6. DISCUSSION
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The present investigation evaluates the effectiveness of the methanol extract and its different fractions derived from *Helicteres isora* roots and the antidiabetic compounds derived from active fraction based on the activity guided phytopharmacological studies on high fat diet and low dose streptozotocin-induced type 2 Diabetes mellitus (HFD-STZ diabetic rats) and streptozotocin (STZ)-induced type 1 diabetic rats. The active constituents were isolated and identified as saponins from the n-butanol fraction.

The isolated saponins were further evaluated for effects on glucose and lipid metabolism regulating genes in the liver and the adipose tissue of C57Bl/KsJ-db/db mice by quantitative real time polymerase chain reaction (qRT-PCR) and reverse transcription polymerase chain reaction RT-PCR.

The high fat diet-fed, STZ-treated rat model provides a novel animal model for type 2 diabetes mellitus that simulates the human syndrome and is suitable for the testing of antidiabetic compounds (Reed et al., 1999; Srinivasan et al., 2005). It has been documented that consumption of high fat or high simple sugar diet can lead to insulin resistance in rats (Grundlege and Thenen, 1982; Storlien et al., 1991; Storlien et al., 1993). It has also been reported that the high fat feeding induces insulin resistance initially in rat liver and adipose tissues; this is followed by impaired glucose metabolism in skeletal tissues. It has been reported that the rats fed high simple carbohydrate or high fat diets had significantly higher fasting plasma insulin and glucose levels compared to control animals, characteristic symptoms of insulin resistance (Kraegen et al., 1991). Low dose streptozotocin combined with high energy intake can effectively induce type 2 diabetes
mellitus through altering the related gene expression (Wang et al., 2007). In the present study, male Sprague-Dawley (SD) rats fed on high fat diet for 4 weeks exhibited the features of insulin resistance characterized by mild hyperglycemia, glucose intolerance, hypertriglyceridemia, hypercholesterolemia, hyperinsulinemia and decreased insulin sensitivity. The results are in agreement with the previous reports (Reed et al., 1999; Srinivasan et al., 2005). HFD-STZ diabetic rats were not insulin deficient but had hyperglycemia and higher insulin levels as compared to the NPD fed rats. In the present study, fat-fed STZ-injected rats became hypertriglyceridemic. This is characteristic abnormality of lipoprotein metabolism in patients with type 2 diabetes (Albrink, 1974). The long-term high-fat diet led to a significant increase in body weight in the present study. The weight difference between the two experimental groups might be due to significant visceral and subcutaneous fat accumulations in rats fed with high-fat diet (Borst and Conover, 2005). The results of present study revealed that the methanol extract (ME) of Helicteres isora roots and the n-butanol fraction (BF) and the isolated saponins (SA) have potent antihyperglycemic and antihyperlipidemic properties in HFD-STZ diabetic rats. The ME showed dose dependent reduction on the glucose levels and the maximum reduction was observed with the highest dose (500mg/kg).

STZ-induced type 1 diabetic rats exhibited significant hypoinsulinemia, hyperglycemia and hyperlipidemia. Administration of methanol extract, n-butanol fraction and isolated saponins to STZ-diabetic rats for 15 days caused significant reduction in blood glucose, triglycerides and cholesterol in the rats. However, there was no change in serum insulin
levels, which ruled out the possibility of stimulating insulin secretion as the mechanism contributing to these effects.

Administration of methanol extract (300mg/kg) to normal control rats did not cause any significant change in the biochemical parameters when compared with normal control rats and in acute toxicity study the methanol extract did not show any mortality, or any remarkable symptoms of toxicity and/or any significant changes in general behavior in rats, at the 1, 2, 3, 4 and 5 g/kg doses, indicating no toxicity in the mentioned dose range.

