Diabetes mellitus (DM) is a disease that results in chronic inflammation and apoptosis in pancreatic islets in either type 1 or type 2 DM patients and is characterized by abnormal insulin secretion or insulin receptor or post-receptor events affecting the metabolism, in addition to damaging the liver, kidney and β-cells of the pancreas (Baynes, 1991). Chronic damage is associated with elevated oxidative or inflammatory activities with a continuum of tissue insults leading to more severe diabetic complications (Ndisang, 2010). Insulin-resistant glucose utilization in peripheral tissues such as muscle and adipose tissues is a universal feature of both insulin-dependent DM (IDDM) and non-insulin-dependent DM (NIDDM). In this process, GLUT and SREBP-1c along with other components play crucial roles (Charron et al., 1989). GLUT-4, a member of the glucose transporter family, is mainly expressed in skeletal muscle, heart and adipose tissues. It plays a critical role in insulin-stimulated glucose transport in these tissues, with glucose uptake occurring when insulin stimulates the translocation of GLUT-4 from the intracellular pool to the plasma membrane (Shepherd & Kahn 1999). GLUT-2 being the primary glucose transporter isoform in the liver plays a pivotal role in glucose homeostasis by mediating bidirectional transport of glucose (Bell et al., 1990). Furthermore, it has been reported that SREBP-1c expression and its nuclear abundance is low in livers of STZ-induced diabetic rats and increases markedly with insulin treatment. This fact presents a direct relation between insulin and fatty acid metabolism (Shimomura et al., 1999). A rise in cytoplasmic free calcium concentration ([Ca$^{2+}$])$_i$ owing to influx through voltage-gated L-type Ca$^{2+}$ channels in the plasma membrane is a central component of the stimulus-secretion coupling mechanism leading to insulin release by pancreatic β-cells (Prentki et al., 1987). This pulsatility of calcium is lost in patients with type 2 DM and thereby affects insulin secretion. Oxidative stress is one of the key mechanisms in the pathogenesis of diabetes-related vascular dysfunction. STZ-induced β-cell damage involves many complicated mechanisms, one of which is production of reactive oxygen species (ROS), particularly NO, either from STZ or neighboring macrophages. Peroxynitrite and proinflammatory cytokines such as TNF-α have been found to cause DNA damage and apoptosis in human and rat Islets (Hadjivassiliou et al., 1998). However, the prevention from selective destruction of insulin-producing β-cells of pancreatic Islets can be one of the targets for either the cure or delay of DM.

Besides drugs in modern medicine, several species of plants have been described in the scientific and popular literature as having hypoglycemic activity. Because of their perceived
effectiveness, minimal side effects in clinical experience and relatively low costs, herbal
drugs are prescribed widely even when their biologically active compounds are unknown.

Research conducted over last several decades has shown that the plants and plant based
therapies have a potential to control diabetes and its complications. For most of the natural
medicines, their mechanisms of action are found to be complex. However several
mechanisms are illustrated depending upon the active constituents present in respective plant
drugs. Since the efficacy of plant drug depends upon its quality and purity, standardization
has been carried according to World health Organization (WHO) guidelines for evaluating
the quality, purity and safety of plant drugs. Chemical tests performed on aqueous extract of
Tamarind seeds confirmed the presence of flavonoids, polyphenolic compounds and tannins
in the drug. The results obtained in present study are in accordance with previous reports of
Sudjaroen et al., (2007). Heavy metal analysis showed TSE do not contain lead, cadmium,
arсенic and mercury as contaminants. Microbial determination in the crude drugs showed that
the *Escherichia coli* are present with in pharmacopoeial limits (Indian Herbal Pharmacopoeia
1999) and other bacteria such as *Salmonella typhi, Pseudomonas auruginosa and Staphylococcus aureus* are nil.

HPLC chromatogram of the aqueous extract of TSE was recorded in gradient conditions to
identify the marker components in the extract. Herbal extracts are a mixture of many
compounds and produce complex chromatograms. However, under the experimental
conditions used in this work, no interference was observed from these constituents. The
presence of catechin and epicatechin along with other chemical constituents has been
reported in Tamarind seed pericarp (Sudjaroen et al., 2007) which is further validated in the
present findings (Fig. 1). In general, the antihyperglycemic nature of TSE is supported by the
fact that the polyphenols considered for their insulin mimetic action (Diasy et al., 2010)
present in the extract, exhibit antioxidant activity and radical scavenging ability.

