymes activities were maintained at near normal level.

Conversion of glucose to glucose-6-phosphate by hexokinase is the important step in the maintenance of glucose homeostasis. In liver, hexokinase is an important regulator of glucose storage and disposal and in pancreas it regulate glycolytic rate and play a central role in control of glucose stimulated insulin secretion (O’Doherty et al. 1999). Previous studies showed that the glycolytic enzyme hexokinase activity was decreased and the gluconeogenic enzymes like glucose-6-phosphatase and fructose-1, 6-bisphosphatase was increased in diabetic rat (Stanley and Kamalakannan, 2006; Pari and Sankaranarayanan, 2009; Kanchana et al. 2011) and db/db mice (Seo et al. 2008). Fructose reported to be a key factor in the development of IR through increased fatty acid and ROS production. The increase in fructose in liver bypasses two highly regulated steps in glycolysis, glucokinase and phosphofructokinase, both of which are inhibited by increasing concentrations of their by products but metabolized by fructokinase (Khitan and Kim, 2013). Recent studies reported that curcumin can modulate enzyme involved in gluconeogenic pathway and enhance the glucose metabolism (Jiménez-Osorio et al. 2016). These results coincide with the present study which highlights that the co-administration of curcumin effectively prevents the alteration in the glycolytic and gluconeogenic enzymes in the tissues of rat liver and kidney in experimental group of animals. This may be due to the action of curcumin on carbohydrate metabolizing enzymes towards the glucose metabolism which helped in regulating the homeostasis.
Table 5.1 Effect of curcumin on metabolizing enzymes in liver of control and experimental group of rats.

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hexokinase</strong> (mM of glucose phosphorylated/h/mg protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.62±0.1</td>
<td>2.59±0.2*</td>
<td>3.62±0.2</td>
<td>5.38±0.3*</td>
</tr>
<tr>
<td><strong>Glucose 6-Phosphatase</strong> (mM of inorganic phosphorous liberated/min/mg protein)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>0.24±0.0</td>
<td>5.69±0.3*</td>
<td>0.24±0.0</td>
<td>1.59±0.2*</td>
</tr>
<tr>
<td><strong>Fructose 1,6-bis phosphatase</strong> (mM of inorganic phosphorous liberated/h/mg protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>116.25±4.5</td>
<td>167.05±11.1*</td>
<td>131.50±35.1</td>
<td>140.39±5.3*</td>
</tr>
</tbody>
</table>

Table 5.2 Effect of curcumin on metabolizing enzymes in kidney of control and experimental group of rats.

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hexokinase</strong> (mM of glucose phosphorylated/h/mg protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.67±0.1</td>
<td>3.34±0.1*</td>
<td>4.76±0.1</td>
<td>5.62±0.2*</td>
</tr>
<tr>
<td><strong>Glucose 6-Phosphatase</strong> (mM of inorganic phosphorous liberated/min/mg protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>28.38±1.7</td>
<td>46.27±2.0*</td>
<td>26.58±1.7</td>
<td>37.19±2.5*</td>
</tr>
<tr>
<td><strong>Fructose 1,6-bis phosphatase</strong> (mM of inorganic phosphorous liberated/h/mg protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.26±1.8</td>
<td>26.97±2.2*</td>
<td>20.69±1.3</td>
<td>18.91±5.5*</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD, n=6, p≤0.05, Comparisons are made between * Group 1 vs Group 2; # Group 2 vs Group 4.
5.6 Effect of curcumin on lipid profile

Lipids are a source and store of energy, as it plays an important physiological role in skeletal muscle, heart, liver and pancreas. Abnormalities in lipid profile is one of the most common complications in diabetes mellitus. Deregulation of fatty acid metabolism develops insulin resistance and type 2 diabetes mellitus. In a diabetic condition, the increase in the level of serum lipids are due to the increased lipolysis of adipose tissue, and thereby cause abnormal lipoprotein concentration. Plasma LDL can undergo reuptake in the liver via specific receptors and get cleared from the circulation. This increase in LDL concentration may be due to defective receptors for LDL. HDL can be protected by reversing cholesterol transport, inhibiting the oxidation of LDL and by neutralizing the atherogenic effects of oxidized LDL. HDL helps to scavenge cholesterol from extra hepatic tissues and decreased HDL can contribute to the increased cholesterol levels. A greater increase of LDL may cause a greater decrease of HDL as there is a reciprocal relationship between the concentration of LDL and HDL (Georg et al. 2000).

Insulin resistance alters the lipid metabolism and lipoprotein composition. The elevated free fatty acid impairs the utilization of lipids by muscle and changes the lipid profiles. Hypertriglyceridemia reduces HDL level which is the main factor for the alteration of lipoprotein in diabetes mellitus. The VLDL and TG enhance the free fatty acid and glucose level (Mukherjee et al. 2013; de Castro et al. 2013). The serum lipid levels are high in diabetes due to the increased mobilization of free fatty acid from peripheral depots for the synthesis of lipid and an uncontrolled action of lipolytic hormone on the fat depot (Satheesh and Pari, 2008; Ahmed et al. 2010).

The level of total cholesterol, triglyceride, LDL and VLDL were significantly increased and the level of HDL was significantly (p<0.05) decreased in high fructose diet fed rats (Group 2) when compared with control (Group1) rats (Figure 5.7 and Table 5.3). Co-administration of curcumin along with fructose
showed a significant decrease in the levels of total cholesterol, triglyceride, LDL and VLDL and increase in the level of HDL when compared to group 2 insulin resistance induced rats.

**Figure 5.7 Effect of curcumin on lipid profile in blood of control and experimental group of rats**

Fructose is a lipogenic sugar and high fructose administration causes increased production of triglycerides leading to lipogenesis which reduces glucose uptake resulting in an increase in the fasting blood glucose level and insulin secretion (Basiano *et al.* 2005). High fructose diet consumption induces / causes insulin resistance, stimulates lipogenesis, resulting in increased levels of TGs, cholesterol, and low-density lipoprotein (Dupas *et al.* 2016), hyper-insulinemia, hyper-triglyceridemia and oxidative stress associated diabetes impairment of beta cell function, leading to hyperglycemia.
Table 5.3 Effect of curcumin on Total cholesterol and Triglycerides in blood of control and experimental group of rats.

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol</td>
<td>142.16±41.2</td>
<td>263.33±36.1*</td>
<td>175.83±2.0</td>
<td>162.16±26.2#</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>106.50±8.1</td>
<td>269.16±28.1*</td>
<td>99.66±13.1</td>
<td>102.66±18.9#</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD, n=6, p≤ 0.05, Comparisons are made between * Group 1 vs Group 2; # Group 2 vs Group 4.