In our study on HFD-STZ diabetic rats, the results of $K_T$ demonstrate that treatment with ME, BF, saponins and pioglitazone results in marked improvement in insulin sensitivity characterized by an increase in glucose disappearance rate during the insulin tolerance test. These results indicate that the effect of the extract, fractions and saponins may be mediated through an overall insulin sensitization in these animals.

The levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents a risk factor for coronary heart disease. The hypercholesterolemia and hypertriglyceridemia occurs in STZ-induced diabetic rats. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides (Taskinen, 1987). In diabetic state, lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridemia. In STZ-induced type 1 diabetic animals, the standard drug insulin (5 I.U. /kg, i.p. daily) for 6 weeks exhibited significant antidiabetic activity with maximum reduction of serum glucose as compared to the diabetic control group. Insulin treatment also significantly reduced serum triglyceride, cholesterol, LDL and VLDL levels. The standard reference treatment with insulin also
decreased the weight loss of diabetic animals and altered the weight towards normalcy during the course of treatment. Administration of methanol extract of *H. isora* and n-butanol fraction for 6 weeks to STZ-type 1 diabetic rats caused significant reduction in the elevated serum glucose and lipid levels. The results indicate that the continued administration of the ME and BF produced a sustained antihyperglycemic effect in STZ-induced diabetic rats. The decrease of blood glucose was more when compared with that of corresponding sub-acute treatment and was marked at the end of 6 weeks treatment. The possible mechanism of these effects may be either the increase in the glucose utilization in the periphery or decrease in the endogenous glucose production in the liver of the STZ-diabetic rats.

In our study, HFD-STZ diabetic rats showed increased concentrations of serum triglyceride, cholesterol, and LDL, whereas HDL was decreased. The elevation in serum triglyceride and cholesterol levels observed in the study on HFD feeding has also been reported in earlier studies (Reed et al., 1999; Tanaka et al., 2001; Srinivasan et al., 2005). Treatment of HFD-STZ diabetic rats with ME, BF and SA showed restoration of these parameters towards the near control levels.

It has been shown that abnormally high serum levels of LDL and low serum levels of HDL are associated with an increased risk for atherosclerosis (Rubin et al., 1991; Korhonen et al., 1996). The atherogenic index, calculated as the ratio of LDL and HDL, is believed to be an important risk factor for diagnosis of atherosclerosis. Our data demonstrate that ME, BF and SA significantly decreased the ratio in HFD-STZ diabetic rats.
An imbalance between free radical production and antioxidant level leads to oxidative stress, which has been observed by us on the basis of depressed antioxidant defense system in the HFD-STZ diabetic group. Administration of ME, BF and SA to HFD-STZ diabetic rats has prevented the buildup of oxidative stress by restoring normal activities of the enzymatic antioxidant SOD and catalase and normal levels of the non-enzymatic antioxidant GSH in the liver; the untreated HFD-STZ diabetic rats showed diminished concentrations of these antioxidants. The diminished antioxidant defense system in HFD-STZ diabetic rats leads to damage so-called lipid peroxidation. We have observed increased concentration of malondialdehyde measured in the form of thiobarbituric acid-reactive substances (TBARS), indices of lipid peroxidation, in the serum and liver of HFD-STZ diabetic animals. Administration of ME, BF and SA caused a significant decrease in the lipid peroxidation levels. The treatment also reduced the lipid peroxidation levels in the liver tissue of the type 1 diabetic rats and raised the levels of cellular antioxidant reduced glutathione (GSH) and those of antioxidant enzymes super oxide dismutase (SOD) and catalase. The results of this study show that H. isora possesses significant antidiabetic activity along with potent antihypertriglyceridemic and antioxidant potential in diabetic conditions.

It has been suggested that in diabetes, oxidative stress plays a key role in the pathogenesis of both microvascular and macrovascular complications (Giugliano et al., 1996). Diabetic subjects with such complications may have a defective cellular antioxidant response against the oxidative stress generated by hyperglycemia, which can predispose the patient to organ damage (Ceriello et al, 2000). Because ROS not only can damage or change the...
functions of proteins directly but also act as subcellular messengers to influence the gene expression. The past decade has seen a rapid growth in the number of genes shown to be influenced by redox changes and in those that exert downstream effects through increased ROS formation (Allen and Tresini, 2000; Hensley et al., 2000).