The Neonatal–STZ Wistar model is a well-characterized model of type 2 diabetes. STZ
rats develop persistent diabetes rapidly after 6 weeks of age, and shows diabetes-like
symptoms such as lack of insulin release in response to glucose, glucose intolerance, and
depletion of pancreatic insulin store (Weir et al., 1981; Porte, 1991; Masiello et al., 1998). In
the current study, TSE was tested after chronic dosing (once a day) in a preclinical model of
STZ-induced type 2 diabetes for period of 4 weeks. The body weights of diabetic rats were
found to be less during the course of the development, may be due to accelerated lipolysis,
while weight gain was significantly observed in rats treated for 4 weeks with TSE and
metformin. The TSE-treated groups had shown significant antihyperglycemic effects associated with increase in plasma insulin activity. The reduced glucose levels suggested that TSE might either promote glucose uptake by inhibiting hepatic gluconeogenesis or by absorption of glucose into the muscle and adipose tissues either through the stimulation of a regeneration process and revitalization of the remaining β-cells to release insulin or through the insulin mimetic response.

As TSE is reported to have antidiabetic action, it was of interest to analyze whether TSE affects Islet [Ca2+]i and insulin release. In the pancreatic β-cell the oscillations in [Ca2+]i may be of particular physiological importance. An elevated concentration of glucose within the β-cell ultimately leads to membrane depolarization and an influx of extracellular calcium. The resulting increase in intracellular calcium is thought to be one of the primary triggers for exocytosis of insulin-containing secretory granules (Weigle, 1987). The present results indicate that the TSE increased Islet [Ca2+]i significantly in the presence or absence of 20 mM D-glucose (Fig. 4). The data are consistent with the role of ATP and ADP as the connecting linkages between increased glucose metabolism and the ionic events that lead to release of insulin. Bormann and Melzig (2000) reported that flavonoids might be responsible for inhibition of metallopeptidases which are involved in degradation of neuropeptides. The ability of TSE to mobilize intracellular Ca2+ in pancreatic Islets both in the absence and presence of glucose might be due to the presence of flavonoids, suggesting inhibition of degradation of neuropeptides in the cells of the Islets of Langerhans and in the basolateral surfaces of pancreatic acinar cells involved in increase of intracellular calcium and enhanced insulin secretion (Adeghate & Donath, 1990).

The Maillard reaction (excess blood glucose + Hb = HbA1c) occurs between lipids and glucose, which results in glycation of phospholipids in the cell membrane or the organelle under hyperglycemic conditions. These glycated lipids are causative agents of oxidative stress (lipid peroxidation) and are related to exacerbation of chronic diabetic complications (Nagai et al., 2008). In the present study, administration of extract for 4 weeks tended to significantly bring down the level of HbA1c in both TSE-treated diabetic groups. This might have been due to improved glycemic control produced by the extract.

The destruction of β-cells in diabetes is mediated by changing expression levels of anti-apoptotic or pro-apoptotic proteins. STZ could induce NO formation and further lead to potential mitochondrial membrane changes, hence the release of cytochrome C, which triggers apoptosis (Hirst & Robson, 2010). In the present study, the diabetic rats showed
significant increase in NO and decrease in insulin secretions and vice versa in normal rats. TSE treatment to diabetic rats shows decrease in NO, but significant increase in insulin secretions. Our results demonstrate that TSE suppresses STZ-induced apoptosis significantly (Fig. 2-I, K, L). These data suggest that TSE possesses potent anti-apoptotic effects when β-cells suffer STZ-induced impairment. Correlations could be found in our study between the total phenolic content and NO scavenging ability of the extract. The results are in agreement with those reported by Komutarin et al. (2004), where the seed coat extract of tamarind seeds suppressed NO in the murine macrophage cell line and freshly isolated peritoneal macrophages.

Significant changes are observed after TSE treatment in the distribution of insulin, glucagon, and somatostatin-positive cells in the Islets of Langerhans. The number of insulin-positive cells decreases markedly in both the TSE-treated and an untreated diabetic rat when compared to control animals, but the decrease is much greater in the untreated rats. The effect may be due to prevention of β-cell death by decreasing the oxidative stress caused by STZ in diabetic rats. Pons & Aoki (1995) reported that the number of glucagon-producing alpha and somatostatin-producing delta cells was increased in diabetes and it appears that somatostatin-producing cells rise in number to compensate for the relative reduction in insulin-secreting cells. This up regulation of alpha cells of pancreas controls the glucagon release to maintain glucose homeostasis by way of the feedback mechanism of somatostatin. This plausible mechanism supports the results of the present study, where there is a decrease in glucagon-positive cells, whereby somatostatin positive cells were noted in TSE-supplemented rats (Fig. 2).