On the other hand conversion of fructose to triose phosphate, a precursor for fatty acid synthesis is rapid and independent of insulin action. Thus it elevates the level of TG which trigger the rapid synthesis of lipids due to unregulated fructose metabolism and result in insulin resistance (Ramesh and saralakumari, 2012). Several studies showed that high cholesterol/ fructose diet induced an increase in lipid and insulin level in rats (Ryan et al. 2009; Collimo et al. 2010; Wada et al. 2010; Samuel, 2011). Other studies demonstrated that administration of curcumin reduced total cholesterol, triglyceride, LDL, VLDL and increased HDL level in high cholesterol diet fed rats (Arafa et al. 2005; Manjunatha and Srinivasan 2008; Zingg et al. 2013). Recent study also reported that curcumin inhibits hyperlipidemia and hepatic fat accumulation in fructose fed wistar rats (Maithilikarpagaselvi et al. 2016). Our results coincide with the previous studies that curcumin is a potent hypolipidemic agent that reduces the lipogenesis and alter the lipogenic enzymes to regulate the homeostatic level prevent the changes in lipid metabolism that caused by the administration of high fructose diet which is evident from the results in group 4 rats when compared to group 2 rats.
5.7 Effect of curcumin on the antioxidants

Antioxidant enzymes are the first line of defense system that protect against cellular and tissue injury under various circumstances. Catalase (CAT) is responsible for the spontaneous scavenging or detoxification of hydrogen peroxide ($H_2O_2$), whereas glutathione peroxidase (GPx) scavenges lipid peroxides. Super oxide dismutase (SOD), a principal antioxidant enzyme for the elimination of superoxide anion, into molecular oxygen and hydrogen peroxide. The hydrogen peroxide produced is further detoxified to water by the enzymes CAT. In addition, GPx is engaged in diminution of highly cytotoxic products like lipid-peroxides and other organic hydroperoxides. Reduced glutathione (GSH) is an essential co-substrate for the activity of GPx that oxidizes the GSH into oxidized glutathione. Glutathione reductase (GRd) recycles oxidized glutathione back to glutathione, through an NADPH-consuming process. The activities of these key enzymatic antioxidants were diminished during oxidative stress (OS). Since diabetic state usually increases oxidative stress and activities of these enzymatic antioxidants were found to be decreased in diabetic rats (Sreekutty and Mini, 2016).

Oxidative stress, defined as an impaired balance between free radical production and antioxidant capacity resulting in accumulation of oxidative products, a well-recognized mechanism that plays important roles in many pathological conditions. Several human diseases have been closely associated with OS, including aging, metabolic syndrome and diabetes. OS induces changes in redox balance resulting in dysregulation of redox biology and plays an important role in liver disease, cardiovascular diseases and diabetes (Lozano et al. 2016). Free radical injury is important contributing factor for the development of Insulin resistance and impaired insulin secretion. Recently it has been suggested that glycation of antioxidant enzymes could alter the structure and function of antioxidant enzymes such that they are unable to detoxify free radicals (Desai et al. 2013).
Fructose diet causes oxidative stress (Shawky et al. 2014), decreases the antioxidant enzymes and produces more ROS which contribute in the insulin resistance (Castro et al. 2015; Ning et al. 2015; Incir et al. 2016). Fructose diet induces systemic oxidative stress which play a pivotal role in the pathogenesis of insulin resistance (Hokayem et al. 2013).

The influence of curcumin on antioxidant in liver and kidney tissues of control and experimental rats are given in Table 5.4 and Table 5.5. The levels of antioxidants (CAT, SOD, GPX, VIT.C and VIT.E) were significantly (p≤0.05) reduced in group 2 insulin resistance induced rats when compared to group 1 control rats. Co-administration of curcumin along with fructose (group 4) caused significant (p≤0.05) increase in the levels of antioxidants when compared with group 2 insulin resistance induced rats.

Hyperglycemia results in the production of ROS leading to increased oxidative stress in a variety of tissues, and thus suggested to be a potential contributor to the development of complications in diabetes. Increased free radical production or reduced antioxidant defense response, both of which occur in the diabetic state may give rise to increased oxidative stress (Evans et al. 2002). Earlier studies showed that in high fructose diet fed rats, hyperglycemia associated with the increased production of free radicals, lipid peroxidation or impaired antioxidant defenses and oxidative stress can lead to damage of cellular organelles and enzymes and development of insulin resistance (Maritim et al. 2003; Olatunji et al. 2007; Armutcu et al. 2007; Pasko et al. 2010; Suwannaphet et al. 2010).

Similarly, fructose-induced hyperglycemia is one of the important factors to increase ROS, lipid peroxidation causing the depletion of the antioxidant defense status in various tissues (Reddy et al. 2009) which coincide with our study as evident in group 2 rats with high fructose diet induced insulin resistance. Previous studies showed the antioxidant effect of curcumin in Streptozotosin induced diabetic rat and curcumin scavenge the reactive oxygen
species (ROS) and inhibit peroxidation which could be useful as preventive agent against diabetes mellitus (Murugan and Pari et al. 2006; Mathews et al. 2012). These results correlate with our study that co-administration of curcumin along with high fructose diet increased level of antioxidants in tissues of rat liver and kidney (Group 4) which indicates curcumin as a free radical scavenger effectively prevented the complication in fructose induced insulin resistance through the reduction in ROS production thus protecting the tissues from oxidative stress.

Table 5.4 Effect of curcumin on antioxidants in liver of control and experimental group of rats.

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase (Units/mg of protein)</td>
<td>108.67±1.81</td>
<td>69.10±2.06*</td>
<td>108.27±1.85</td>
<td>107.82±1.66#</td>
</tr>
<tr>
<td>Super oxide dismutase (Units/mg protein)</td>
<td>1526.39±18.29</td>
<td>685.25±4.44*</td>
<td>1337.80±11.87</td>
<td>1135.74±8.20#</td>
</tr>
<tr>
<td>Glutathione peroxidase (Units/mg protein)</td>
<td>142.00±1.86</td>
<td>21.48±1.11*</td>
<td>93.34±1.81</td>
<td>137.81±2.32#</td>
</tr>
<tr>
<td>Vitamin C (µM/mg of tissue)</td>
<td>1.60±0.06</td>
<td>0.70±0.02*</td>
<td>1.6±0.06</td>
<td>1.15±0.02#</td>
</tr>
<tr>
<td>Vitamin E (µM/mg of tissue)</td>
<td>1.56±0.03</td>
<td>0.83±0.04*</td>
<td>1.54±0.04</td>
<td>2.22±0.008#</td>
</tr>
<tr>
<td>Glutathione reductase (mg/100g of tissue)</td>
<td>1.59±0.04</td>
<td>0.06±0.003*</td>
<td>1.43±0.04</td>
<td>1.06±0.04#</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD, n=6, p≤0.05. Comparisons are made between * Group 1 vs Group 2; # Group 2 vs Group 4
Table 5.5 Effect of curcumin on antioxidants in kidney of control and experimental group of rats.