A strong association between oxidative stress and insulin resistance in the development of vascular dysfunction leading to hypertension has been documented (Kashiwagi et al., 1999). Several mechanisms have been suggested as being involved in the oxidative stress in diabetics, such as glucose autooxidation, protein glycation, formation of advanced glycation products and polyol pathway (West, 2000). These reports suggest that antioxidant therapy may be beneficial for diabetic patients.

In the histopathological study, treatment with the methanol extract and n-butanol fraction reduced and normalized the histopathologic changes in liver, kidney and pancreas when compared with STZ diabetic animals; these results indicate the effectiveness of the drug in the prevention and management of long term complications of diabetes mellitus as the histopathological changes in the kidney viz., glomerular mesangial cell hyperplasia and renal tubular cell vacuolation with hyperaemia observed in the STZ diabetic rats were reversed with the treatment. Similarly in the HFD-STZ diabetic rats, treatment with H. isora methanol extract and n-butanol fraction and saponins reduced and normalized the histopathologic changes, reduced fatty inclusions and preserved the normal architecture of cells in liver, kidney and pancreas when compared with HFD-STZ diabetic control animals. HFD-STZ diabetic and STZ diabetic rats had significant increases in serum
creatinine, urea, ASAT and ALAT levels. Treatment caused significant decreases in all these safety markers. These markers are sensitive measures for liver and kidney functions.

Taken together, our results suggest that *H. isora* appears to have multiple influences on glucose and lipid metabolism that strongly counteract the untoward effects of a high fat diet. Treatment with the methanol extract, n-butanol fraction and the isolated saponins produced a significant reduction in elevated levels of glucose and lipids in experimental models of type 1 and type 2 Diabetes mellitus.

The results of C57BL/KsJ-db/db mice study demonstrate that the methanol extract and isolated active constituents saponins are effective hypolipidemic and hypoglycemic agents in *db/db* mice, obese and diabetic animals with insulin resistance. Insulin resistance profoundly contributes to the pathophysiology of type-2 diabetes and causes reduced glucose utilization and increased glucose production in the liver, leading to hyperglycemia. Insulin resistance, in both human and animal models, is commonly associated with several abnormalities in the lipid metabolism, including increased plasma free fatty acid levels, hypertriglyceridemia, hypercholesterolemia, and enhanced lipogenesis in liver. Recent works show that insulin resistance develops as a consequence of the effects of inflammatory and hormonal factors, endoplasmic reticulum (ER) stress, and accumulation of by-products of nutritional ‘overload’ in insulin-sensing tissues (Muoio and Newgard, 2008).

In our study, methanol extract and saponins significantly lowered the serum non esterified fatty acids (NEFA) and triglyceride levels when compared with control group. Elevated plasma free fatty acid levels have been found to increase hepatic triglyceride production.
Furthermore, lowering of serum cholesterol levels has been observed in our study by methanol extract and saponins. The treatment also reduced the serum glucose and insulin levels when compared with the control \(db/db\) mice.

The treatment altered the expression of the genes encoding the regulatory enzyme of gluconeogenesis and glycogenolysis in the liver of the \(db/db\) mice, suppressed the mRNA expression of glucose-6-phosphatase (G6Pase). No significant change in the expression level of phosphoenolpyruvate carboxykinase (PEPCK) was observed.