It has been reported that acute marked hyperglycemia caused by glucose-injection induced delayed gastric emptying in diabetic rats, healthy volunteers, and type 1 diabetic patient (Chang et al., 1996). Therefore, the diabetic rats considered in the present study for assessing the gastrointestinal functions showed blood glucose levels of more than 300 mg/dL. Delayed gastric emptying affects the postprandial glucose profile as it impairs the delivery of nutrients to the small intestine. Conversely, hyperglycemia tends to slow gastric emptying. In diabetic patients, there is evidence for both hyperglycemia and delayed gastric emptying and these two causes are linked by the likelihood that prolonged hyperglycemia causes autonomic neuropathy (Horowitz et al., 2002). The STZ model of diabetes exhibit various stages of type 2 diabetes such as impaired glucose tolerance, mild to severe (based on dose) hyperglycemia, lowered plasma and pancreatic insulin. Also the β-cells in STZ rats bear resemblance to the
insulin secretary characteristics found in type 2 diabetic human patients (Portha et al., 1974; Lee et al., 2003). In the present study, STZ induced diabetic rats (mortality 30%) had mild gastroparesis with slow gastric emptying and intestinal transit rate in comparison to normal control rats, thus indicating that STZ induced diabetic rats could be used as the animal model for diabetic gastroparesis. These dysfunctions of gastrointestinal transit in diabetic animals were significantly improved by the administration TSE whereas metformin reduced the gastric emptying rate. Metformin results are in accordance with the findings of Maida et al. (2011), where it reduced the gastric emptying with improvement in GLP-1. Recent studies have revealed that, exogenous GLP-1 or GLP-1 derivatives cause a delay in gastric emptying and intestinal transit rates, which was considered to be partially responsible for the inhibition of postprandial hyperglycemia (Nauck, et al., 1997). However, TSE was unable to ameliorate GLP-1 mRNA when compared to metformin and normal rats thus excluding the possibility of its antidiabetic action through GLP-1. The improvement in gastric emptying rate and small intestinal transit time may be a secondary response to the insulin mimetic action of TSE, as it has been reported that insulin increases the gastric emptying directly by its hypoglycaemic effect (Schvarcz et al., 1995) and indirectly on stomach through the vagus nerve (Quigley and Templeton, 1930).

Several clinical studies have demonstrated an association between hypoadiponectinemia and the development of insulin resistance and type 2 diabetes. Plasma adiponectin levels correlate positively with HDL cholesterol and negatively with triglyceride levels (Ouchi et al., 2003). A decrease in adiponectin concentration may therefore result in an increased blood lipid concentration, as shown in our study (Fig. 2). TSE exhibited a significant hypolipidemic activity, decreasing total cholesterol, triglycerides and LDL cholesterol in serum with improved adiponectin level in the diabetic rats. The decrease in serum triglycerides and cholesterol may be associated with the epicatechin content of TSE (Chan et al., 1997). The results obtained in this study are in accordance with those obtained by Martinello (2006), showing the hypolipidemic and antioxidant action of *T. indica*. In the current investigation, the improved level of adiponectin by TSE treatment in diabetes may focus light on the connecting link between TSE and adiponectin induced AMP activated protein kinase. Since, activation of protein kinase results in stimulation of glucose uptake in muscle and inhibition of hepatic glucose production, cholesterol and triglyceride synthesis (Ouchi et al., 2000).

Maiti et al. (2004) reported the antidiabetic potential of Tamarind seeds by targeting the liver and kidney as the site of action. Supplementation of TSE for 4 weeks restored the level
of transaminases and liver glucose-6-phosphatase activity in conjunction with insulin. Furthermore, the antihyperglycemic activity of TSE was correlated with restoration of pancreatic β-cell mass in STZ diabetic rats for 8 weeks of treatment (Mahmoudzadeh-Saheb et al., 2010). As the dysregulation of GLUT-2 and GLUT-4 controlling mechanism can result in the pathophysiologic states associated with diabetes and insulin resistance (Villanueva-Penacarrillo et al., 2001), we attempted to explore this aspect of diabetes for antihyperglycemic action of *T. indica*.