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase (Units/mg of protein)</td>
<td>58.55±0.7</td>
<td>17.53±1.6*</td>
<td>49.44±0.9</td>
<td>70.42±0.69#</td>
</tr>
<tr>
<td>Superoxide dismutase (Units/mg protein)</td>
<td>1518.34±8.7</td>
<td>678.73±10.7*</td>
<td>1345.78±16.9</td>
<td>1134.16±11.86#</td>
</tr>
<tr>
<td>Glutathione peroxidase (Units/mg protein)</td>
<td>169.20±1.0</td>
<td>17.53±1.0*</td>
<td>102.94±1.9</td>
<td>124.07±2.18#</td>
</tr>
<tr>
<td>Vitamin C (µM/mg of tissue)</td>
<td>1.80±0.4</td>
<td>0.72±0.6*</td>
<td>1.8±0.4</td>
<td>1.27±0.03#</td>
</tr>
<tr>
<td>Vitamin E (µM/mg of tissue)</td>
<td>4.03±0.1</td>
<td>1.35±0.4*</td>
<td>3.52±0.5</td>
<td>2.63±0.07#</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD, n=6, *p≤0.05, Comparisons are made between * Group 1 vs Group 2; # Group 2 vs Group 4.

5.8 Effect of curcumin on the lipid peroxidation

Lipid peroxidation is a free-radical mediated degradation or propagation of oxidative insult to polyunsaturated fatty acids and the process has been terminated through enzymatic means or by free radical scavenging activity by antioxidants. The effect of curcumin on lipid peroxidation in liver and kidney tissues of control and experimental group of rats are shown in Table 5.6 and Table 5.7. The results showed that the levels of the lipid peroxidation was significantly (*p≤0.05) increased in group 2 insulin resistance induced rats when compared to group 1 control rats. Co-administration of curcumin along with fructose (group 4) caused significant (*p≤0.05) decrease in the level of lipid peroxidation when compared with group 2 rats.

Induction of diabetes caused a significant elevation in lipid peroxidation which may be attributed to the enhanced production of ROS or lipid peroxidation
products such as TBARs and Hydroperoxides (Sreekutty and Mini, 2016). These results correlate with our study that co-administration of curcumin along with high fructose diet (Group 4) decreased the level of TBARS and lipid hydroperoxides in tissues of rat liver and kidney. This indicates curcumin as a free radical scavenger effectively prevented the fructose induced insulin resistance in diabetic complications through the reduction in ROS production and peroxidation levels thus protecting the tissues from oxidative stress.

Table 5.6 Effect of curcumin on lipid peroxides in liver of control and experimental group of rats.

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiobarbituric acid reactive substances (mM/100g tissue)</td>
<td>0.05±0.004</td>
<td>0.31±0.03*</td>
<td>0.04±0.006</td>
<td>0.20±0.004#</td>
</tr>
<tr>
<td>Hydroperoxides (mM/100g of tissue)</td>
<td>737.12±63.57</td>
<td>1115.18±223.04*</td>
<td>599.56±71.90</td>
<td>779.10±100.92#</td>
</tr>
</tbody>
</table>

Table 5.7 Effect of curcumin on lipid peroxides in kidney of control and experimental group of rats.

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiobarbituric acid reactive substances (mM/100g tissue)</td>
<td>0.14±0.02</td>
<td>0.34±0.02*</td>
<td>0.12±0.01</td>
<td>0.08±0.003#</td>
</tr>
<tr>
<td>Hydroperoxides (mM/100g of tissue)</td>
<td>540.10±10</td>
<td>1271.17±354.15*</td>
<td>517.81±46.5</td>
<td>640.24±73.76#</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD, n=6, p≤0.05, Comparisons are made between * Group 1 vs Group 2; # Group 2 vs Group 4.
5.9 Effect of curcumin on total protein, albumin, urea, uric acid and creatinine level

Kidney function used to be analyzed by measuring blood urea, creatinine, uric acid, total protein and albumin which are the main metabolic product of protein and nucleotides. Kidney damage is mainly caused by the accumulation of the metabolic waste products in the body and leads to increased levels in blood especially in pathological conditions like diabetes. As a result, the plasma levels of urea and creatinine are elevated in the diabetic hyperglycemia that is considered as significant markers of renal impairment (Mirmohammadlu et al. 2015). Renal damage caused by abnormal glucose regulation in diabetes which causes elevated glucose level, increased level of glycosylated proteins and increased oxidative stress (Sreekutty and Mini, 2016).

The effect of curcumin on total protein and albumin in blood of control and experimental rats are shown in Table 5.8. The results showed that the total protein and albumin levels were significantly ($p \leq 0.05$) reduced in the group 2 insulin resistance induced rats when compared with group 1 control rats. Co-administration of curcumin along with fructose (Group 4) caused significant ($p \leq 0.05$) increase in the levels of total protein and albumin when compared to group 2 rats.

Figure 5.8 and figure 5.9 shows the effect of curcumin on urea, uric acid and creatinine in blood of control and experimental rats. The levels of urea, uric acid and creatinine were significantly ($p \leq 0.05$) increased in the group 2 insulin resistance induced rats when compared with group 1 control rats. Co-administration of curcumin along with fructose (Group 4) caused significant ($p \leq 0.05$) reduction in the levels of urea, uric acid, creatinine when compared with group 2 rats.

High fructose diet induces IR and oxidative stress which causes alteration in the renal markers such as urea, uric acid, creatinine and proteins by excessive
production of ROS (Mallaiah et al. 2014; Khitan and kim, 2013). Interestingly, fructose has the property of inducing high plasma levels of uric acid (Perheentupa and Raivio, 1967) as a result of fructose metabolism, it undergoes phosphorylation by the enzyme fructokinase into fructose-1-phosphate using ATP as a phosphate donor. The end result is in an accumulation of the phosphorylated substrate and therefore depletion of hepatic ATP with a consequent increase in the degradation of nucleotides to uric acid. Furthermore, the insulin resistance induced by high fructose intake appears to be mediated by the elevated uric acid levels (Nakagawa et al. 2006; Reungjui et al. 2007; Soltani et al. 2013).