These results indicate that the active constituent saponins and methanol extract affect the last step in gluconeogenesis and glycogenolysis. Glucose is formed from gluconeogenic precursors in liver and renal cortex, and also from glycogen in liver. Both gluconeogenesis and glycogenolysis result in the formation of glucose 6-phosphate (Glc-6-P), which has to be hydrolysed by glucose-6-phosphatase (G6Pase) before being released as glucose into the circulation. G6Pase thus plays a critical role in blood glucose homoeostasis. The mRNA levels of hepatic PEPCK and G6Pase are elevated in \(db/db\) mice at 12 weeks of age (Friedman et al., 1997). Slight changes in the expression of these genes can alter the blood glucose levels (Kahn, 1997).

Glucose-6-phosphatase (G6Pase), found mainly in the liver and the kidneys, plays the important role of providing glucose during starvation. Starvation and diabetes cause 2–3-fold increase in G6Pase activity in the liver. Northern blots have shown that these conditions are associated with 2–4-fold increase in G6Pase mRNA (Schaftingen and Gerin, 2002). These effects are probably due to the reciprocal changes in insulinaemia and glucagonaemia. Insulin causes a decrease in the activity of G6Pase in the liver in vivo. It
also decreases G6Pase mRNA both in vivo and in hepatoma cells, where the effect appears to be transcriptional (Hombuckle et al., 2001). In the present study, statistically insignificant increase in the expression of ACOX was observed with methanol extract and pioglitazone treatment whereas there was no change in the saponins treated db/db mice group. The protein encoded by this gene is the first enzyme of the fatty acid beta-oxidation pathway, which catalyzes the desaturation of acyl-CoAs to 2-trans-enoyl-CoAs. It donates electrons directly to molecular oxygen, thereby producing hydrogen peroxide.

Among various glucose transport systems, the liver plays a dual role, as glucose uptake occurs from circulation when gluconeogenesis and glycogenolysis are low; however, glucose is released when gluconeogenesis and glycogenolysis are activated (Oka et al., 1990). Hepatic Glut2 expression is higher in obese and diabetic animals, such as db/db mice (Friedman et al., 1997), in the present study treatment with methanol extract or saponins did not cause any change in the expression of Glut2 in the liver of db/db mice. However, pioglitazone treatment caused statistically insignificant reduction in the expression of Glut2. In contrast to the hepatic Glut2, the adipocyte Glut4 expression was significantly higher in all the treated groups when compared with the control db/db mice group. In general, glucose transport in the liver and adipocytes is regulated by different mechanisms (Oka et al., 1990). Adipocyte Glut4 overexpression is known to alleviate insulin resistance and pancreatic defects in db/db mice, resulting in a markedly improved glycemic control (Gibbs et al., 1995). The ability of adipose tissue to produce inter-organ regulatory factors is dependent on the metabolic state. Mice with an adipose-specific knockout of the GLUT4 glucose transporter have impaired insulin sensitivity in muscle and
liver (Abel et al., 2001). Interestingly, food deprivation also causes a form of insulin resistance and is associated with a decrease in adipose Glut4 expression (Sivitz et al., 1989). The rate-limiting step in the uptake and metabolism of glucose by insulin target cells is glucose transport, which is mediated by specific glucose transporters of the plasma membrane. In normal muscle cells and adipocytes, the glucose transporter isoform is Glut4, a 12-transmembrane domain protein that mediates transport of glucose in the direction of glucose gradient (Douen et al., 1990; Barret et al., 1999). Insulin promotes Glut4 incorporation into plasma membrane, and this translocation from intracellular compartments appears to fail in the insulin resistance present in some form of diabetes (Klip et al., 1990; King et al., 1992; Zierath et al., 1996; Garvey et al., 1998). Therefore, the improved glycemic control observed in \textit{db/db} mice in our study may partly be due to increase in the Glut4 expression.

PPAR \(\gamma\) has been reported to regulate the expression of genes involved in glucose metabolism. In our study treatment with saponins significantly increased the PPAR \(\gamma\) expression in the adipose tissue of the \textit{db/db} mice without altering the hepatic PPAR \(\alpha\) expression. PPAR \(\gamma\) can directly activate hepatic glucokinase expression and may improve glucose homeostasis in type-2 diabetes (Kim et al., 2004).