GLUT-4 expression is down-regulated when there is relative insulin deficiency, such as in STZ-induced diabetes (Charron et al., 1999). GLUT-4 protein level was measured by immunoblot analysis in the homogenate of skeletal muscles and was decreased in the diabetic control group (Fig. 3). After treatment with TSE, the contents of GLUT-4 protein and GLUT-4 mRNA were restored to near normal values in skeletal muscles. The decrease in GLUT-4 levels is essentially one of the main reasons of hyperglycemia in the diabetic state, which is due to decreased uptake of glucose by the skeletal muscles. Restoration of GLUT-4 levels would, therefore, enhance the uptake of glucose in the skeletal muscles and thus help to combat hyperglycemic conditions. Glucose unresponsiveness associated with GLUT-2 impairment is typically demonstrated in type 2 diabetes (Thorens et al., 1992). The effects of insulin on SREBP-1c have been corroborated by *in vivo* studies showing that SREBP-1c expression and nuclear abundance were low in the livers of STZ-induced diabetic rats, and markedly increased after insulin treatment (Valerio et al., 2006). Therefore, the stimulating action of TSE on GLUT-2 protein and SREBP-1c mRNA expression might be due to the insulin secretagogue effect.

In present study, the hypo-adiponectinemia in diabetic rats may partly explain the decreased phosphorylation of the 5′ adenosine monophosphate-activated protein kinase (AMPK-α Thr172) signalling pathway (Yamauchi et al., 2002). AMPK has been reported to be an important mediator of glucose metabolism and increases glucose transport by stimulating the translocation of GLUT-4. Our study shows that the protein expression of membrane GLUT-4 is decreased, indicating that glucose metabolism would be reduced in diabetic rat. Thus, it can be hypothesized that the decreased GLUT-4 may be due to the reduced phosphorylation of AMPK in the diabetes, which was ultimately improved by TSE treatment to the diabetic rats.

Mammalian intestinal α-glucosidase inhibitors have become exciting candidates to slow down the digestion of carbohydrates and in turn mitigate postprandial hyperglycaemic
excursions. It catalyzes the final step in the digestive process of carbohydrates. Recent studies have suggested that polyphenols such as flavonoid (Gao and Kawabata, 2005) and tannin (Toda et al., 2000) have potent α-glucosidase inhibitory activities contributing to the suppression of postprandial hyperglycaemia. These findings led us to substantiate the anti-hyperglycaemic action of Tamarind seeds, which have been reported to possess polyphenols and tannins (Siddhuraju, 2007) in significant amount. Its aqueous extract showed α-glucosidase inhibitory activity in vitro (Fig. 2). The present results revealed that TSE possessed α-glucosidase inhibition (42%) at highest concentration (10,000 µg/mL) in dose dependent manner. These findings are complementary to the reports by Funk and Melzig (2005), where different plant materials including Tamarind leaf extract showed strong α-amylase inhibitory activity. The in vivo anti-hyperglycaemic effect of TSE therefore, can be corroborated with its α-glucosidase inhibitory action in conjunction with other mechanisms described above.

8. Conclusion

The proposed mechanism of the anti-hyperglycaemic action of TSE has been diagrammatically represented in Fig. 26. The data presented in this study support and thus accepts our primary hypothesis that the antidiabetic action of Tamarind seeds comprises an array of actions and provides direct evidence for the involvement of glucose transporters (GLUT-2, GLUT-4) in controlling glucose homeostasis. In addition, the β-cell-preserving action of TSE along with the insulin mimetic potential (mediated by increase in cytosolic Ca\(^{2+}\) of Islets) also helps to control hyperglycemic excursions in diabetes. Furthermore, the study has also provided a plausible mechanistic explanation for the effect of seeds and fruits of *T. indica* on lipid metabolism as the SREBP-1c mRNA concentration in the liver in conjunction with adiponectin were improved. The Present work also illustrates beneficial role of Tamarind seed in controlling hyperglycaemic excursions by targeting α-glucosidase and gastrointestinal functions. Thus, we can conclude that TSE along with its anti-diabetic action may further be explored for the treatment of GI dysfunctions associated with diabetes. The present study on the antidiabetic potential of Tamarind seeds provides a strong rationale for the study of compounds and functionalities involved in its antihyperglycemic action, thereby offering a foundation for a new herbal drug in diabetes therapy.
Fig. 26. Proposed mechanism of action of Tamarind seeds for antihyperglycemic activity in type 2 diabetes rats