Table 5.8 Effect of curcumin on Total protein and Albumin in blood of control and experimental group of rats.

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total protein (g/dl)</strong></td>
<td>11.83±0.55</td>
<td>9.14±0.90*</td>
<td>11.08±0.78</td>
<td>10.90±0.47*</td>
</tr>
<tr>
<td><strong>Albumin (g/dl)</strong></td>
<td>3.33±0.17</td>
<td>2.19±0.19*</td>
<td>3.30±0.28</td>
<td>3.41±0.32*</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD, n = 6, *p<0.05, comparisons are made between * Group 1 vs Group 2; # Group 2 vs Group 4

It would seem that uric acid inhibits endothelial function by impairing nitric oxide (NO) induced vasodilation which is necessary for insulin to stimulate glucose uptake into the tissues as evidenced with the results in this study. This effect of uric acid on endothelial function has also been reported in the human (Mercuro et al. 2004; Johnson et al. 2013). Thus intake of fructose is associated with hyperuricaemia, albuminuria and progression into kidney disease. High fructose consumption increases urea, uric acid, creatinine in serum which causes impairment of the glomerular function (Lanaspa et al. 2014).
Figure 5.8 Effect of curcumin on urea and uric acid in blood of control and experimental group of rats.

Values are represented as mean ± SD, n = 6, p ≤ 0.05, comparisons are made between * Group 1 vs Group 2; # Group 2 vs Group 4

Figure 5.9 Effect of curcumin on creatinine in blood of control and experimental group of rats.

Values are represented as mean ± SD, n = 6, p ≤ 0.05, comparisons are made between * Group 1 vs Group 2; # Group 2 vs Group 4
On the other hand studies showed the renoprotective effect of curcumin in carbon-tetrachloride, Streptozotosin and alloxan induced renal damage in rats (Murugan and Pari. 2007; Venkatanarayana et al. 2012; Kim et al. 2016). This correlates with our study that the administration of curcumin along with fructose diet altered the renal functional markers through reduced ROS production to optimize the renal production in the insulin resistance.

5.10 Multivariate analysis

To explore the biochemical multidimensional data, unsupervised statistical method was executed between the experimental groups. The PLS-DA plots’ for blood, kidney and liver showed a clear differentiation of Group 2 from others (Fig. 5.10 a–c). The loading coefficient map of blood (Figure 5.11 a) indicate a significantly elevated concentrations of creatinine, glucose, insulin, LDL, total cholesterol, TG and VLDL which shows that these factors are predominantly responsible for the separation of Group 2 from other groups. Similar results were obtained in previous studies suggest that high fructose diet influence in blood alters diabetic markers, renal markers and lipid profile causing insulin resistance (Schaefer et al. 2009; Kolderup et al. 2015; Mazloom et al. 2013). Of these factors, glucose, insulin, creatinine, total cholesterol, TG and VLDL scored ≥1 in VIP plot (Figure 5.11 b), indicate the potential contribution for insulin resistance. Further, the loading coefficient of kidney (Figure 5.12 a), biochemical parameters showed that TBARS is the most contributing factors for insulin resistance with the VIP score ≥1 (Figure 5.12 b). This report of our study correlate with the previous studies which showed that TBARS levels are increased in insulin resistance of kidney tissues (Hernández-Salinas et al. 2015; Vinayagam and Xu, 2015). In liver (Figure 5.13 a), the glucose 6-phosphatase, TBARS and vitamin C were identified as the most contributing factors for insulin resistance with VIP score ≥1 (Figure 5.13 b).
Figure 5.10 Multivariate PLS-DA analysis.

Blood (a), kidney (b) and liver (c) shows a significant differentiation (p ≤ 0.05) between the groups. The observations were coded according to groups: green Group 1; blue Group 2; red Group 3; yellow Group 4.

Similar reports were obtained in the earlier studies suggested that glucose 6-phosphatase, TBARS are main factor influenced in the insulin resistance of liver (Hakayem et al. 2013; Botezelli et al. 2012). Similarly, the separation of Group 4 samples was attributed to albumin and HDL as the major protecting factor of insulin resistance determined by VIP plot (Figure 5.14 a). Subsequent analysis showed that CAT, GSH, hexokinase are the major protective components of kidney (Figure 5.14 b) and GSH, hexokinase and vitamin E are the important protective factor of liver from insulin resistance (Figure 5.14 c). Overall, these
biochemical changes confirm the likely importance of fructose and the biochemical factors in causing insulin resistance.

5.10.1 Correlation analyses

Pearson correlation analysis was carried for Group 2 and 4 rats on the major contributing biochemical factors (VIP score ≥1) in blood and tissues. In Group 2 blood (Figure 5.15 a), albumin, creatinine, glucose, insulin, total cholesterol, TG, and VLDL were positively associated with insulin resistance. However, reinstation of these molecules was noticed (Figure 5.15 b) except LDL and total cholesterol in response to co-administration of curcumin (Group 4). Similarly, TBARS of kidney (Figure 5.16) and glucose 6-phosphatase, TBARS and vitamin C of liver (Figure 5.17) was reinstated in Group 4 compared to Group 2 rats. In addition to these changes, most of the molecules such as albumin and HDL-Cholesterol of blood, CAT, GSH, hexokinase of kidney, GSH, hexokinase and vitamin E of liver showed a positive association in Group 4 rats upon co-administration with curcumin. Curcumin a multifaceted nature polyphenol compound which regulate the metabolic changes to homeostasis in insulin resistance condition (Ak and Gulcin, 2008; Zhang et al. 2013; Ghorbani et al. 2014; Pulido-Moran et al. 2016).

These reports correlated with our results in group 4 rats fed curcumin co-administered along with fructose diet which showed that the homeostatic effect of curcumin to regulate the metabolic pathways. Overall our results of multivariate and correlation analysis were argument with the previous studies which suggest the alteration in these vital molecule albumin, creatinine, glucose, insulin, total cholesterol, TG, and VLDL in blood TBARS and glucose 6-phosphatase in kidney and liver may influence insulin resistance.
Figure 5.11 Loading coefficient and VIP plot for blood parameters of Group 2 rats

The loading coefficient map showing (a—blood) that insulin, glucose, VLDL, total cholesterol, LDL, triglyceride and creatinine were predominantly responsible for the classification of groups. b. The VIP scores for the biochemical parameters analyzed in (Group 2 blood) showing glucose, insulin, triglyceride, creatinine and VLDL with VIP ≥ 1.