Methanol extract and pioglitazone treatment caused reduction in the expression of Fatty acid binding protein 4 (FABP4), while there was no change with the saponins treatment in the \textit{db/db} mice. FABP4 also known as A-FABP (Adipocyte FABP) was first detected in mature adipocytes and adipose tissue. This protein has also been termed adipocyte P2 (aP2) because of its high sequence similarity (67%) to peripheral myelin protein 2 (M-
FABP/FABP8) (Hunt et al., 1986). Expression of A-FABP (FABP4) is highly regulated during differentiation of adipocytes, and its mRNA is transcriptionally controlled by fatty acids, PPARγ agonists and Insulin (Haunerland and Spener, 2004; Makowski and Hotamisligil, 2005; Chmurzynska, 2006).

A-FABP is the best-characterized isoform among the entire FABP family with the most striking biology. A-FABP-deficient mice exhibited reduced hyperinsulinemia and insulin resistance in the context of both dietary and genetic obesity, but the effect of A-FABP on insulin sensitivity was not observed in lean mice (Hotamisligil et al., 1996; Uysal et al., 2000). Adipocyte/macrophage FABPs, A-FABP and E-FABP act at the interface of metabolic and inflammatory pathways. These FABPs exert a dramatic impact on obesity, insulin resistance, type 2 diabetes, fatty liver disease, atherosclerosis and asthma. The creation of pharmacological agents to modify FABP function may therefore provide tissue-specific or cell-type-specific control of lipid signalling pathways, inflammatory responses and metabolic regulation, thus offering a new class of multi-indication therapeutic agents. FABP-mediated lipid metabolism is closely linked to both metabolic and inflammatory processes through modulating critical lipid-sensitive pathways in target cells, especially adipocytes and macrophages. A-FABP offers highly attractive therapeutic opportunities for a broad range of pathologies in metabolic diseases by addressing an evolutionary bottleneck in the metabolic design of humans (Furuhashi and Hotamisligil, 2008).

Adipsin is a circulating glycoprotein of the serine protease family, which is predominantly synthesized and secreted by adipose tissue (Cook et al., 1987). This protein, the expression of which shows marked changes in pathophysiological states associated
with variations in adipose tissue mass, presents the properties of a putative systemic regulator of energy balance. The expression of adipsin mRNA has been reported to be suppressed in two strains of genetically obese mice, \( ob/ob \) and \( db/db \) (Flier et al., 1987). Adipsin deficiency is a distal effect of the \( db \) mutation that is not required for obesity to develop as adipsin expression is not impaired in the young suckling \( db/db \) pup at the time when the obese phenotype is emerging. Adipsin's impairment is rather a secondary feature brought about by the neuroendocrine factors that develop in the post weaning obese mice. One likely candidate is the severe hyperinsulinemia of these animals (Dugail et al., 1990). Treatment with methanol extract and saponins in our study produced significant increase in the expression of the adipsin. Pioglitazone also caused significant increase in the expression of adipsin when compared with control \( db/db \) mice.

The adipsin-ASP (acylation stimulating protein) system, therefore, appears to be involved in the regulation of triglyceride metabolism in adipocytes, the function of this system is to increase the rate of triglyceride synthesis in adipocytes rather than, as was originally suggested, to increase the rate at which lipolysis occurs (Flier et al., 1987). The increase in triglyceride synthesis induced by ASP is achieved by translocation of glucose transporters from intracellular vesicles to the cell surface, thereby increasing specific membrane glucose transport (Germinario et al., 1993). By contrast, membrane transport of fatty acids is not directly affected by ASP. However, net fatty acid uptake does increase secondary to stimulation of the enzyme, diacylglycerol acyltransferase, which controls the rate limiting step in the synthesis of a triglyceride molecule (Yasruel et al., 1991). Related to glucose metabolism, ASP increases glucose transport in adipocytes and myotubes through
translocation of Glut1, Glut3 and Glut4 (Germinario et al., 1993; Maslowska et al., 1997; Tao et al., 1997)