Figure 5.12 Loading coefficient and VIP plot for kidney parameters of Group 2 rats

The loading coefficient map showing (a—kidney) that glucose 6-phosphatase, fructose 1,6-bisphosphatase, hydroperoxides and TBARS were predominantly responsible for the classification of groups. b. The VIP scores for the biochemical parameters analyzed in (Group 2 kidney) showing hexokinase, catalase, glutathione reductase and TBARS with VIP ≥ 1.
Figure 5.13 Loading coefficient and VIP plot for liver parameters of Group 2 rats

The loading coefficient map showing (a—liver) that glucose 6-phosphatase, fructose 1,6-bisphosphatase, hydroperoxides, vitamin C and TBARS were predominantly responsible for the classification of groups. b. The VIP scores for the biochemical parameters analyzed in (Group 2) liver showing glucose 6-phosphatase, glutathione reductase, catalase, superoxide dismutase, glutathione peroxidase and TBARS with VIP ≥ 1.

Figure 5.14 VIP score plots for blood, kidney and liver parameters of Group 4 rats

a. The VIP scores for the biochemical parameters of blood analyzed in Group 4 showing that HDL, urea, albumin and uric acid with VIP ≥ 1. b. The VIP scores for the Group 4 kidney showing fructose 1,6-bisphosphatase, hexokinase, glutathione reductase and catalase with VIP ≥ 1. c The VIP scores Group 4 liver showing that vitamin E, hexokinase, glutathione reductase and hydroperoxides with VIP ≥ 1.
Figure 5.15 Correlation co-efficient plot for blood parameters of Group 2 rats versus Group 4 rats

[Image of correlation coefficient plots for blood parameters]

Correlation coefficient plots for the blood parameter in the Group 2 (a) and Group 4 rats (b) shows the association between biochemical parameter with the Group 2 and 4 rats, respectively. The biochemical parameter with positive value represents positively correlated and the negative value represents the negative association to the analyzed rats.

Figure 5.16 Correlation co-efficient plot for kidney parameters of Group 2 versus Group 4 rats

[Image of correlation coefficient plots for kidney parameters]

Correlation coefficient plots for the kidney parameters in the Group 2 (a) and Group 4 rats (b) shows the association between biochemical parameter with the Group 2 and Group 4 rats, respectively. The biochemical parameter with positive value represents positively correlated and the negative value represents the negative association to the analyzed rats.
Correlation coefficient plots for the liver parameters in the Group 2 (a) and Group 4 rats (b) shows the association between biochemical parameter with the Group 2 and Group 4 rats, respectively. The biochemical parameter with *positive value* represents positively correlated and the *negative value* represents the negative association to the analyzed rats.

### 5.11 Molecular docking study by Auto Dock 4

Peroxisome proliferator-activated receptor (PPARγ) is a member of the nuclear receptor superfamily of ligand activated transcription factors. The most clinically used PPARγ agonists, rosiglitazone and pioglitazone, which belong to the thiazolidinedione (TZD) class of anti-diabetic drugs, are able to manage obesity-linked insulin resistance and type 2 diabetes (Miyazaki *et al.* 2002; Gerstein *et al.* 2006). A large number of studies have revealed a broad spectrum of action for PPARγ agonists beyond the control of glucose and lipid metabolism, including anti-inflammatory properties (Moller and Berger, 2003; Collino *et al.* 2010) A molecular docking study was carried out to investigate binding efficiency of curcumin agonist for PPARγ and to study the most contributing factors. Curcumin is an agonist for PPARγ that regulates the carbohydrate metabolism through the expression of genes associated with
diabetes. PPARγ, a ligand inducible transcription factor, binds with naturally occurring molecules and increases insulin sensitivity which regulates the transcription of genes and also has anti-diabetic capacity.

**Figure 5.18 Molecular docking study of PPAR gamma.**

(A) PIOGLITAZONE WITH PPAR gamma  (B) CURCUMIN WITH PPAR gamma

A. The binding efficiency of pioglitazone with PPAR gamma. B. The binding efficiency of curcumin with PPAR gamma of particular amino acids in the binding sites.

**Table 5.9 Active site of the PPARγ with Pioglitazone and curcumin**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Active site</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPAR γ with Pioglitazone</td>
<td>Thr (461), Glu (460), Met (463), Thr (459), Ile (456), Leu (465), Gln (470), Leu (453), Tyr (473), Lys (457)</td>
</tr>
<tr>
<td>PPAR γ with Curcumin</td>
<td>Ile (341), Arg (288), Ser (289), Ala (292), Leu (333), Ile (326), Leu (330), Met (329)</td>
</tr>
</tbody>
</table>
Table 5.10  Binding energy of the PPARγ with Pioglitazone and curcumin

<table>
<thead>
<tr>
<th>Rank</th>
<th>Binding energy of Curcumin with PPAR γ</th>
<th>Binding energy of Curcumin with Pioglitazone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>-9.44</td>
<td>-7.92</td>
</tr>
<tr>
<td>2.</td>
<td>-9.53</td>
<td>-7.42</td>
</tr>
<tr>
<td>3.</td>
<td>-9.89</td>
<td>-7.3</td>
</tr>
<tr>
<td>4.</td>
<td>-7.55</td>
<td>-8.42</td>
</tr>
<tr>
<td>5.</td>
<td>-10.04</td>
<td>-9.35</td>
</tr>
<tr>
<td>6.</td>
<td>-5.6</td>
<td>-6.11</td>
</tr>
<tr>
<td>7.</td>
<td>-9.98</td>
<td>-8.27</td>
</tr>
<tr>
<td>8.</td>
<td>-8.94</td>
<td>-8.64</td>
</tr>
<tr>
<td>9.</td>
<td>-10.16</td>
<td>-6.7</td>
</tr>
<tr>
<td>10.</td>
<td>-8.94</td>
<td>-9.49</td>
</tr>
</tbody>
</table>

PPARγ, one of three known isoforms, a regulator of lipid and glucose metabolism responsible for metabolic disorders, possess anti-inflammatory effects and also molecular targets for drugs against several metabolic disorders (Kim and Ahn, 2004; Grygiel-Gorniak, 2014). The results are ranked according to least binding energies for score.