Adiponectin is adipose-specific adipocytokine. Many clinical studies have demonstrated that low plasma concentrations of adiponectin (hypoadiponectinaemia) associate closely with obesity-related diseases, including atherosclerotic cardiovascular diseases, type 2 Diabetes mellitus, hypertension and dyslipidaemia. Accumulating experimental evidence indicates that adiponectin possesses anti-atherogenic, anti-inflammatory and anti-diabetic properties and may also participate in the mechanism of metabolic syndrome and other diseases (Okamoto et al., 2006). In the present study, pioglitazone treatment caused significant increase in the expression of adiponectin while methanol extract and saponins didn’t cause any change in the expression of adiponectin when compared with control db/db mice. There was no significant change observed in the mRNA expression of lipoprotein lipase (LPL) with any of the treatment when compared with control db/db mice. These results indicate that the active constituent saponins and methanol extract do not affect LPL for hypolipidemic effects. LPL hydrolyzes the triacylglycerol component of circulating lipoprotein particles. Since insulin is the principal factor responsible for up-regulating LPL activity in adipose tissue (Kraemer et al., 1998), both insulin-deficient and -resistant forms of diabetes are associated with reduced LPL activity, and thus, the development of hypertriglyceridemia (Chen et al., 1980; Terrettaz et al., 1994). A peptide known as angiopoietin like 3 (ANGPTL3), which is predominantly expressed in the liver and is involved in lipid metabolism, has been identified (Koishi et al., 2002). This hormone decreases triglyceride clearance by inhibiting LPL activity, resulting in hypertriglyceridemia
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DISCUSSION

(Shimizugawa et al., 2002). Thus, the regulation of ANGPTL3, which suppresses LPL activity, is a pivotal issue in elucidating the link between diabetes and hyperlipidemia. ANGPTL3 regulation in the livers of diabetic mice demonstrated ANGPTL3 to be increased in both insulin-deficient and insulin-resistant diabetic states suggesting that ANGPTL3 is a link between diabetes and dyslipidaemia, with ANGPTL3 elevation promoting hyperlipidemia (Inukai et al., 2004). Both ANGPTL3 and Angiopoetin like 4 (ANGPTL4) appear to be involved in regulating lipid storage and breakdown (Oike et al., 2005). However, there was no significant change in the relative expression levels of ANGPTL3 or angiopoetin like 4 with all the treatments, when compared with control db/db mice.

As such, these coordinated responses of the methanol extract and saponins treatments seem to play an important role in regulating the lipid and glucose metabolism in db/db mice. Accordingly, both exhibit antidiabetic properties and are able to reduce certain diabetic complications related to hyperlipidemia in db/db mice.

In-vitro studies

The results of the in-vitro studies indicate that the saponins and sapogenin activate the PI3K/AKT pathway in C2C12 skeletal muscle cells as well as in the HepG2 hepatocytes. The phosphatidylinositol 3-kinase (PI3K) / protein kinase B (Akt) pathway is crucial signaling cascade triggered by insulin and growth factors. The PI3K pathway serves to protect cells against apoptosis. This effect may be important for the survival of tumour cells. The pathway plays a pivotal role for the effects of insulin, and impaired signaling through PI3K may predispose to the development of diabetes. The serine/threonine kinase Akt, also known as protein kinase B (PKB), is a central node in cell signaling downstream of growth
factors, cytokines, and other cellular stimuli and is one of the most important and versatile protein kinases at the core of human physiology and disease. Aberrant loss or gain of Akt activation underlies the pathophysiological properties of a variety of complex diseases, including type-2 diabetes and cancer (Manning and Cantley, 2007).