The docking accuracy was evaluated in terms of the root mean square deviation (RMSD) and the prediction was considered successful if the RMSD value was less than 1.8˚A. The best ten energy poses for the curcumin against protein target was determined. The results are ranked according to least binding energies for score. The top ranked binding efficiency of curcumin with PPARγ showed -9.44 kcal/mol (Table 5.10). Curcumin showed better interaction with PPARγ at their active site (Table 4.9). For Instance, Ile(341), Arg(288), Ser(289), Ala(292), Leu(333), Ile(326), Leu(330) and Met(329) contributes
curcumin to binding region. Overall, the docking results showed that curcumin is potentially involved in binding with the PPARγ as agonist that increase the concentration of most contributing proteins that are associated with diabetes.

5.12 Effect of curcumin on nitric oxide

Nitric oxide (NO) is an important key metabolic and vascular regulator secreted by endothelial cells which is involved in many physiological roles especially vasodilation. Reduced levels of NO are beneficial for physiological and cellular functions where as increased levels of NO may contribute to detrimental effects in the cells. Earlier studies demonstrated that metabolic changes and hyperglycemia cause impairment of NO production and also the correlation between diabetes and the level of NO, but it is still controversial (Zemancikova and Torok, 2014; Adela, et al. 2015). Recent evidence indicates that fructose induced insulin resistance in pancreatic β-cells leads to type 2 diabetes through lipogenesis and according to the lipotoxicity hypothesis, chronically elevated free fattyacid (FFA) may directly damage the cells of the pancreas by causing increased NO production (Mukherjee et al. 2013). The high level of NO synthesis as a biomarker for the nitro-oxidative stress which is measured by the stable end product or metabolite of NO such as nitrite or nitrate (Bulboaca et al. 2016).

The results showed that the level of NO (figure 5.19) was significantly increased in group 2 insulin resistance induced rats when compared with group 1 control rats. Co-administration of curcumin along with fructose (group 4) showed a significant decrease in the levels of NO close to near normal when compared to group 2 rats.

Earlier reports have shown that high fructose diet induce hyperglycemia and stimulated the NO production (Atlas et al. 2010; Yang et al. 2010; Padiya et al. 2011). But still reports claims that fructose feeding has been shown to diminish the production of nitric oxide by lowering the activity and expression of eNOS.
(Tappy and Le, 2010; Palanisamy and Venkataraman, 2013) in our study the increase in the level of NO metabolites in group 2 rats may be due to the metabolic changes as a result of fructose induced oxidative stress (Pazzini et al. 2015). The increased NO production was enhanced by the induction of hyperglycemia and hyperlipidemia upon administration of fructose diet which contribute with high oxidative stress.

**Figure 5.19 Effect of curcumin on NO in blood of control and experimental group of rats.**

On the other hand, curcumin has been reported to be a scavenger of NO (Sreejayan and Rao, 1997) and Curcumin significantly reduces and restored the production of nitric oxide by reducing oxidative stress and restoring antioxidant enzymes (Soto-Urquieta et al. 2014; Amalraj et al. 2016). Curcumin has the potency to reduce the nitrates or nitrate through the decrease the oxidative stress (Bulboaca et al. 2016) which correlates with the result of the present study which shows that curcumin effectively normalizes the increase in NO level.
through may be increase in the antioxidant system which protects from cellular damage.

5.13 Effect of curcumin on genes associated with IR (PGC-1α, PDX-1, FOXO and ET-1)

The mRNA expression profile of genes associated with IR namely, PGC-1α, PDX-1, FOXO and ET-1 were analyzed in terms of their fold changes. Consumption of large amounts of fructose or sucrose increases lipogenesis and circulating triglycerides in humans and animal. Therefore, it is possible that the high fructose diets could cause changes in expression of genes involved in insulin resistance, metabolism and response to oxidative stress. Hence, the present study examined the expression levels of the above mentioned genes in group 2 fructose induced IR condition as well as co-administration of curcumin (group 4) in rat tissues using real time PCR.

5.13.1 Effect of curcumin on Endothelin-1 gene expression

Endothelin-1 (ET-1) is a potent endogenous vasoconstrictor, mainly secreted by endothelial cells and their actions are mediated through two types of receptors: ETA and ETB. Apart from a vasoconstrictor, ET-1 also stimulates the production of reactive oxygen species. It is claimed that ET-1 induces proinflammatory mechanisms thereby increasing superoxide anion production and cytokine secretion. Since, increased endothelial ET-1 expression enhances an increase in the expression of genes associated with lipid synthesis in the vasculature and accelerates the progression of atherosclerosis. Also increased circulating levels of ET-1 has been found in patients with diabetes, and a positive correlation between plasma ET-1 levels and type 2 diabetes has been demonstrated both in animal models of diabetes and in patients with diabetes (Cardillo et al. 2002). It is also reported that ET-1 induces a reduction in insulin sensitivity and may take part in the development of the metabolic syndrome (Kalani, 2008). The production of ET-1 may play an important role in disease-
related vascular complications, including those associated with type 1 and type 2 diabetes (Denise et al. 2010).

Figure 5.20 represents the fold change of ET-1 mRNA expression in pancreatic tissue of control and experimental group of rats. The fold change of ET-1 mRNA expression was found to be upregulated around 1 fold in group 2 insulin resistance induced rats when compared to group 1 control rats. Whereas in group 4 rats co-administered with curcumin, the ET-1 mRNA expression was significantly ($P \leq 0.05$) reduced around 1.5 fold near to that of normal condition.

Increased ET-1 expression is thought to arise from fructose-induced hyperinsulinemia, since insulin has been shown to stimulate the production and secretion of ET-1 *in vitro* and *in vivo* and upregulate the ET-1 expression in rats (Klein and Kiat et al. 2015) similarly in pancreatic islet cells, ET-1 stimulate the release of insulin causes hyperinsulinemia (De Carlo et al. 2000). Abnormal level of ET-1 plays a major role in exhibiting endothelial dysfunction and causes impaired endothelial NO production which participate in the pathogenesis of cardiovascular and diabetic complications (Muniyappa and Sowers, 2013). Recent study demonstrated that curcumin attenuate the ET-1 expression thereby prevented the cell death in brain tissues (Stankowska, 2015). These results correlate with the present study that the co-administration of curcumin has the potential to normalize/prevent the expression of ET-1.
Figure 5.20 Effect of curcumin on ET-1 mRNA expression in control and experimental group of rats

Effect of curcumin on ET-1 mRNA expression in pancreas of high fructose diet induced insulin resistance in rats. Total RNA was isolated and subjected to real time RT-PCR analysis. * indicates the significant ($p \leq 0.05$) down regulation compared to group 2.