In response to growth factors, Akt signaling regulates nutrient uptake and metabolism in a cell-intrinsic and cell-type-specific manner through a variety of downstream targets. One of the most important physiological functions of Akt is to acutely stimulate glucose uptake in response to insulin. Akt2, the primary isoform in insulin-responsive tissues, has been found to associate with glucose transporter 4 (Glut4)-containing vesicles upon insulin stimulation of adipocytes (Calera et al., 1998), and Akt activation leads to Glut4 translocation to the plasma membrane (Kohn et al., 1996). The Rab-GAP AS160 (also known as TBC1 domain family member 4; TBC1D4) is an important direct target of Akt involved in this process (Eguez et al., 2005; Sano et al., 2007). Five putative Akt sites are phosphorylated on AS160 in response to insulin, of which S588 and T642 score the highest using the Scansite program. Importantly, mutation of these two sites to alanine significantly blocks insulin-stimulated Glut4 translocation (Sano et al., 2003). The other candidate Akt substrates involved in various steps of Glut4 translocation have been identified, including PIKfyve (Berwick et al., 2004). Glut1 is the main glucose transporter in most cell types, and unlike Glut4, it appears to be regulated primarily through alterations in expression levels. Activation of mTORC1, through Akt-mediated phosphorylation of TSC2 and PRAS40, can contribute to both HIFα-dependent transcription of the Glut1 gene and cap dependent translation of Glut1 mRNA (Zelzer et al., 1998; Taha et al., 1999).
Akt activation can also alter glucose and lipid metabolism within cells. Upon entry into the cell, glucose is converted to its active form glucose 6-phosphate through the action of hexokinases. Akt has been demonstrated to stimulate the association of hexokinase isoforms with the mitochondria, where they more readily phosphorylate glucose, but the direct target of Akt is currently unknown (Robey and Hay, 2006). Glucose 6-phosphate can be stored by conversion to glycogen or catabolized to produce cellular energy through glycolysis. The Akt signaling can regulate both of these processes. Particularly important in muscle and liver, Akt-mediated phosphorylation and inhibition of GSK3 prevents GSK3 from phosphorylating and inhibiting its namesake substrate glycogen synthase, thereby stimulating glycogen synthesis. Akt activation also increases the rate of glycolysis (Eistrom et al., 2004), and this is probably a major factor contributing to the highly glycolytic nature of tumor cells. Akt’s ability to enhance the rate of glycolysis is due, at least in part, to its ability to promote the expression of glycolytic enzymes through HIFα (Semenza et al., 1994; Majumder et al., 2004; Lum et al., 2007). In a cell-context-dependent manner, Akt-mediated phosphorylation and inhibition of FOXO1 also contribute to glucose homeostasis, as FOXO1 promotes hepatic glucose production and regulates the differentiation of cells involved in metabolic control (Accili and Arden, 2004). In hepatocytes, Akt can also inhibit gluconeogenesis and fatty acid oxidation through direct phosphorylation of S570 on PGC-1α (Li et al., 2007), which is a coactivator that can coregulate genes with FOXO1 and other transcription factors. Akt signaling regulates lipid metabolism through phosphorylation and inhibition of GSK3. As described above, phosphorylation of substrates by GSK3 often targets them for proteasomal degradation,
and GSK3 has been shown to promote degradation of the sterol regulatory element-binding proteins (SREBPs), which are transcription factors that turn on the expression of genes involved in cholesterol and fatty acid biosynthesis (Sundqvist et al., 2005). Therefore, Akt-mediated inhibition of GSK3 promotes SREBP stability and enhances lipid production. In our Western blot study, incubation with saponins and sapogenin increased the phospho-Akt (Ser473) protein levels relative to the Akt total protein in a time dependent manner. Similarly, increase in the phospho-GSK-3α/β (Ser21/9) protein relative to GSK-3β total protein was observed, which is the substrate of Akt. Activation of Akt was also observed in the immunofluorescence study. There was increase in the fluorescence of the phospho-Akt (Ser473) at 1 h, 2 h, 4 h and 6 h incubation with saponins and the sapogenin. Hence, the activation of Akt and the subsequent phosphorylation and inactivation of GSK-3α/β is responsible for the observed antihyperglycemic and antihyperlipidemic effects of the saponins observed in our studies on STZ-induced type 1 diabetic rats, HFD-STZ diabetic rats and C57BL/KsJ-db/db mice.

In the immunofluorescence study, translocation of the glucose transporter Glut4 to the myotube cell surface from the intracellular compartment was observed at 2 h, 4 h and 6 h incubation with the saponins and the sapogenin. The rate-limiting step in the uptake and metabolism of glucose by insulin in target cells is glucose transport, which is mediated by specific glucose transporters of the plasma membrane. In normal muscle cells and adipocytes, the glucose transporter isoform is Glut4, a 12-transmembrane domain protein that facilitates transport of glucose in the direction of glucose gradient (Douen et al., 1990; Barret et al., 1995). Insulin promotes Glut4 incorporation into plasma membrane, and this
translocation from intracellular compartments appears to fail in the insulin resistance present in some form of diabetes (Zierath et al., 1996; Garvey et al., 1998). Insulin resistance in type 2 diabetes is manifested by decreased insulin stimulated glucose transport and metabolism in adipocytes and skeletal muscle resulting in down-regulation of the major insulin-responsive glucose transporter, Glut4 (Kellerer et al., 1999). Molecular basis of insulin resistance depends on impaired insulin signal transduction with key defects in the glucose transport. The skeletal muscle has a paramount role in energy balance and is the primary tissue for insulin-stimulated glucose uptake and disposal (Smith and Muscat, 2005). In our Western blot study on C2C12 cells saponins and sapogenin increased the Glut4 protein as well as mRNA expression levels. In our study on HFD-STZ diabetic rats and obese C57BL/KsJ-db/db mice, these effects of saponins and the sapogenin appears to be helpful in mediating the antihyperglycemic effect in the insulin resistant animals.

In the light of above discussion, it can be concluded that:

- Treatment with the extracts and fractions of *H. isora* roots produced a significant reduction in elevated levels of glucose and lipids in experimental models of type 1 and type 2 Diabetes mellitus, the possible mechanism of these effects may be either the increase in the glucose utilization in the periphery or decrease in the endogenous glucose production in the liver of the diabetic rats.

- Treatment had no effect on serum insulin levels in type 1 Diabetes mellitus indicating that it does not release insulin.
The treatment produced reduction in serum insulin levels and improved insulin sensitivity in high fat diet fed and low dose STZ-treated type 2 diabetic rats.

In type-2 diabetic and obese C57BL/KsJ-db/db mice treatment with methanol extract and isolated saponins caused a significant reduction in the serum glucose and lipid levels and increased the expression of adipisin, ACOX, PPAR γ and Glut4 while reduced expression of FABP4 and G6Pase, whereas there was no effect on the expression levels of adiponectin, LPL, PEPCK, Glut2, ANGPTL3, ANGPTL4 and PPAR α.

In HepG2 cells and C2C12 skeletal muscle cells saponins and sapogenin activates PI3K/AKT pathway hence regulates glucose transport and also stimulates glycogen synthesis through phosphorylation and inactivation of GSK-3α and β. The activation of phospho-Akt (Ser473) and translocation of glucose transporter Glut4 to the myotube cell membrane was observed in immunofluorescence study on C2C12 myotubes.

Our results provide the molecular basis for understanding the effects of the active constituents saponins and the isolated sapogenin and the root extract having antidiabetic and antihyperlipidemic effects. For further studies, it is suggested that the saponins and the sapogenin should be further investigated as potential lead molecules for their antidiabetic and antihyperlipidemic effects, and should be evaluated for their effects on the NF-κB activation and adipocytokine changes in different in-vivo and in-vitro models and also for their effects on diabetic complications.