5.13.2 Effect of curcumin on PDX-1 gene expression

The Pancreatic duodenal homeobox -1 (PDX-1) a master regulator of transcription in β-cells and its expression is critical to the development of pancreas and normal β-cell function in adults. PDX-1 is a transcription factor that is expressed in beta and delta cells of the islets of Langerhans and in dispersed endocrine cells of the duodenum and involved in regulating the expression of a number of key beta-cell genes. PDX-1 acts as a master regulator of β cell fate by simultaneously activating the genes essential for β cell identity and repressing those associated with α cell identity. Impaired expression of PDX-1 as a consequence of hyperglycemia or increased lipid concentrations are reported to be associated with diabetes.

The mRNA expression of PDX-1 in pancreatic tissue of control and experimental rats were shown in figure 5.21. In the present study, the PDX-1 mRNA expression was significantly ($p \leq 0.05$) down regulated in group 2 rats high
fructose diet administered rats when compared to group 1 rats. The expression of PDX-1 mRNA was maintained at the near normal level with the co-administration of curcumin (group 4) when compared to group 2 rats.

Figure 5.21 Effect of curcumin on PDX-1 mRNA expression in control and experimental group of rats

Effect of curcumin on PDX-1 mRNA expression in pancreas of diet induced insulin resistance in rats. Total RNA was isolated and subjected to real time RT-PCR analysis. *indicates the significant ($p \leq 0.05$) down regulation compared to group 2.

Earlier study showed that the PDX-1 expression in pancreas was down regulated in high fructose diet fed rats due to glucolipotoxicity (Balakumar et al. 2016), which correlates with our results in group 2 rats. The recent study showed that curcumin stimulates the expression of PDX-1 (Song et al. 2015). Hence, our results also supports the ameliorative effect of PDX-1 expression in order to regulate the glucose metabolism by curcumin.

5.13.3 Effect of curcumin on PPARγ (PGC-1α) gene expression

Peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α), the master regulator of mitochondrial biogenesis and energy expenditure and several metabolic functions have been attributed to PGC-1α such as fasting induces
hepatic PGC-1α expression, thereby increasing gluconeogenesis, whereas in skeletal normally and cardiac muscle exercise increases PGC-1α mediated mitochondrial biogenesis and respiration. Thus, PGC-1α expression seems finely tuned to reflect cellular energy needs, with conditions of increased energy demands inducing its expression. PGC-1α performs all these tasks by regulating the activity of a large number of transcription factors and modulates a number of genes involved in metabolic pathways as gluconeogenesis and fatty acid synthesis and oxidation or glycolysis (Fernandez-Marcos and Auwerx, 2011; Mandave et al. 2017). Hence the present study was conducted to determine the ameliorative effect of curcumin on regulation of PPARγ mRNA expression in type 2 diabetes.

**Figure 5.22 Effect of curcumin on PGC-1α mRNA expression in control and experimental group of rats.**

![Figure 5.22](image)

Effect of curcumin on PGC1α mRNA expression in pancreas of diet induced insulin resistance in rats. Total RNA was isolated and subjected to real time RT-PCR analysis. * indicates the significant ($p \leq 0.05$) down regulation compared to group 2.

Figure 5.22 shows the results of mRNA expression of PGC-1α. It was found that the levels of the PGC-1α mRNA expression was significantly ($p \leq 0.5$) down regulated in group 2 rats high fructose diet administered rats when compared to group 1 rats. The expression of PGC-1α mRNA were restored the changes evidenced in the group 2 rats. This might be due to the co-administration of
curcumin which stimulated the fatty acid uptake and decreased the lipid synthesis.

Fructose diet alters the expression of PGC-1α, transcriptional regulator reduces expression and activity which contributes the insulin resistance (Hokayem et al. 2013). Generally, PPARs modulate expression of the genes involved in metabolism of lipids and its activation stimulates lipid oxidation and lipogenesis, which induces differentiation of adipocytes and increases insulin sensitivity in mature adipocytes (Mohammadi et al. 2016). Based on the results of the present study, the role of PPARγ in the improvement of insulin resistance, it is possible that the increase in the PPARγ expression could be an alternative mechanism for antihyperglycemic effect of curcumin PGC 1α and β has been reported to coactivate PPAR thereby reduce lipogenesis followed by oxidative stress and regulate the transcription factors involved in the insulin resistance. Docking study of curcumin with PPAR shows more binding affinity compare this with a known antidiabetic drug pioglitazone which showed that curcumin a potent regulator of PPAR (Reddy et al. 2015). Curcumin mediates the metabolism and expression of genes through activation of PPARγ (Luo et al. 2016). Curcumin induce PPARγ activation which increases fatty acid uptake and decrease triglycerides and alters the hyperglycemia, hyperlipidemia and improve insulin resistance to maintain the homeostatic action of the metabolism (Aggarwal, 2010; Bei et al. 2012). These reports correlate with our study that curcumin reduces the complication of the insulin resistance through activate the expression of PPARγ.

5.13.4 Effect of curcumin on FOXO1 gene expression

Forkhead box protein O1 (FOXO1) is a transcription factor that regulates genes involved in the control of cell proliferation and cell death, as well as those in the regulation of oxidative stress and metabolism. FOXO1 is O-GlcNAcylated (O-N-acetylglucosamine) in response to high glucose, resulting in the stimulation of its transcriptional activity on the glucose 6-phosphatase (G6Pase) promoter in
hepatocytes and an increase in Glucose 6-Phosphatase expression. Earlier study suggested that, in hyperglycemic conditions, O-GlcNAcylation of FoxO1 promotes glucose production by the liver and thereby participates in glucotoxicity. O-GlcNAcylation of FOXO1 plays a pivotal role in the adverse effects of hyperglycemia in pancreatic cells (Kuo et al. 2008). Generally, the coactivation of FOXO1 by PGC-1α has been implicated in the regulation of hepatic gluconeogenic gene expression. Similarly, pancreatic FOXO1 reported to regulate β-cell proliferation and function through inhibiting PDX-1 (Li et al. 2013).

In the present study the FOXO1 mRNA expression was significantly ($p \leq 0.05$) upregulated in group 2 rats when compared to group 1 control rats. The expression of FOXO1 mRNA expression was down regulated in group 4 rats co-administered with curcumin when compared to group 2 rats (Fig. 5.23).

High consumption of fructose intake influence cellular and molecular mechanisms and thereby affects β-cell dysfunction and insulin resistance. In these circumstances, transcription factors of β-cell function (PDX1) as well as hepatic gluconeogenesis (FOXO1) were adversely affected in diet-induced insulin-resistant rats (Balakumar et al. 2016). It has been reported that curcumin inhibit the phosphorylation and translocation of FOXO1 and enhance the insulin sensitivity (Han et al. 2012; Tian et al. 2015). These reports suggested that curcumin exhibited protective effect against oxidative stress induced by insulin resistance through inhibiting the FOXO1 phosphorylation. PPARγ activation by curcumin normalize and restorative action on ET-1 system in diabetes. As previously mentioned that docking study revealed that curcumin stimulated the expression of insulin sensitizing genes. FOXO1 phosphorylation are negatively affected by glucolipotoxicity.

Earlier studies reported that ET-1, FOXO1, PDX-1 and PGC-1α genes influence IR through up and down regulation of glycolytic and gluconeogenic enzymes (Babu et al. 2013; Kandula et al. 2016).
Figure 5.23 Effect of curcumin on FOXO1 mRNA expression in control and experimental group of rats

Effect of curcumin on FOXO1 mRNA expression in pancreas of diet induced insulin resistance in rats. Total RNA was isolated and subjected to real time RT-PCR analysis. * indicates the significant (p ≤ 0.0) down regulation of mRNA expression compared to group 2.

Similarly, these reports correlates with our study that the above mentioned genes are being up and down regulated in order to maintain the glucose homeostasis upon the co-administration of curcumin in experimental rats. Thus, curcumin acts as a protector that improve hyperglycemia in diabetes induced rats by regulating gene expression of glucose regulating enzymes through PPARγ receptor. Overall, PPARγ activation by curcumin regulates the changes in the genes associated with diabetes and IR caused by administration of high fructose diet in experimental group of rats.

5.14 Histology

IR causes oxidative damage to the tissues by inducing alteration of metabolites, and affects the normal biological functions (Wagnerberger et al. 2013). High fat diets leading to accumulation of triglycerides and lipids in organ and tissues which cause insulin resistance (Auberval et al. 2014). Fructose diet induces insulin resistance, hyperglycemia, oxidative stress were contribute in the kidney damage (Dinicolantonio et al. 2016). The histological staining methods are
necessary to study the impairment of the tissues. Heamatoxylin and eosin stains are shows the morphological study of the tissue and Oil red O staining method used to determine the lipid droplets in the tissues (Lozono et al. 2016).

5.14.1 Effect of curcumin on liver stained with Hematoxylin and Eosin

Plate 5.1 shows the histological findings of liver stained with Hematoxylin and Eosin. Control group depicts the normal architecture of liver and Group 2 rats fed with high fructose diet shows portal congestion with periportal steatosis (fatty change) confirms the pathological condition. Group 3 Curcumin treated rats shows no histological changes. Group 4 rats co-treated with high fructose diet and curcumin resulted in reduced centrilobular congestion with mild microvesicular steatosis when compared to group 2 rats.

Plate 5.1 Effect of curcumin on histological changes in Liver of control and experimental group of rats.

Plate 5.1 shows the representative photographs of H&E stain on Liver (× 400); Group 1 control; Group 2 fructose; Group 3 curcumin; Group 4 curcumin + fructose
Plate 5.2 Effect of curcumin on histological changes in kidney of control and experimental group of rats.

Plate 5.2 shows the representative photographs of H&E stain on kidney (× 400); Group 1 control; Group 2 fructose; Group 3 curcumin; Group 4 curcumin + fructose.

5.14.2 Effect of curcumin on kidney stained with Hematoxylin and eosin

Plate 5.2 shows the histology of kidney stained with hematoxylin and eosin. Group 1 shows the normal architecture of the kidney. In group 2 rats administered high fructose diet, necrosis of the proximal convoluted tubules (tubular necrosis) was observed. No characteristic histological changes were seen in Group 3. A mild glomerular congestion with cloudy changes of proximal tubule was seen in Group 4 rats co-administered with curcumin.

5.14.3 Effect of curcumin on liver stained with Oil red O

The histological analysis showed in Plate 5.3 represents that there are no lipid droplets in the group 1 and group 3 rats whereas group 2 rats showed the infiltration of lipid accumulated in portal centrilobular cells. The group 4 rats expressed the recovery of fatty changes in the liver with the co-administration of curcumin. These histological analysis revealed that the co-treatment of curcumin
protects the organs from the abnormal changes caused by the high fructose diet and thus co-treatment of curcumin along with fructose effectively prevent the high fructose diet induced insulin resistance. Several studies showed that high fat and high fructose diets induce insulin resistance and tissue damage. Excess fructose diet increases the lipid synthesis and metabolic diseases which cause lipotoxic cellular dysfunction and cause accumulation of lipids induces damages in the liver and kidney (De Castro *et al.* 2013; Abo-youssef, 2015; Hao *et al.* 2015).

High consumption of fructose impair insulin sensitive tissues (Baena *et al.* 2016). Previous studies reported the accumulation of lipids, hepato-cellular damage in liver and glomerular congestion of kidney in high fat and high fructose diet fed rats (Lee *et al.* 2015; Lozano *et al.* 2016). High fructose (HF) diet-induced hyperglycemia and liver injury in mice (Yang *et al.* 2012; Hu *et al.* 2015). The high-fat diet (HFD) induces changes in renal lipid metabolism due to an imbalance between lipogenesis and lipolysis in the kidneys, as well as systemic metabolic abnormalities and subsequent renal lipid accumulation and renal injury.

The glomerular and tubule interstitial lesions associated with chronic glomerulopathy, nephrotic syndrome, chronic renal failure, diabetic nephropathy, obesity-associated renal disease, and aging nephrosclerosis are related to renal tissue lipid accumulation. In rats, the HFD causes renal injury preceded by endothelial dysfunction and hypertension, both induced by increased oxidative stress, an exacerbated inflammatory response, and disruption of the renal filtration barrier (Roza *et al.* 2016). The recent study demonstrated that the curcumin has the potential to reduce lipid deposition in the liver (Wang *et al.* 2016; Lee *et al.* 2016). And curcumin supplementation to the high dietary fructose suppresses the renal injury in albino adult rat (Abdel-Kawi *et al.* 2016).
Plate 5.3 Effect of curcumin on histological changes in Liver of control and experimental group of rats.

Plate 5.3 shows the representative photographs of the Oil Red O stain on liver tissue (× 100). Group 1 control; Group 2 fructose; Group 3 curcumin; Group 4 curcumin + fructose

These reports correlates with our study in Group2, high fructose diet fed rats with those observations. The histological analysis of liver and kidney revealed that the co-administration of curcumin protects the organs from the abnormal changes caused by the high fructose diet and thus co-administration of curcumin along with fructose effectively prevent the damages caused with high fructose diet induced insulin resistance in rats.